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Hirsutane-Type Sesquiterpenes with Uncommon Modifications from Three Basidiomycetes

Johannes C. Liermann,[†] Anja Schüffler,*[,‡] Beate Wollinsky,[‡] Judith Birnbacher,[‡] Heinz Kolshorn,[§] Timm Anke,[⊥] and Till Opatz*[,†]

[†]*Institut für Organische Chemie, Universität Hamburg, Martin-Luther-King-Platz 6, D-20146 Hamburg, Germany,* [‡]*Lehrbereich Biotechnologie, Technische Universität Kaiserslautern, Paul-Ehrlich-Strasse 23, D-67663 Kaiserslautern, Germany,* [§]*Institut für Organische Chemie, Universität Mainz, Duesbergweg 10-14, D-55128 Mainz, Germany, and* [⊥]*Institut für Biotechnologie und Wirkstoff-Forschung e.V., Erwin-Schroedinger-Strasse 56, D-67663 Kaiserslautern, Germany*

opatz@chemie.uni-hamburg.de; schueffl@ibwf.de

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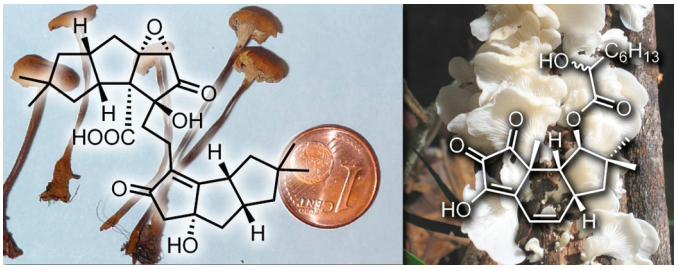

From three basidiomycetes, *Xeromphalina* sp., *Stereum* sp., and *Pleurocybella porrigens*, six triquinane sesquiterpenes with unprecendented modifications and a rearranged sesquiterpene related to coriolin C have been isolated. Their isolation, structure elucidation, and biological evaluation are described.

Introduction

About 100 000 species of fungi have been described although it is expected that the number of unidentified fungi is several hundred fold higher.[1] The broad variety of species and habitats is a challenge for studies of their chemical ecology, and fungi continue to be sources of new natural products with specialized biological activities.[2] In recent years, our ongoing search for new bioactive fungal metabolites not only has yielded virtually all classes of natural compounds, such as isoprenoids,[3,4] glycosides,[5] cyclic peptides,[6] and polyketides,[7] but also has often furnished natural compounds with unexpected modifications[8,9] or unusual dimers.[10,11]

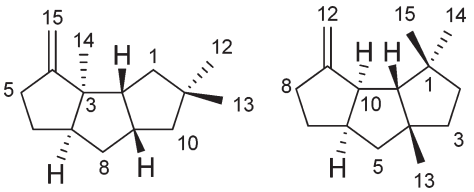

FIGURE 1. Two common linear triquinane scaffolds.

Basidiomycetes are a rich source for structurally diverse sesquiterpenes,[12] with the linear triquinanes as a prominent compound class of more than 70 reported examples discovered since 1947.[13] Linear triquinanes are categorized according to their ring scaffold with the hirsutanes[13] and capnellenes[14] being the most common ones (Figure 1). There are

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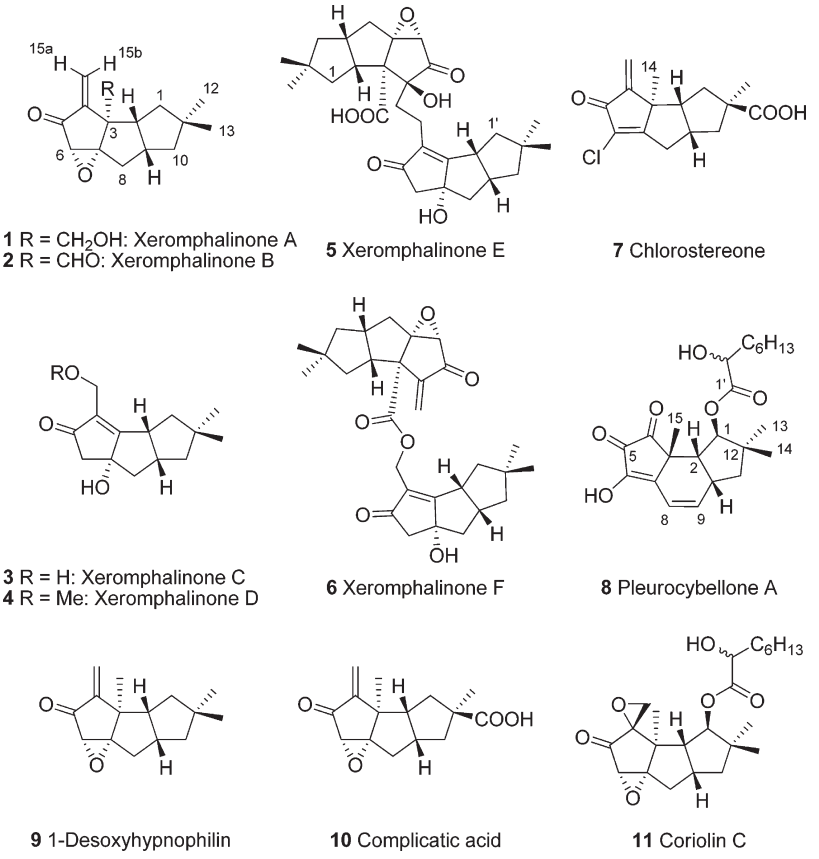
CHART 1



about 30 reported examples for each skeleton. Modifications of these scaffolds are manifested in oxygenation, unsaturation, degradation to norsesquiterpenes, or rearrangement to form different ring skeletons like protoilludenes.[15] Many linear triquinanes possess antibiotic and cytotoxic activities.[12]

In this paper, we describe the isolation, structure elucidation, and biological evaluation of seven new sesquiterpenoids originating from three basidiomycetes.

Results and Discussion

Six compounds were isolated from *Xeromphalina* sp. and were named xeromphalinones A−F (**1**−**6**). From the MS and UV data, it was assumed that they might be triquinane sesquiterpenoids so far undescribed from this genus. To our knowledge, only the fungal pigment xerocomic acid has been reported as a metabolite from *Xeromphalina* sp.[16]

According to high-resolution MS, xeromphalinone A (**1**) and B (**2**) had elemental compositions of $C_{15}H_{20}O_3$ and $C_{15}H_{18}O_3$, respectively, and exhibited great similarity with respect to their UV and IR spectra as well as their MS fragmentation pattern (Chart 1). Moreover, both compounds gave similar ^{1}H NMR spectra (Table 1) which in either case revealed an *exo* methylene group and two methyl singlets. ^{13}C NMR spectra (Table 2) revealed one ketone carbonyl around δ_C 195 and three aliphatic methylene groups for both compounds. In addition, xeromphalinone A (**1**) gave two methylene protons at δ_H 3.94 and 3.81, coupled with an hydroxyl resonance at δ_H 1.94 in an ABX spin system. These signals were missing in the spectra of xeromphalinone B (**2**) and a sharp singlet at δ_H 9.80 as well as a corresponding methine at δ_C 200.2 were found instead, obviously resulting from the oxidized form of alcohol **1**. HMBC data revealed that the methyl singlets corresponded to a geminal dimethyl fragment neighbored by two methylene groups. The methylene groups gave COSY correlations to two methine groups, forming a five-membered ring. Another five-membered ring could be assigned to be anellated by using the COSY and HMBC correlations given in Figure 2. HMBC correlations from the *exo* methylene and the angular side chain protons could be used to establish the last ring containing an epoxide as judged from the elemental composition and the absence of further hydroxy protons exchanging with residual water (NOESY). In the 2D NMR spectra, similar correlations were found for both compounds which have been summarized in Figure 2. The relative configuration could be established by NOESY, and the absolute configuration of both compounds can be safely assumed to be identical with that of the closely related compound **9**, the specific rotation of which matches well the values found for **1** and **2**.[17] While the oxidation of the geminal methyl group C-13 is commonly found in

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TABLE 1. ^{1}H NMR (400 MHz) of Xeromphalinones A−D (**1**−**4**) and Chlorostereone (**7**) and Coupling Constants (*J*, in Hz)

	1[a]	**2**[a]	**3**[b]	**4**[a]	**7**[a]
1	1.64, dd (12.3, 10.5) 1.51, ddd (12.3, 8.7, 1.9)	1.63, ddd (12.7, 8.4, 1.8) 1.51, dd (12.7, 10.5)	1.76, m	1.86, dd (11.9, 9.0) 1.76, m	2.41, ddd (13.1, 8.6, 1.6) 1.63, dd (13.1, 8.6)
2	2.53, ddd (11.3, 10.5, 8.7)	2.69, td (10.5, 8.4)	3.55, q (9.2)	3.53, q (9.0)	2.50, dt (11.0, 8.6)
3					
4					
5					
6	3.44, s	3.47, s	2.40, d (17.8) 2.34, d (17.8)	2.56, d (17.9) 2.48, d (17.9)	
7					
8	2.07, dd (13.7, 9.1) 2.02, dd (13.7, 8.2)	2.33, dd (13.5, 9.0) 2.17, dd (13.5, 8.3)	1.96, m 1.68, dd (13.7, 9.2)	2.04, d (14.0) 1.75, m	2.96, dd (15.6, 8.4) 2.27, dd (15.6, 7.0)
9	2.76, ddddd (11.3, 10.6, 9.1, 8.2, 7.7)	2.89, m	2.99, dqd (9.9, 9.2, 1.0)	3.03, pseudo quin (9.0)	2.81, m
10	1.79, ddd (12.2, 7.7, 1.9) 1.30, dd (12.2, 10.6)	1.84, ddd (12.5, 7.8, 1.8) 1.31, dd (12.5, 10.0)	1.80, 1.72, m	1.79, m	2.57, ddd (12.6, 7.4, 1.6) 1.37, dd (12.6, 11.8)
11					
12	1.12, s	1.10, s	1.06, s	1.09, s	1.43, s
13	0.91, s	0.91, s	0.99, s	1.00, s	
14	3.94, dd (11.2, 4.0) 3.81, dd (11.2, 6.8)	9.80, s			1.22, s
15	6.18, s 5.42, s	6.37, s 5.70, s	4.19, br d (14.3) 4.15, br d (14.3)	4.13, d (12.6) 4.08, d (12.6)	6.04, s 5.27, s
OH-7			3.10, br s	−[c]	
OH-14	1.93, dd (6.8, 4.0)				
OH/OCH_3-15			2.86, br s	3.36, s	

[a]In $CDCl_3$. [b]In MeCN-d_3. [c]Not observed.

TABLE 2. ^{13}C NMR (101 MHz) of Xeromphalinones A−D (**1**−**4**) and chlorostereone (**7**)

	1[a]	**2**[a]	**3**[b]	**4**[a]	**7**[a]
1	39.8, CH_2	41.1, CH_2	47.2, CH_2	47.0, CH_2	37.0, CH_2
2	49.8, CH	52.5, CH	43.3, CH	43.0, CH	48.7, CH
3	50.5, qC	61.9, qC	183.8, qC	185.3, qC	50.9, qC
4	150.1, qC	145.6, qC	135.9, qC	133.3, qC	151.0, qC
5	197.1, qC	195.5, qC	209.0, qC	207.7, qC	189.8, qC
6	60.8, CH	59.0, CH	51.3, CH_2	51.0, CH_2	126.7, qC
7	76.3, qC	74.8, qC	85.3, qC	85.5, qC	180.2, qC
8	31.7, CH_2	32.1, CH_2	42.1, CH_2	41.7, CH_2	30.8, CH_2
9	39.3, CH	40.4, CH	46.5, CH	45.9, CH	44.9, CH
10	48.7, CH_2	48.5, CH_2	49.2, CH_2	48.8, CH_2	46.2, CH_2
11	42.9, qC	43.1, qC	43.5, qC	43.3, qC	54.9, qC
12	29.1, CH_3	28.8, CH_3	28.7, CH_3	28.6, CH_3	24.4, CH_3
13	26.9, CH_3	27.0, CH_3	26.6, CH_3	26.7, CH_3	182.9, qC
14	63.9, CH_2	200.2, CH			23.6, CH_3
15	115.7, CH_2	124.8, CH_2	55.7, CH_2	65.1, CH_2	115.7, CH_2
OCH_3-15				59.1, CH_3	

[a]In $CDCl_3$. [b]In MeCN-d_3.

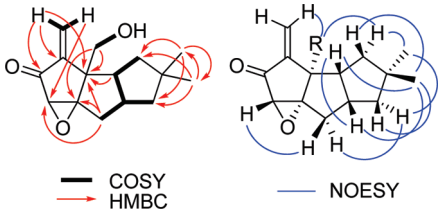

FIGURE 2. Significant 2D NMR correlations for **1** and **2**.

triquinanes, to the best of our knowledge, xeromphalinones A (**1**) and B (**2**) are the first examples for hirsutane triquinanes in which the angular methyl group C-14 is oxidized.

Xeromphalinone C (**3**) was less lipophilic than **1** or **2**, and mass spectrometric analyses indicated an elemental composition of $C_{14}H_{20}O_3$, so one carbon of the original sesquiterpene body was missing. Analysis of 2D NMR spectra showed that the right half of the molecule was identical with

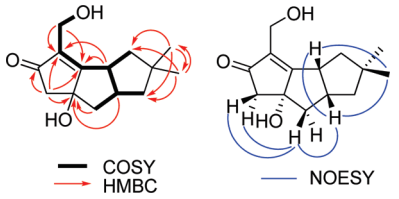

FIGURE 3. Significant 2D NMR correlations for **3**.

that of **1** and **2** while the left-most ring was found to be modified. The angular carbon C-7 was found at δ_C 85.3 and should carry an oxygen substituent as in **1** and **2**. A methylene AB spin system showed strong HMBC correlations to both C-7 and a ketone carbonyl at δ_C 209.0 and should be located in between these two carbons as judged by the geminal coupling constant of 17.8 Hz and the absence of other scalar couplings. The ketone and two quaternary carbons at δ_C 135.9 and 183.4 form a hydroxymethylated enone system constituting the third ring. Therefore, xeromphalinone C (**3**) belongs to the norhirsutanes, and the existence of an analogous capnellene-based compound has been reported in 2008,[18] the NMR data of which are in accordance with those recorded for **3**. The relative configuration was assigned by using NOESY to be in analogy with **1** and **2** (Figure 3). The absolute configuration of **3** was assumed to be identical with that of the other xeromphalinones due to the close structural relationship.

Xeromphalinone D (**4**) had an elemental composition of $C_{15}H_{22}O_3$, and its ^{13}C NMR (table 2) spectroscopic data were in striking accordance with those of **3** except for the methylene C-15, which was moderately shifted downfield to δ_C 65.1. An additional methoxy group was found resonating at δ_H 3.36, forming an ether with the oxygen located at C-15. Therefore, xeromphalinone D (**4**) is the methyl ether of **3**.

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TABLE 3. ^{1}H (400 MHz) and ^{13}C NMR (101 MHz, $CDCl_3$) of Xeromphalinones E (**5**) and F (**6**) in $CDCl_3$

	xeromphalinone E (**5**)		xeromphalinone F (**6**)	
	δ_H, *J* in Hz	δ_C, mult	δ_H, *J* in Hz	δ_C, mult
1	1.71, m	44.1, CH_2	1.69, m	43.6, CH_2
	1.44, dd (13.1, 10.0)		1.23, dd (12.4, 10.7)	
2	2.77, ddd (11.3, 10.0, 8.3)	45.5, CH	2.63, td (10.7, 8.2)	53.3, CH
3		62.0, qC		59.6, qC
4		81.0, qC		146.6, qC
5		209.1, qC		195.8, qC
6	3.26, s	58.7, CH	3.43, s	61.2, CH
7		74.3, qC		75.0, qC
8	2.51, dd (12.5, 8.9)	33.6, CH_2	2.45, dd (13.0, 9.3)	32.3, CH_2
	1.92, dd (12.5, 8.1)		2.03, dd (13.0, 8.4)	
9	2.66, m	38.5, CH	2.79, m	39.9, CH
10	1.71, m	48.1, CH_2	2.78, m	48.1, CH_2
	1.18, dd (12.2, 10.7)		1.31, dd (12.3, 9.8)	
11		42.3, qC		42.6, qC
12	1.06, s	29.1, CH_3	1.08, s	28.9, CH_3
13	0.91, s	26.8, CH_3	0.88, s	26.9, CH_3
14		172.6, qC		170.6, qC
15	2.13, td (13.3, 4.8)	34.9, CH_2	6.32, s	126.5, CH_2
	1.89, m		5.93, s	
1′	1.79, 1.66, m	46.4, CH_2	1.83, 1.71, m	46.7, CH_2
2′	3.26, q (9.2)	42.5, CH	3.49, q (9.2)	42.6, CH
3′		185.4, qC		186.6, qC
4′		135.7, qC		130.6, qC
5′		211.3, qC		206.5, qC
6′	2.45, d (18.2)	50.5, CH_2	2.58, d (18.0)	50.6, CH_2
	2.40, d (18.2)		2.47, d (18.0)	
7′		84.6, qC		85.3, qC
8′	2.04, br d (13.9)	41.1, CH_2	2.08, d (14.0)	41.6, CH_2
	1.63, dd (13.9, 9.2)		1.73, m	
9′	2.97, pseudo quin (9.2)	46.0, CH	3.02, pseudo quin (9.2)	45.9 CH
10′	1.80, 1.72, m	48.5, CH_2	1.77, m	48.7, CH_2
11′		43.4, qC		43.4, qC
12′	1.06, s	28.5, CH_3	1.07, s	28.4, CH_3
13′	0.97, s	26.9, CH_3	0.96, s	26.3, CH_3
15′	2.31, td (13.3, 4.8)	18.0, CH_2	4.87, d (12.5)	56.7, CH_2
	1.72, m		4.82, d (12.5)	

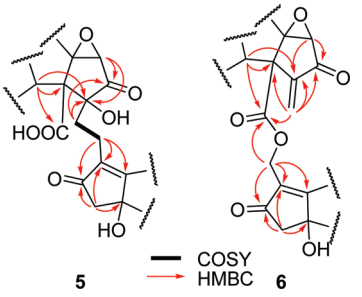

FIGURE 4. Significant 2D NMR correlations for **5** and **6**.

While compounds **1**−**3**, **5**, and **6** were identified in the culture fluid by HPLC/MS before workup, xeromphalinone D (**4**) could only be detected when methanol was used during isolation.

As was apparent from its elemental composition of $C_{29}H_{38}O_7$, xeromphalinone E (**5**) had a more complex structure. ^{1}H, ^{13}C, and 2D NMR spectra (table 3) allowed identification of two substructures which corresponded closely to the aforementioned discrete xeromphalinones. One half of the molecule was related to xeromphalinones A (**1**) and B (**2**) with C-14 having the oxidation state of a carboxylic acid. The exocyclic double bond was, however, missing, and the quaternary C-4 had a chemical shift of δ_C 81.0 typical for a tertiary alcohol. HMBC data (Figure 4) showed that a methylene group (δ_C 34.9) was attached to that quarternary carbon which itself was part of an ethylene bridge. The other methylene group was attached to the same pentacyclic enone as in xeromphalinones C (**3**) and D (**4**), the scaffold of which constituted the second half of the dimeric compound **5**. The relative configuration was established by NOESY to be consistent with that of compounds **1** and **3**. The absence of NOE correlations between H-6 and H-15 suggested the depicted (*S*)-configuration of C-4 although that assignment remained tentative in lack of further supporting data.

Xeromphalinone F (**6**) promised to have a similar dimeric structure considering its elemental composition of $C_{29}H_{36}O_6$. Again, two xeromphalinone substructures could be identified from the NMR data (Table 3), but the exocyclic double bond was intact in this case. As already observed in **5**, C-14 was oxidized to a carboxyl function as became apparent from its chemical shift (δ_C 170.6), and an HMBC correlation (Figure 4) to this carbon was found from two methylene protons at δ_H 4.87 and 4.82, belonging to the alcohol component of an ester. This methylene group was attached to an enone system belonging to the same norhirsutane scaffold found in xeromphalinone C (**3**).

The formation of **6** is remarkable only to the extent that an accidental esterification of the two fragments appears unlikely. In contrast, the origin of the ethylene bridge between the two halves of **5** is somewhat enigmatic since it seems to be

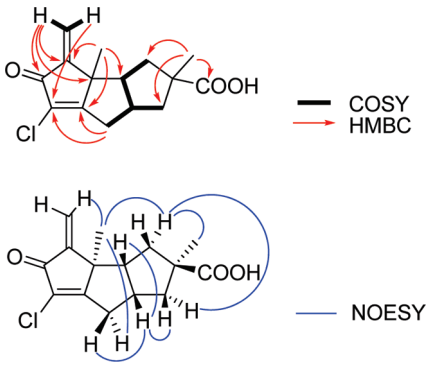

FIGURE 5. Significant 2D NMR correlations for **7**.

formed against the natural reactivity pattern by linking two electrophilic carbons.

From *Stereum* sp., chlorostereone (**7**) and complicatic acid (**10**)[19] were isolated. This well-investigated genus is a rich source for sesquiterpenoids, especially for hirsutane-based compounds like hirsutic acid C[13] or the hirsutenols A−F.[20,21] Mass spectra of **7** showed an isotopic pattern typical for the presence of one chlorine atom which could be confirmed by high-resolution mass spectrometry, giving an elemental composition of $C_{15}H_{17}ClO_3$. Again, NMR spectra (Tables 1 and 2) showed an *exo* methylene group, two methyl singlets, three aliphatic methylenes, and two methines. HMBC data indicated that the methyl groups were located at different positions in the hirsutane skeleton. The methyl group at δ_H 1.43 showed an HMBC correlation to a quaternary carbon at δ_C 182.9, which corresponds to one of the geminal methyl groups from the hirsutane scaffold oxidized to a carboxylic acid. The aliphatic parts were assigned by COSY, and the left-most ring was assigned by using HMBC correlations from the *exo* methylene protons and the angular methyl group to contain an α-chlorinated enone. The relative configuration was determined by NOESY as shown in Figure 5, and the absolute configuration should be identical with that of the synthetic nonchlorinated analogue,[22] the optical rotation of which is in accordance with that of **7**. Chlorostereone (**7**) probably originates from the same biosynthetic pathway as complicatic acid (**10**) also isolated from the same organism. To our knowledge, **7** is the first example for a chlorinated triquinane from a terrestrial fungus. Two chlorinated triquinanes, chloriolins B and C, have been described from an unidentified fungus found in the Indo-Pacific sponge *Jaspis* aff. *johnstoni*.[23]

From *Pleurocybella porrigens*, pleurocybellone A (**8**), a new compound, and the known coriolin C (**11**)[24,25] were

TABLE 4. ^{1}H (400 MHz) and ^{13}C NMR (101 MHz, $CDCl_3$) of Pleurocybellone A (**8**) in $CDCl_3$

	δ_H, J in Hz	δ_C, mult
1	4.73, d (10.8)	80.0, CH
2	2.71, dd (10.8, 7.4)	46.3, CH
3		44.2, qC
4		199.6, qC
5		181.4, qC
6		152.4, qC
7		142.5, qC
8	6.75, dd (9.8, 2.6)	117.4, CH
9	6.14, dd (9.8, 3.1)	143.7, CH
10	3.05, m	35.3, CH
11	2.09, dd (13.7, 10.3)	42.6, CH_2
	1.46, dd (13.7, 4.0)	
12		40.2, qC
13	0.97, s	24.5, CH_3
14	0.96, s	28.6, CH_3
15	1.31, s	24.4, CH_3
1′		176.1, qC
2′	4.18, dd (7.3, 4.0)	70.7, CH
3′	1.76, 1.60, m	33.6, CH_2
4′	1.46, 1.29, m	24.5, CH_2
5′	1.31, 1.27, m	29.2, CH_2
6′	1.25, m	31.9, CH_2
7′	1.28, m	22.7, CH_2
8′	0.86, pseudo-t (6.9)	14.2, CH_3

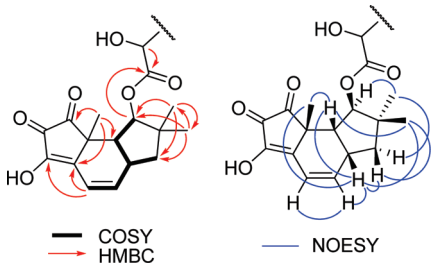

FIGURE 6. Significant 2D NMR correlations for **8**.

isolated. Previous investigations of this fungus yielded particularly amino acids[26,27] and fatty acids[28,29] but to our knowledge no sesquiterpenoid metabolites have been described so far. Mass spectrometric analyses of **8** gave an elemental composition of $C_{23}H_{32}O_6$. Eight carbon atoms belonged to an α-hydroxy octanoyl side chain, leaving 15 carbons for the sesquiterpenoid core. The ^{1}H NMR spectrum (Table 4) showed two protons at δ_H 6.75 and 6.14, coupled with a coupling constant of 9.8 Hz, indicating a (*Z*)-configured double bond. COSY and NOESY indicated that the proton at δ_H 6.14 was neighbored by a methine group belonging to an aliphatic five-membered ring that also carried two geminal methyl groups and an oxygen function. This oxygen function was acylated to form the hydroxyoctanoate described before as became apparent from an HMBC correlation between the methine proton at δ_H 4.74 and the ester carbonyl at δ_C 175.6. The other olefinic proton at δ_H 6.75 gave an HMBC correlation to a quaternary carbon at δ_C 141.0, which also correlated with the angular methyl group at δ_H 1.33. This methyl group was additionally coupled with a carbonyl at δ_C 199.6 and the second methine in the aliphatic

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TABLE 5. Antimicrobial Activities of Compounds 1−8 (inhibition zone in mm, 50 μg/disc[a])

	Penicillium notatum	*Paecilomyces variotii*	*Mucor miehei*	*Nematospora coryli*	*Micrococcus luteus*	*Enterobacter dissolvens*	*Bacillus brevis*	*Bacillus subtilis*
1	13	29	40	12	25	11	36	37
2	13	20	40	12	22	11	34	30
3								
4				10				
5								
6	+		13	+	15		19	18
7	+		15 i	35 i			16	16
8	+	+	+	15 i	22		27	25

[a]Disk diameter: 6 mm; + inhibition zone < 10 mm; i: inhibition zone incomplete; all other tests negative.

five-membered ring, the latter constituting a six-membered ring containing the double bond. HMBC correlations from δ_H 6.75 allowed the detection of a quaternary carbon at δ_C 152.4 adjacent to C-7, and together with the remaining carbonyl at δ_H 181.4, the left-most ring can be constituted as an enolized 1,2,3-cyclopentanetrione. This enolized trioxoindane-like bicyclic ring system is also reported for palbinone with nearly identical ^{13}C NMR shifts.[30,31] The relative stereochemistry was assigned by NOESY (Figure 6). Since the right part of the molecule is identical with that of **11** also isolated from *P. porrigens*, the depicted absolute configuration was tentatively assigned. Because both pleurocybellone A (**8**) and coriolin C (**11**) are produced by the same fungus and have an α-hydroxyoctanoate side chain, it is very likely that they are closely related in their biogenesis.

Biological Activities and Reactivity with Cysteine. In accordance with previous examples like pleurotellol and structurally related hirsutanes[32] or the creolophins,[33] all isolated metabolites lacking the exocyclic enone moiety exhibited no or reduced antibiotic activities against fungi and bacteria (Table 5). The cytotoxicities (IC_{50} value) against Jurkat cells of xeromphalinone A (**1**), B (**2**), F (**6**), chlorostereone (**7**), and pleurocybellone A (**8**) ranged from 1 to 5 μg/mL (2 to 20 μM). For xeromphalinones C (**3**), D (**4**), and E (**5**) the IC_{50} values exceeded 50 μg/mL (above 100 μM). The formation of cysteine adducts was observed by HPLC-MS for xeromphalinones A (**1**), B (**2**), and F (**6**) as well as for chlorostereone (**7**) and pleurocybellone A (**8**).

Experimental Section

Materials and Methods. Melting points were determined with a Dr. Tottoli apparatus and are uncorrected. Optical rotations were measured at 589 nm. UV and IR spectra were measured with a spectrophotometer and an FTIR spectrometer, respectively. NMR spectra were recorded with a 400 MHz spectrometer. The chemical shifts were referenced to the residual solvent signal ($CDCl_3$: δ_H 7.26 ppm, δ_C 77.16 ppm; MeCN-d_3: δ_H 1.94 ppm, δ_C 1.32 ppm).[34] ESIMS and HRESIMS spectra were recorded on quadrupole TOF mass spectrometers. FABMS spectra were measured with Xe-FAB ionization, using *m*-nitrobenzyl alcohol or glycerol as the matrix. HRFABMS data were measured with PEG 300 or 600 as an internal reference. HPLC/MS analyses were performed with a HPLC/MSD system.

Producing Organism. The producing fungi (*Xeromphalina* sp. IBWF07014, *Stereum* sp. IBWF01082, and *Pleurocybella porrigens* IBWF96022) are deposited in the culture collection of the Institute of Biotechnology and Drug Research (IBWF e.V.), Kaiserslautern, Germany. For maintenance, the fungi were grown on YMG agar slants (yeast extract 4 g/L, malt extract 10 g/L, glucose 4 g/L, the pH value was adjusted to 5.5 before autoclaving). Solid media contained 2% agar.

Fermentation and Isolation of the Triquinanes. Xeromphalinones A−F (1−6): The fungus IBWF07014 was grown in YMG medium in a 20 L fermenter at 22−24 °C with agitation (130 rpm) and aeration (3 L/min). For inoculation, a well-grown shake culture (250 mL) in the same medium from a 500 mL Erlenmeyer flask was used. During the fermentation, the carbon source was depleted after 14 days. Then, the culture fluid was separated from the mycelia through filtration. The xeromphalinones were isolated as shown in Scheme 1 in the Supporting Information. The purification of the compounds was achieved by preparative HPLC (Nucleosil 100-5, C18, 250 × 21 mm) with the eluents MeCN and 0.1% HCOOH.

Compound **4** was found to be an artifact derived from **3** by the extraction with MeOH (later discontinued). This was proven by HPLC/MS analysis (see text).

Chlorostereone (7): *Stereum* sp. IBWF01082 was grown in malt medium (malt extract 40 g/L, the pH value was adjusted to 5.5 before autoclaving) in a 20 L fermenter at 22−24 °C with agitation (130 rpm) and aeration (3 L/min). For inoculation, a well-grown shake culture (250 mL) in the same medium was used. During fermentation, the carbon source was depleted after 16 days. The fermentation was stopped and the culture fluid was separated from the mycelia through filtration. The culture fluid (14 L) was extracted twice with EtOAc and the combined extracts were dried. This crude extract (3.6 g) was applied onto silica gel (0.063−0.2 mm). Elution with cyclohexane/EtOAc (1:1) yielded 785.4 mg of an intermediate. With a MeCN/H_2O gradient (30% to 55% MeCN in 25 min, flow: 20 mL/min) 8.3 mg of chlorostereone (**7**) (54% MeCN; 24 min) was isolated by preparative HPLC (RP 18, 7 μm, 250 × 25 mm).

Along with chlorostereone (**7**), complicatic acid (**10**)[19] was purified from crude extracts.

Pleurocybellone A (8): *Pleurocybella porrigens* IBWF96022 was grown in YMG medium in a 100 L fermenter at 24 °C with agitation (150 rpm) and aeration (15 L/min). For inoculation, a 10 L portion of a well-grown 20 L fermentation in the same medium was used. During fermentation, the carbon source was depleted after 62 h. The fermentation was stopped and the culture fluid was separated from the mycelia through filtration. The culture fluid (78 L) was absorbed to DIAION HP 21 and eluted with MeOH. This crude extract (17.9 g) was applied onto silica gel (0.063−0.2 mm). Elution with cyclohexane/EtOAc (7:3) yielded 2.85 g of intermediate. The purification of 0.366 g of pleurocybellone A (**8**) (rt 19 min) was achieved by preparative

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HPLC (Nucleosil 100-7, RP18, 250 × 21 mm) with H_2O/acetonitril gradient (57% to 70% MeCN in 23 min; flow: 20 mL/min). In addition, coriolin C (**11**)[24,25] was isolated.

Biological Activity and Reactivity with Cysteine. The antimicrobial activities against bacteria and fungi were determined by using the agar diffusion assay as described previously.[35] The cytotoxicity of the compounds was assessed by using Jurkat cells (ATCC TIB 152). The assay was performed as described before.[36] The formation of cysteine adducts was tested by using 10 mM of the compound (diluted in MeCN:H_2O 1:1) and incubation for 1 h at room temperature with 20 mM L-cysteine (diluted in H_2O) and was verified by HPLC-MS analysis.

Xeromphalinone A (1): yellow oil; $[\alpha]^{26}_D$ −130 (*c* 0.28, $CDCl_3$); UV (MeCN) λ_{max} (log ε) 233 (3.71) nm; IR (KBr) ν 3437, 2954, 1727, 1639, 1261, 1049 cm^{-1}; NMR data see Tables 1 and 2; APCIMS (pos.) *m*/*z* (%) 249.1 (100) $[M + H]^+$, 231.1 (38) $[M - OH]^+$, 219.1 (62) $[M - CHO]^+$, 201.1 (93) $[M - CHO - H_2O]^+$; HRESIMS *m*/*z* 271.1309 ($C_{15}H_{20}O_3$+Na^+ requires 271.1310).

Xeromphalinone B (2): yellow oil; $[\alpha]^{26}_D$ −177 (*c* 0.40, $CDCl_3$); UV (MeCN) λ_{max} (log ε) 228 (3.58), 298 (2.56) nm; IR (KBr) ν 3430, 2930, 1732, 1633, 1368, 1112 cm^{-1}; NMR data see Tables 1 and 2; APCIMS (pos.) *m*/*z* (%) 247.1 (60) $[M + H]^+$, 219.1 (75) $[M - CO + H]^+$, 201.1 (100) $[M - CO - OH]^+$; HRESIMS *m*/*z* 269.1160 ($C_{15}H_{18}O_3 + Na^+$ requires 269.1154).

Xeromphalinone C (3): yellow crystals, mp 161−162 °C; $[\alpha]^{24}_D$ −156 (*c* 0.28, MeCN); UV (MeCN) λ_{max} (log ε) 231 (4.12) nm; IR (KBr) ν 3430, 2954, 1712, 1664, 1383, 1056 cm^{-1}; NMR data see Tables 1 and 2; FABMS (pos.) *m*/*z* (%) 259.1 (16) $[M + Na]^+$, 237.1 (100) $[M + H]^+$, 129.1 (52) $[M - OH]^+$, 201.1 (33) $[M - H_2O - OH]^+$; HRFABMS *m*/*z* 237.1486 ($C_{14}H_{20}O_3 + H^+$ requires 237.1491).

Xeromphalinone D (4): yellow oil; $[\alpha]^{22}_D$ −110 (*c* 0.28, $CDCl_3$); UV (MeCN) λ_{max} (log ε) 229 (4.06) nm; IR (KBr) ν 3423, 2925, 1708, 1667, 1383, 1057 cm^{-1}; NMR data see Tables 1 and 2; ESIMS *m*/*z* (%) 523.3 (52) $[2M + Na]^+$, 273.1 (100) $[M + Na]^+$, 219.1 (50) $[M - OCH_3]^+$; HRESIMS *m*/*z* 273.1464 ($C_{15}H_{22}O_3 + Na^+$ requires 273.1461).

Xeromphalinone E (5): greenish oil; $[\alpha]^{22}_D$ −126 (*c* 0.41, $CDCl_3$); UV (MeCN) λ_{max} (log ε) 234 (4.23), 308 (2.37) nm; IR (KBr) ν 3434, 2953, 1708, 1667, 1383, 1057 cm^{-1}; NMR data see Table 3; ESIMS *m*/*z* (%) 1019.5 (27) $[2M + Na]^+$, 521.3 (69) $[M + Na]^+$; 499.3 (100) $[M + H]^+$, 481.3 (27) $[M - OH]^+$; HRESIMS *m*/*z* 521.2513 ($C_{29}H_{38}O_7 + Na^+$ requires 521.2510).

Xeromphalinone F (6): brownish oil; $[\alpha]^{22}_D$ −151 (*c* 0.47, $CDCl_3$); UV (MeCN) λ_{max} (log ε) 229 (4.32) nm; IR (KBr) ν 3436, 2952, 1731, 1208, 1057 cm^{-1}; NMR data see Table 3; ESIMS *m*/*z* (%) 983.5 (75) $[2M + Na]^+$, 503.2 (100) $[M + Na]^+$; 481.3 (23) $[M + H]^+$, 463.2 (48) $[M - OH]^+$; HRESIMS *m*/*z* 503.2407 ($C_{29}H_{36}O_6 + Na^+$ requires 503.2404).

Chlorostereone (7): yellow oil; $[\alpha]^{26}_D$ +28.1 (*c* 0.43, $CDCl_3$); UV (MeCN) λ_{max} (log ε) 259 (3.77), 330 (2.67) nm; IR (KBr) ν 3390, 2945, 1706, 1467, 1029 cm^{-1}; NMR data see Tables 1 and 2; APCIMS (neg.) *m*/*z* (%) 281.1 (39) $[M(^{37}Cl) - H]^-$, 279.1 (100) $[M(^{35}Cl) - H]^-$; HRESIMS *m*/*z* 303.0774 ($C_{15}H_{17}{}^{35}ClO_3 + Na^+$ requires 303.0764).

Pleurocybellone A (8): red oil; $[\alpha]^{25}_D$ +32.8 (*c* 0.25, $CDCl_3$); UV (MeOH) λ_{max} (log ε) 235 (4.00), 381 (3.86) nm; IR (KBr) ν 3436, 2929, 1738, 1701, 1602, 1431, 1089 cm^{-1}; NMR data see Table 4; APCIMS (neg.) *m*/*z* (%) 403.2 (100) $[M - H]^-$; HRESIMS *m*/*z* 427.2121 ($C_{23}H_{32}O_6 + Na^+$ requires 427.2097).

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Supporting Information Available: Purification scheme of **1**−**6** and ^1H and ^{13}C NMR spectra for **1**−**8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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