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Hygrophorones A–G: fungicidal cyclopentenones from *Hygrophorus* species (Basidiomycetes)

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Dedicated to Professor Dr. Dr. h. c. W. Steglich on the occasion of his 70th birthday.

Abstract

Twenty new 5-(hydroxyalkyl)-2-cyclopentenone derivatives (hygrophorones) could be isolated from *Hygrophorus latitabundus*, *H. olivaceoalbus*, *H. persoonii*, and *H. pustulatus*. Their fungicidal activity was exemplarily tested. The hygrophorones have structural similarities to the antibiotic pentenomycin. Chemically, hygrophorones are 2-cyclopentenones with hydroxy or acetoxy substituents at C-4 and/or C-5. An odd-numbered 1′ oxidized alkyl chain (C_{11}, C_{13}, C_{15}, or C_{17}) is attached at C-5. In addition, from *H. persoonii* the new γ-butyrolactone derivative [5-(*E*)-2-hydroxytetradexylidene-5*H*-furan-2-one] could be isolated. Some hygrophorones are responsible for the color reaction of the stipes of these fungi upon treatment with potassium hydroxide solution. Structural elucidations are based on 1D (^{1}H, ^{13}C) and 2D (COSY, NOESY, HSQC, HMBC) NMR spectroscopic analyses as well as HR-FT-ICR-MS investigations.

Keywords: *Hygrophorus latitabundus*; *Hygrophorus olivaceoalbus*; *Hygrophorus persoonii*; *Hygrophorus pustulatus*; Basidiomycetes; Fungi; Natural Products; Hygrophorones; Cyclopentenone derivatives

1. Introduction

The genus *Hygrophorus* (German: Schnecklinge; English: wax caps) includes about 60 species. They are obligate symbionts (mycorrhiza) with deciduous or coniferous trees. Fruit bodies of the wax caps are characterized in particular by a very slimy pileus surface with thick and waxy lamellae. *Hygrophorus persoonii* Arnolds, *H. pustulatus* (Pers.) Fr., *H. latitabundus* Britz., and *H. olivaceoalbus* (Fr.: Fr.) Fr. belong to the section *Olivaceoumbrini* (Bataille) Konr. et M. in *Hygrophorus* (Bas et al., 1980). The color reaction of the stipe with potassium hydroxide solution is an important taxonomic feature. The stipes of *H. persoonii* and *H. latitabundus* turn yellow to brown when a drop of potassium hydroxide solution is applied. Also *H. pustulatus* shows these color reaction, which was unknown so far. Interestingly, *H. olivaceoalbus* does not show this color reaction although it belongs to the same section in *Hygrophorus*. Field observations revealed that fruit bodies of this genus are hardly ever attacked by parasitic fungi. Both, the color reaction as well as the ecological observations prompted us to look for their chemical basis.

2. Results and discussion

Hygrophorones, named after the producing genus *Hygrophorus,* are soluble in petroleum ether, methanol, or chloroform but not in water. Therefore they can easily be extracted from intact or rapidly cut fungal material with petroleum ether. The yield of extraction is higher with fresh or frozen fungal material than with lyophilized fruit bodies. All hygrophorones are sensitive to bases and strong acids. They can easily be detected on tlc plates either by developing them with hydroxide solution or by heating, where upon hygrophorones color yellow to brown. The second method is easier to apply and more sensitive.

The petroleum ether extract of *H. persoonii* yielded six new compounds (**1**–**6**) after separation by SPE and

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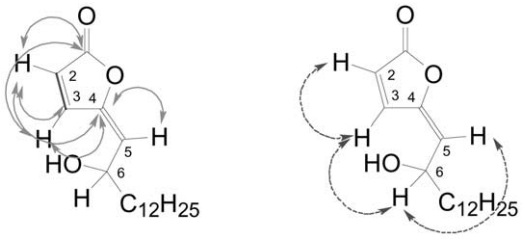
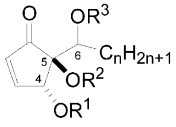


Fig. 1. Important HMBC (left) and NOE (right) crosspeaks for **27**.

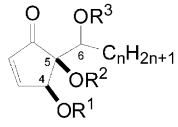
HPLC. The ^{1}H NMR spectrum (Table 1) of 4,6-di-*O*-acetyl hygrophorone A^{12} (**1**) showed signals of two acetate groups [δ ^{1}H = 2.136 (3H, *s*), 1.996 (3H, *s*)] which could be observed in ^{13}C NMR (δ ^{13}C = 170.5, 20.9; 169.9, 20.7), too (Table 2). A peak at δ ^{13}C = 202.3 ppm in the ^{13}C NMR spectrum indicated the presence of a carbonyl group, signals at δ ^{13}C = 134.4 ppm and 156.8 ppm belong to a carbon–carbon double bond. Interpretation of the HMBC spectrum showed that a five-membered ring was formed by the C=C double bond, the C atom of the carbonyl group, and two C atoms (δ ^{13}C = 79.7 ppm and 81.4 ppm). Furthermore, the acetate groups were attached to C-4 and C-6. A long unbranched and saturated alkyl chain (–$C_{12}H_{25}$) was connected to the latter. Correlation peaks in the HMBC spectrum between the proton of the OH group and C-1, C-4, and C-5 indicated that the OH group was attached at C-5. HMBC correlation peaks of H-6 and C-1, C-4, and C-5 evidenced the connection of C-6 to C-5. COSY and NOESY measurements were consistent with the proposed structure. The molecular formula of 4,6-di-*O*-acetyl hygrophorone A^{12} (**1**) was deduced from its high resolution ESI-FT-ICR mass spectrum as $C_{22}H_{36}O_6$.

The ^{1}H NMR spectra of 4-*O*-acetyl hygrophorone A^{12} (**2**) and 6-*O*-acetyl hygrophorone A^{12} (**3**) resemble the spectrum of **1**. While shift and coupling pattern of H-2 and H-3 of **2** and **3** were similar to **1**, H-6 of **2** [δ ^{1}H = 3.725 ppm (1H, *dt*, *J* = 9.6 Hz, *J* = 3.0 Hz)] and H-4 of **3** [δ ^{1}H = 4.835 ppm (1H, *ddd*, *J* = 6.7 Hz, *J* = 2.0 Hz, *J* = 1.8 Hz)] showed a high field shift and an additional coupling. Moreover, the signal for one acetate group was replaced by a signal for an OH group [**2**: δ ^{1}H = 2.39 ppm (1H, *br d*, *J* = 3.0 Hz), **3**: δ ^{1}H = 3.012 ppm (1H, *d*, *J* = 6.7 Hz)]. The molecular formula $C_{20}H_{34}O_5$, which could be obtained for both molecules by ESI-FT-ICR-MS confirmed **2** and **3** were the monodeacetylated derivatives of **1**. While the acetoxy group of **2** was linked to C-4, in **3** this group was connected to C-6.

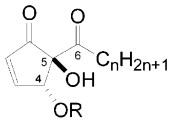
4,6-Di-*O*-acetyl hygrophorone A^{14} (**4**), 4-*O*-acetyl hygrophorone A^{14} (**5**) and 6-*O*-acetyl hygrophorone A^{14} (**6**) are homologues of **1**, **2**, and **3** with two additional methylene groups in the alkyl chain. Deacetylation of **1** with diluted NaOH in MeOH gave the diacetylated derivatives **2** and **3** as well as hygrophorone A^{12} (**7**) with three free OH groups and its methanol adduct (**31**). The 4,5,6-tri-*O*-acetyl hygrophorone A^{12} (**8**) could easily be got by acetylating of **1** with an excess of acetanhydride in pyridine.



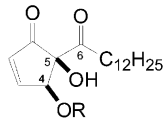
1: R^1 = Ac R^2 = H R^3 = Ac n = 12
2: R^1 = Ac R^2 = H R^3 = H n = 12
3: R^1 = H R^2 = H R^3 = Ac n = 12
4: R^1 = Ac R^2 = H R^3 = Ac n = 14
5: R^1 = Ac R^2 = H R^3 = H n = 14
6: R^1 = H R^2 = H R^3 = Ac n = 14
7: R^1 = H R^2 = H R^3 = H n = 12
8: R^1 = Ac R^2 = Ac R^3 = Ac n = 12



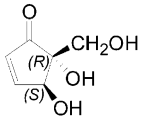
9: R^1 = H R^2 = H R^3 = H n = 14
10: R^1 = H R^2 = H R^3 = H n = 16
11: R^1 = Ac R^2 = H R^3 = H n = 14
12: R^1 = H R^2 = H R^3 = Ac n = 14
13: R^1 = Ac R^2 = H R^3 = Ac n = 14
14: R^1 = Ac R^2 = Ac R^3 = Ac n = 14



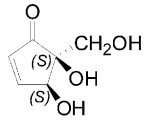
19: R = Ac n = 12
20: R = H n = 12
21: R = Ac n = 14



17: R = Ac
18: R = H



15: Epipentenomycin



16: Pentenomycin

Table 1
^{1}H NMR data of H1–H7 for selected hygrophorones in $CDCl_3$, 500 MHz. Signals of the alkyl chains: 1.20–1.33 *m* $(CH_2)_n$, 0.880 *t* (7.0) CH_3

Pos.	**1** δ ^{1}H	mult. *J* (Hz)	**2** δ ^{1}H	mult. *J* (Hz)	**3** δ ^{1}H	mult. *J* (Hz)	**7** δ ^{1}H	mult. *J* (Hz)	**8** δ ^{1}H	mult. *J* (Hz)	**9** δ ^{1}H	mult. *J* (Hz)	**11** δ ^{1}H	mult. *J* (Hz)
2	6.441	*dd* (6.2/1.8)	6.437	*dd* (6.1/1.6)	6.316	*dd* (6.1/1.8)	6.366	*dd* (6.1/1.6)	6.489	*dd* (6.4/1.8)	6.301	*dd* (6.0/1.3)	6.438	*dd* (6.2/1.3)
3	7.462	*dd* (6.2/2.1)	7.561	*dd* (6.1/2.2)	7.524	*dd* (6.1/2.0)	7.646	*dd* (6.1/2.0)	7.347	*dd* (6.4/2.1)	7.644	*dd* (6.0/2.3)	7.588	*dd* (6.2/2.7)
4 H	5.721	*dd* (2.1/1.8)	5.801	*dd* (2.2/1.6)	4.835	*ddd* (6.7/2.0/1.8)	4.873	*dd* (2.0/1.6)	6.244	*dd* (2.1/1.8)	4.727	*dd* (2.3/1.3)	5.722	*dd* (2.7/1.3)
4 OH/OAc	2.136	*s* OAc	2.171	*s* OAc	3.012	*d* (6.7) OH	*n.d.*		2.135	*s* OAc	3.073	*br s* OH	2.156	*s* OAc
5 OH/OAc	3.247	*br s* OH	3.185	*br s* OH	2.958	*s* OH	*n.d.*		1.983*	*s* OAc	2.196	*br s* OH	*n.d.*	
6 H	5.017	*m*	3.725	*dt* (9.6/3.0)	5.145	*dt* (10.4/2.9)	3.859	*m*	5.176	*t* (6.5)	3.777	*br d* (10.1)	3.818	*m*
6 OH/OAc	1.996	*s* OAc	2.39	*br d* (3.0) OH	2.045	*s* OAc	*n.d.*		2.123*	*s* OAc	3.718	*br s* OH	*n.d.*	

Pos.	**12** δ ^{1}H	mult. *J* (Hz)	**13** δ ^{1}H	mult. *J* (Hz)	**14** δ ^{1}H	mult. *J* (Hz)	**17** δ ^{1}H	mult. *J* (Hz)	**18** δ ^{1}H	mult. *J* (Hz)	**19** δ ^{1}H	mult. *J* (Hz)
2	6.300	*dd* (6.1/1.2)	6.438	*dd* (6.2/1.2)	6.470	*dd* (6.3/1.5)	6.537	*dd* (6.0/1.6)	6.466	*dd* (6.0/1.3)	6.591	*dd* (6.1/1.6)
3	7.640	*dd* (6.1/2.4)	7.550	*dd* (6.2/2.8)	7.432	*dd* (6.3/2.8)	7.802	*dd* (6.0/2.6)	7.857	*dd* (6.0/2.4)	7.701	*dd* (6.1/2.1)
4 H	4.793	*dd* (2.4/1.2)	5.785	*dd* (2.8/1.2)	5.967	*dd* (2.8/1.5)	5.734	*dd* (2.6/1.6)	4.850	*ddd* (7.2/2.4/1.3)	5.827	*dd* (2.1/1.6)
4 OH/OAc	*n.d.*		2.151	*s* OAc	2.094*	*s* OAc	2.138	*s* OAc	3.046	*d* (7.2) OH	2.089	*s* OAc
5 OH/OAc	*n.d.*		2.730	*s* OH	2.102*	*s* OAc	4.064	*s* OH	4.646	*s* OH	4.622	*br s* OH
6 H	5.177	*dd* (10.1/2.8)	5.086	*dd* (9.7/3.6)	5.227	*dd* (10.1/2.9)	–	–	–	–	–	–
6 OH/OAc	2.000	*s* OAc	2.062	*s* OAc	2.002	*s* OAc	–	–	–	–	–	–
7A	1.7	*m*	1.7	*m*	1.69	*m*	2.595	*ddd* (17.9/8.1/6.7)	2.452	*dt* (17.6/7.4)	2.591	*ddd* (17.9/7.6/7.1)
7B	1.7	*m*	1.7	*m*	1.83	*m*	2.439	*ddd* (19.7/8.1/6.7)	2.390	*dt* (17.6/7.3)	2.320	*ddd* (17.9/7.6/7.0)

Pos.	**20** δ ^{1}H	mult. *J* (Hz)	**22** δ ^{1}H	mult. *J* (Hz)	**25** δ ^{1}H	mult. *J* (Hz)	**27** δ ^{1}H	mult. *J* (Hz)	**28** δ ^{1}H	mult. *J* (Hz)
1 H	–	–	*5.707*	*ddd* (2.1/2.0/1.1)	5.659	*ddd* (2.2/1.5/1.0)	–	–	–	–
1 OH/OAc	–	–	2.108	*s* OAc	2.018	*s* OAc	–	–	–	–
2	6.446	*dd* (6.1/1.7)	6.159	*ddd* (6.1/2.1/1.1)	6.058	*ddd* (6.0/2.2/1.2)	6.256	*dd* (5.6/1.8)	6.230	*dd* (5.5/0.7)
3	7.722	*dd* (6.1/2.0)	6.190	*ddd* (6.1/1.9/1.1)	6.192	*ddd* (6.0/2.0/1.0)	7.794	*dd* (5.6/0.8)	7.372	*dd* (5.5/0.4)
4 H	4.942	*dd* (2.0/1.7)	5.724	*ddd* (2.0/1.9/1.1)	4.932	*ddd* (2.0/1.4/1.4)	–	–	–	–
4 OH/OAc	*n.d.*		2.018	*s* OAc		*n.d.*	–	–	–	–
5 OH/OAc	*n.d.*		4.003	*br s*		*n.d.*	5.756	*ddd* (8.1/1.8/0.8)	5.326	*ddd* (8.4/0.7/0.4)
6 H	–	–	–	–	–	–	4.52	*m*	4.793	*m*
6 OH/OAc	–	–	–	–	–	–	*n.d.*		*n.d.*	
7A	2.643	*ddd* (18.3/8.0/6.9)	2.659	*ddd* (17.8/9.0/6.0)	2.698	*ddd* (17.7/8.2/6.7)				
7B	2.464	*ddd* (18.3/8.0/6.5)	2.589	*ddd* (17.8/8.8/6.1)	2.541	*ddd* (17.7/8.2/6.6)				

n.d. = not detected; *assignment uncertain, may be interchanged.

Table 2
^{13}C NMR data of selected hygrophorones in $CDCl_3$, 500 MHz (in ppm)

Pos.	1	2	3	7	8	9	12	13	14	17	18	19	20	22	27
1	202.3	204.7	204.5	205.7	197.6	207.3	205.9	204.2	200.4	201.2	201.3	198.6	199.5	77.5**	169.1
2	134.4	134.9	132.5	133.1	135.4	133.5	132.9	135.4	135.6	135.4	134.1	136.0	133.4	133.8	121.0
3	156.8	161.4	158.1	162.7	154.8	163.5	162.8	157.7	154.7	160.0	164.8	158.2	162.7	134.5	140.5
4	79.7	79.3	78.8	79.5	75.6	71.4	71.5	72.5	70.7	73.6	71.7	80.4	79.5	86.2	150.3
4-$O_2\underline{C}$-CH_3	170.5	170.2	–	–	170.2	–	–	169.8	169.2	170.1	–	169.5	–	169.7	–
4-O_2C-$\underline{C}H_3$	20.9	20.8	–	–	20.9*	–	–	20.7	20.3*	20.3	–	20.8	–	21.0	–
5	81.4	81.4	82.8	83.4	84.3	75.8	76.1	77.1	73.0	82.6	82.4	87.9	91.5	83.6	117.3
5-$O_2\underline{C}$-CH_3	–	–	–	–	169.4*	–	–	–	168.6	–	–	–	–	–	
5-O_2C-$\underline{C}H_3$	–	–	–	–	20.8*	–	–	–	20.1*	–	–	–	–	–	
6	74.1	74.4	75.4	75.6	72.1	73.3	73.4	73.8	79.1	205.3	205.7	202.7	205.8	207.7	68.3
6-$O_2\underline{C}$-CH_3	169.9	–	171.2	–	169.5*	–	170.6	170.4	169.6	–	–	–	–	–	–
6-O_2C-$\underline{C}H_3$	20.7	–	21.1	–	20.6*	–	20.5	20.7	20.5	–	–	–	–	–	–
7	28.7	***	***	***	28.8	31.2	***	***	***	37.0	37.1	39.0	39.2	38.3	38.1
8-(ω-1)	31.8	***	***	***	31.9	31.9	***	***	***	31.9	31.9	32.0	32.0	32.0	32.0
	29.63				29.63	29.68				29.62	29.61	29.72	29.74	29.75	29.72
	29.59				29.61	29.67				29.60	29.59	29.70	29.73	29.73	29.71
	29.57				29.59	29.65				29.56	29.5	29.66	29.68	29.71	29.69
	29.5				29.53	29.64				29.4	29.4	29.50	29.52	29.59	29.62
	29.4				29.45	29.61				29.32	29.33	29.43	29.45	29.57	29.57
	29.34				29.39	29.56				29.30	29.26	29.42	29.44	29.45	29.5
	29.28				29.32	29.51				28.95	29.0	29.12	29.0	29.37	29.4
	25.9				25.8	29.41				23.0	23.1	23.3	23.0	23.7	25.3
	22.6				22.7	29.35				22.7	22.7	22.8	22.8	22.8	22.8
						26.1									
						22.7									
ω	14.1	14.0	14.0	14.0	14.1	14.1	14.0	14.0	14.0	14.1	14.1	14.3	14.3	14.3	14.2

*Assignment uncertain, may be interchanged with similar signal; **1-OAc: 170.4 and 20.8 ppm; ***Partially unresolved CH_2-signals between 22–32 ppm.

From the light petrol extract of *H. olivaceoalbus* the hygrophorones B^{14} (**9**) and B^{16} (**10**) could be isolated. The results of 1D (^1H, ^{13}C) and 2D (COSY, HSQC, HMBC, NOE) NMR experiments as well as the result of the high resolution mass spectroscopy fit perfectly to a cyclopentenone structure with three hydroxy groups at C4, C5, and C6, as well as a pentadecanyl (**9**) and a heptadecanyl (**10**) side chain at C5, respectively.

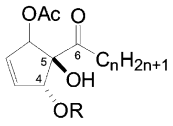
Acetylation of **9** with acetic anhydride in pyridine gave, depending on reaction time and reagent excess, 4-mono-*O*-acetylated (**11**), 6-mono-*O*-acetylated (**12**), 4,6-di-*O*-acetylated (**13**) and 4,5,6-tri-*O*-acetylated (**14**) derivatives. The esters can be separated by HPLC. Proton NMR spectra of the acetates from *H. olivaceoalbus* (**9**–**14**) differ from the spectra of hygrophorones isolated or derived from *H. persoonii* (**1**–**8**). The coupling constants of H-3 and H-4 ($^3J_{H3-H4}$) are larger (2.3–2.9 Hz) then those of constitutionally identical derivates from *H. persoonii* (1.6–1.8 Hz). The coupling constants of H-2 and H-4 ($^4J_{H2-H4}$) are smaller (1.1–1.3 Hz) compared with derivatives from *H. persoonii* (1.6–1.8 Hz). This can be compared to the corresponding coupling constants of known 4,5-*trans* configured epipentenomycin (**15**) of 2.1 Hz ($^3J_{H3-H4}$) and 1.6 Hz ($^4J_{H2-H4}$) (Baute et al., 1991), and of the 4,5-*cis* configured pentenomycin (**16**, Umino et al., 1973) 2.7 Hz ($^3J_{H3-H4}$) and 1.2 Hz ($^4J_{H2-H4}$) (Seepersaud et al., 2000). Therefore, in analogy we assume that the hygrophorones isolated from *H. persoonii* (**1**–**6**) and its derivatives (**7**, **8**) are 4,5-*trans* configurated; and those from *H. olivaceoalbus* (**9**–**14**) accordingly must be 4,5-*cis* configurated. This agrees with the results of NOESY measurements. While a NOE between the H atom of the OH group at C-5 and the vicinal H at C-4 could be observed in **1**, **2**, and **3**; no NOE could be detected between OH-5 and H-4 in **9** and **13**.

The petrol ether extract from *H. pustulatus* yielded 4-*O*-acetyl hygrophorone C^{12} (**17**) and hygrophorone C^{12} (**18**) which could be isolated by SPE and HPLC. The ^1H NMR spectra of **17** and **18** showed many similarities to the NMR spectra of the other hygrophorones. The ^{13}C NMR spectra displayed a signal of an additional carbonyl function (**17**: δ $^{13}C = 205.3$ ppm, **18**: δ $^{13}C = 205.7$ ppm). HMBC investigations showed this to be C-6, formed by an oxidation of the alcohol group to a ketone. ESI-FT-ICR-MS as well as COSY and NOESY spectra confirmed these suggested structures. Derivatives **17** and **18** are side chain oxidized forms of **1** and **3**, respectively. Interestingly, no longer chain homologues of **17** or **18** could be isolated so far.

The petrol ether extract of *H. latitabundus* is proven to be a rich source of cyclopentenone derivatives. The

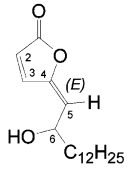
^{1}H and ^{13}C NMR spectra of 4-*O*-acetyl hygrophorone D^{12} (**19**) and hygrophorone D^{12} (**20**) resemble those of **17** and **18**, respectively. A detailed structure elucidation proved them to be diastereomers with *cis* and *trans* configuration at C-4/C-5, respectively. The NOE difference spectra of **17** and **19** differed in fundamental correlation peaks. While for **17** strong NOE correlations between H-4 and H-7A/H-7B and no NOE signal between H-4 and the OH proton could be observed, a strong NOE signal between H-4 and the OH proton and only a very weak NOE signals between H-4 and H-7A/H-7B could be observed for **19**. The shortest distance of H-4 to H-7 is 3.7 Å for the *cis* and 4.1 Å for the *trans* derivate. The distance of H-4 to the proton of the OH group is 4.1 Å for the *cis* and 3.3 Å for the *trans* isomer (Tripos force field analysis; Clark et al., 1989). Therefore **17** should be *cis* and **19** *trans* configured. Also for the side chain oxidized hygrophorones (**17**–**20**) the coupling constant $^{4}J_{H2-H4}$ is bigger for the 4,5-*trans* substance than for the 4,5-*cis* substance as well as $^{3}J_{H3-H4}$ is smaller for 4,5-*cis* then for 4,5-*trans*. Furthermore, compound **18** forms a methylboronate as indicated by a molecular ion at *m*/*z* 334 in the EI mass spectrum obtained by GC–MS. Key fragments at *m*/*z* 197 ($C_{13}H_{25}O^{+}$) and *m*/*z* 138 ($C_6H_7BO_3^{+}$) are generated by an α-cleavage. In contrast to this, **20** does not form a methylboronate for steric reasons. This is in agreement with the results of the NOE experiments: hygrophorones from *H. pustulatus* (**17**, **18**) are *cis* configured. 4-*O*-Acetyl hygrophorone D^{12} (**19**) and hygrophorone D^{12} (**20**) from *H. latitabundus* are *trans* configured. 4-*O*-Acetyl hygrophorone D^{14} (**21**) is a homologue of **19** with two more methylene units in the side chain. Higher or lower homologues of hygrophorone D^{12} (**20**) were only detected with at least one *O*-acetyl group.

The ^{13}C NMR spectrum of 1,4-di-*O*-acetyl hygrophorone E^{12} (**22**) showed the presence of two acetate groups (δ $^{13}C = 170.4$, 20.8; 169.7, 21.0), which expectedly show in the ^{1}H NMR spectrum too [δ $^{1}H = 2.108$ (3H, *s*), 2.018 (3H, *s*)]. HMBC correlation peaks between the ester carbonyl carbon atoms and H-1 and H-4, respectively, showed their position at O-1 and O-4. Lowfield signals in the ^{13}C NMR spectrum (δ $^{13}C = 134.6$, 133.8) indicated the existence of a double bond. Strong HMBC correlation peaks between C-1 and H-2 and between C-4 and H-3 as well as weaker correlation peaks between C-1 and H-3 and between C-4 and H-2 gave evidence that the double bond was located between C-2 and C-3. A signal in the ^{13}C NMR at δ $^{13}C = 207.7$ showed the existence of a keto-group. HMBC correlation peaks between the quaternary C atom at δ $^{13}C = 83.6$ and H-2, H-3, and H-4 showed this C atom is connected to C-1 and C-4. An expected HMBC correlation peak between C-5 and H-1 could not be detected, probably due to a small vicinal coupling constant. Correlation peaks of C-6 with H-1 and H-4 confirmed that a five-membered ring was built by C-1, C-2, C-3, C-4, and C-5. Another HMBC correlation peak between C-6 and H-7 showed that the alkyl chain is connected to C-6. An OH-group [δ $^{1}H = 4.003$ (1H, *br s*)] was connected to C-5. The molecular formula of $C_{22}H_{36}O_6$ was determined by ESI-FT-ICR mass measurements. 1,4-Di-*O*-acetyl hygrophorone E^{10} (**23**) is the lower homologue of **22** with a two methylene groups shorter side chain, whereas 1,4-di-*O*-acetyl hygrophorone E^{14} (**24**) is the higher homologue containing two more CH_2-groups. 1-*O*-Acetyl hygrophorone E^{12} (**25**) and 1-*O*-acetyl hygrophorone E^{10} (**26**) are the monodeacetylated forms of **22** and **23**.

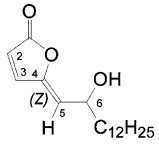


22: R = Ac n = 12
23: R = Ac n = 10
24: R = Ac n = 14
25: R = H n = 12
26: R = H n = 10

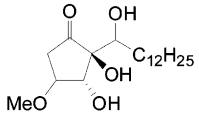
The petrol ether extract of *H. persoonii* contained the two γ-butyro lactones hygrophorone F^{12} (**27**) and hygrophorone G^{12} (**28**) as minor components. They could be separated by SPE, HPLC, and further column chromatography. Although the ^{1}H NMR spectra resample those of the other hygrophorones, big differences are shown in the ^{13}C NMR and HMBC spectra.



27



28



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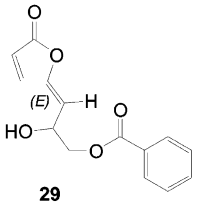

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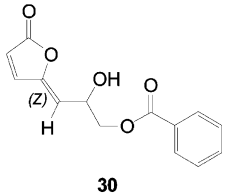

30

The ^{13}C NMR spectrum of **27** showed a signal for an ester carbonyl group (δ $^{13}C = 169.1$ ppm). Moreover, four signals for olefinic C atoms (δ $^{13}C = 117.3$ ppm, 121.0, 140.5, 150.3) were found. APT and HSQC measurements indicated C-4 (δ $^{13}C = 150.3$ ppm) to be quaternary. The molecular formula $C_{18}H_{30}O_3$ was obtained by ESI-FT-ICR-MS. The HMBC spectrum (Fig. 1) indicated the second double bond to be in conjugation to the double bond of the α,β-unsaturated carbonyl group. The lowfield chemical shift of C-4 in the ^{13}C

NMR and its quaternarity gave evidence that a five-membered lactone ring was closed to C-4.

An observed NOE correlation peak between H-3 and H-6, a missing NOE correlation peak between H-3 and H-5 (Fig. 1), as well as the large coupling constants between H-2 and H-5 ($^5J_{2,5}=1.8$ Hz) as well as H-3 and H-5 ($^4J_{3,5}=0.8$ Hz) proved a transoid position of H-3 and H-5. Therefore the exocyclic double bond should have *E*-configuration. An OH-group and a long unbranched and saturated alkyl chain were connected to C-6. The molecular formula as well as ^{13}C NMR measurements determined a chain length of 12 carbon atoms. Comparison of the NMR data with (4*E*)-7-benzoyloxy-6-hydroxy-2,4-hexadien-4-olide (**29**; Tuchinda et al., 1991) confirmed this structure.

From the diastereomeric compound **28** only traces were detectable. It can not be excluded that **28** is an isolation artifact caused by UV induced isomerisation. Structure elucidation of the basic skeleton was possible only with ^1H NMR. According to these limited data, compound **28** is the *Z*-isomer of **27**. In comparison to **27** the ^1H NMR signals of H-3 and H-5 were shifted to higher field values, while H-6 was shifted to lower field caused by the shielding of the OH group and the oxygen of the ring, respectively. In contrast to **27** the NOE correlations between H-3 and H-5 could be observed and those between H-3 and H-6 are missing. Moreover, the coupling constants between H-2 and H-5 ($^5J_{2,5}=0.7$), and H-3 and H-5 ($^4J_{3,5}=0.4$) were significantly smaller as for **27**. A comparison with the NMR data of (4*Z*)-7-benzoyloxy-6-hydroxy-2,4-hexadien-4-olide (**30**) (Tuchinda et al., 1991) confirms this suggestion.

3. Bioactivity evaluation

Initial tests of fungicidal activity were carried out by the method of Gottstein et al. (1982). An aliquot of a methanol solution of the hygrophorones containing 20 or 40 μg substance was spotted on 0.5 mm thin layer silica plates and sprayed with an aqueous, nutritive suspension of the phytopathogen *Cladosporium cucumerinum* Ell. et Arth. After two days in a wet chamber (>95% humidity) the plate was overgrown with a dark gray colored mycel. Areas with sufficient fungicidal effects were recognizable as white spots (inhibition area). A relative quantitative estimation can be deduced from the size and intensity of the spots. The size of the inhibition area of selected hygrophorones is given in Table 3. All hygrophorones tested exhibit at least some antifungal activity against *C. cucumerinum*. 4-*O*-Acetyl hygrophorone A^{12} (**2**) was the most active one in the series of these new cyclopentenone derivatives.

Table 3
Inhibition area in mm^2 of selected hygrophorones after application of 20 or 40 μg. A larger area correlates with higher activity

	1	**2**	**4**	**17**	**18**	**19**	**20**	**21**	**25**	**27**
20 μg	23	188	2	86	55	55	83	14	1	43
40 μg	40	217	6	148	90	82	170	15	28	64

4. Experimental

4.1. General methods

1D (^1H, ^{13}C) and 2D (HSQC, HMBC, COSY, NOESY) NMR spectra were obtained from a Varian Unity 500. Chemical shifts were referenced to internal TMS ($\delta=0$, ^1H) and $CDCl_3$ ($\delta=77.0$, ^{13}C), respectively.

Preparative HPLC were performed on a Merck-Hitachi L-6250 low pressure gradient pump with a L-4250 UV Detector or on a Varian ProStar 218 system with a PrepStar 330 photodiode array detector.

The high resolution ESI mass spectra were obtained from a Bruker Apex III Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer (Bruker Daltonics, Billerica, USA) equipped with an Infinity™ cell, a 7.0 Tesla superconducting magnet (Bruker, Karlsruhe, Germany), an RF-only hexapole ion guide and an external electrospray ion source (Agilent, off axis spray). The sample solutions were introduced continuously via a syringe pump with a flow rate of 120 μl h^{-1}.

The GC–MS measurements of the methylboronated compound **18** were performed with a GC–MS system (Voyager, ThermoQuest): 70 eV EI, source temp. 200 °C; column DB-5MS (J&W, 30 m×0.25 mm, 0.25 μm film thickness), injection temperature 250 °C, interface temperature 300 °C, carrier gas He, flow rate 1.0 ml/min, constant flow mode, splitless injection, column temperature program: 60 °C for 1 min, then raised to 300 °C at a rate of 10 °C min^{-1}, isothermally at 300 °C for 20 min. The methylboronation as well as methylboronation/trimethylsilylation of **18** and **20** were carried out by a procedure described previously (Takatsuto et al., 1982).

IR spectra were measured on a Bruker IFS 28, CD and UV spectra on a Jasco J-710. The specific rotation was measured with a JASCO DIP-1000 polarimeter.

4.2. Fungi material

H. latitabundus was collected under *Pinus sylvestris* near Bad Bibra, Saxony-Anhalt, Germany in November 2002 (leg./det. M. Huth). *H. olivaceoalbus* was collected under *Picea abies* near Kelheim, Bavaria, Germany in September 2001 (leg./det. N. Arnold). *H. persoonii* was collected under *Quercus robur* near Ingolstadt, Bavaria, Germany in September 2000 (leg./det. N. Arnold).

H. pustulatus was collected under *Picea abies* near Harzgerode, Saxony-Anhalt, Germany in November 2001 (leg. T. Lübken/det. Arnold). Voucher specimens are deposited at the Leibniz-Institute of Plant Biochemistry Halle, Germany (IPB).

4.3. Extraction and purification

4.3.1. Hygrophorus persoonii

Frozen fruit bodies of *H. persoonii* (229 g) were extracted thrice with light petrol (40–60 °C). The light yellow solution was concentrated to dryness in vacuo to get an oily residue (1.453 g) which was first adsorbed on Chromabond Diol cartridge and fractionated by sequential extraction with 50%, 70%, and 100% aqu. MeOH. The 70% eluate (337 mg) was purified by preparative HPLC using a LiChrospher 100 RP-18 column (10 μm, 250°4 mm ID, Merck, Germany) using H_2O/MeOH (30/70) (A) and MeOH (B) as eluents (linear gradient: 0–45 min, 70–100% B, flow rate of 5.0 ml/min) to get 51.1 mg 4,6-di-*O*-acetyl hygrophorone A^{12} (**1**, rt = 32.8 min) and 12.6 mg 4,6-di-*O*-acetyl hygrophorone A^{14} (**4**, rt = 38.7 min). Fractions from 28.0 to 31.0 min contained 1.3 mg 4-*O*-acetyl hygrophorone A^{12} (**2**) and 1.7 mg 6-*O*-acetyl hygrophorone A^{12} (**3**) which were further purified by recurring HPLC. Fractions from 34.0 to 37.0 min contained 1.2 mg of a mixture of 4-*O*-acetyl hygrophorone A^{14} (**5**) and 6-*O*-acetyl hygrophorone A^{14} (**6**), which was not further purified. Fractions from 33.0 to 34.0 min contained hygrophorone F^{12} (**27**) together with G^{12} (**28**). Further purification by column chromatography on Lichroprep Diol with $CHCl_3$ yielded 0.9 mg **27** and traces of **28**.

Ten drops of NaOH in MeOH (ca. 0.1%) were given to a solution of 9.7 mg **1** in MeOH (2 ml) and allowed to stire overnight. The solution was diluted with 8 ml water and given on a Chromabond diol cartridge which was conditioned with 50% MeOH. The cartridge was eluted with 50% MeOH and 100% MeOH. The 100% eluate was further purified by HPLC using a Nucleosil 100 RP-18 column (7 μm, 250×7 mm ID, Macherey-Nagel, Germany) using water (A) and methanol (B) as eluents (isocratic flow, 25% A and 75% B, flow rate = 27.6 ml/min) to get a mixture (18.45–21.00 min, 2.6 mg) of **7** and its methanol adduct **31** which were not further separated. A solution of 13.6 mg **1** in pyridine (1.5 ml) and some drops of acetic anhydride was allowed to stir overnight. After removing the solvents in vacuo the peracetylated derivative **8** was obtained quantitatively.

4.3.2. Hygophorus olivaceoalbus

Frozen fruit bodies of *H. olivaceoalbus* (570 g) were extracted thrice with light petrol (40–60 °C). The light yellow solution was concentrated to dryness in vacuo. The oily residue (451 mg) was initially fractionated by solid-phase extraction from a Chromabond Diol cartridge with 70% and 100% aqu. MeOH. The 70%-eluate (76.6 mg) was purified by semipreparative HPLC using a Nucleosil 100 RP-18 column (7 μm, 250°7 mm ID, Macherey-Nagel, Germany) with H_2O (A) and MeOH (B) as solvents (linear gradient: 0–20 min, 80–100% B flow rate 27.6 ml/min) to yield 17.3 mg hygrophorone B^{14} (**9**, rt = 10.5 min) and 6.4 mg hygrophorone B^{16} (**10**, rt = 13.8 min).

Three drops of acetic anhydride were given to a solution of 2.8 mg **9** in pyridine (1 ml). After stirring overnight, pyridine was removed under reduced pressure. The residue was purified by HPLC using a Nucleosil 100 RP-18 column (7 μm, 250×7 mm ID, Macherey-Nagel, Germany) with H_2O (A) and MeOH (B) as solvents (linear gradient: 0–20 min, 90%–100% B flow rate 27.6 ml/min) to obtain two fractions. Fraction I at 6.8–7.8 min gave 2.4 mg of **13** contaminated with **11** (10%, according to ^{1}H NMR) and fraction II at 8.0–9.0 min with 0.9 mg of **14**. The first fraction was not further purified.

A solution of 1 mg **9** in pyridine (1 ml) and one drop of acetic anhydride was allowed to stir for 2 h. The residue (1.1 mg) after removing the solvent contained **12** and **13** in a 2:1 ratio (determined by ^{1}H NMR) and was not further separated.

4.3.3. Hygrophorus pustulatus

Frozen fruit bodies of *H. pustulatus* (280 g) were extracted thrice with light petrol (40–60 °C). The light yellow solution was concentrated to dryness in vacuo. The oily residue (62.6 mg) was fractionated by semipreparative HPLC using a LiChrospher 100 RP-18 column (10 μm, 250×4 mm ID, Merck, Germany) using H_2O (A) and MeOH (B) as solvents (linear gradient: 0–45 min, 60%–100% B, flow rate 5.0 ml/min) to yield 1.0 mg 4-*O*-acetyl hygrophorone C^{12} (**17**, rt = 24.8 min) and 1.5 mg hygrophorone C^{12} (**18**, rt = 27.1 min).

4.3.4. Hygrophorus latitabundus

Frozen fruit bodies of *H. latitabundus* (697 g) were extracted thrice with light petrol (40–60 °C). The light yellow solution was concentrated to dryness in vacuo. The oily residue (187 mg) was initially fractionated by solid-phase extraction from a Chromabond Diol-cartridge with 50%, 70%, and 100% aqu. MeOH. The 70% eluate was further fractionated by preparative HPLC using a LiChrospher 100 RP-18 column (10 μm, 250×4 mm ID, Merck, Germany) with with H_2O (A) and MeOH (B) as solvents (linear gradient: 0–50 min, 79%–82.5% B, flow rate 27.6 ml/min and 82.5%–100% B; flow rate 5 ml/min). Fractions from 18 to 22.5 min contained 1.0 mg hygrophorone D^{12} (**20**), 26–30 min 10.9 mg 4-*O*-acetyl hygrophorone D^{12} (**19**), 31–40 min 7.6 mg 1,4-di-*O*-acetyl hygrophorone E^{12} (**22**), 51–55 min 9.7 mg 4-*O*-acetyl hygrophorone D^{14} (**21**).

Another 486 mg of the light petrol extract (obtained by exhaustive extraction of 490 g frozen fruit bodies) were separated by column chromatography (RP18, 4×28, H_2O/MeOH = 15/85) into three main fractions. Fraction I: 540–750 ml (12.1 mg), fraction II: 1300–1900 ml (31.0 mg), and fraction III: 2170–2490 ml (30.6 mg). Fraction I was a mixture of 1,4-di-*O*-acetyl hygrophorone E[10] (**23**) and 1-*O*-acetyl hygrophorone E[10] (**26**) which was not further separated. Fraction II was separated by HPLC using a LiChrospher 100 RP-18 column (10 μm, 250×4 mm ID, Merck, Germany) with H_2O (A) and MeOH (B) as solvents (linear gradient: 0–50 min, 79%–82.5% B, flow rate 27.6 ml/min and 82.5%–100% B; flow rate 5 ml/min) to yield a mixture (2.2 mg) of **22** and 1-*O*-acetyl hygrophorone E[12] (**25**) which was not further separated. Fraction III was also separated by HPLC using a LiChrospher 100 RP-18 column (10 μm, 250×4 mm ID, Merck, Germany) with H_2O (A) and MeOH (B) as solvents (linear gradient: 0–50 min, 79–82.5% B, flow rate 27.6 ml/min and 82.5%–100% B; flow rate 5 ml/min) to yield 4.5 mg 1,4-di-*O*-acetyl hygrophorone E[14] (**24**).

4.3.5. 4,6-Di-O-acetyl hygrophorone A[12] (1)

4,5-*trans*-4-Acetoxy-5-hydroxy-5-(1-hydroxytridecyl)-2-cyclopenten-1-one: colorless oil; $[\alpha]_D^{23}$ +53.0° (MeOH; *c* 0.940); ν_{max}^{film} cm^{-1} 3470 (br, w), 2955 (m), 2924 (s), 2854 (s), 1746 (s), 1729 (s), 1653 (vw), 1595 (vw), 1465 (m), 1435 (w), 1372 (m), 1329 (vw), 1233 (s), 1192 (w), 1118 (w), 1100 (m), 1070 (m), 1035 (m), 912 (w), 828 (vw), 769 (vw), 719 (vw); ^{1}H NMR (500 MHz, $CDCl_3$) see Table 1; ^{13}C NMR (125 MHz, $CDCl_3$) see Table 2; ESI-FT-ICR-MS: *m*/*z* 419.23986 ($[M+Na]^+$, calc. for $C_{22}H_{36}NaO_6^+$ 419.24041).

4.3.6. 4-O-Acetyl hygrophorone A[12] (2)

4,5-*trans*-4-Acetoxy-5-hydroxy-5-(1-hydroxytridecyl)-2-cyclopenten-1-one: colorless oil; ^{1}H NMR (500 MHz, $CDCl_3$) see Table 1; ^{13}C NMR (125 MHz, $CDCl_3$) see Table 2; ESI-FT-ICR-MS: 377.23084 ($[M+Na]^+$, calc. for $C_{20}H_{34}NaO_5^+$ 377.22984).

4.3.7. 6-O-Acetyl hygrophorone A[12] (3)

4,5-*trans*-4,5-Dihydroxy-5-(1-acetoxytridecyl)-2-cyclopenten-1-one: colorless oil; ^{1}H NMR (500 MHz, $CDCl_3$) see Table 1; ^{13}C NMR (125 MHz, $CDCl_3$) see Table 2; ESI-FT-ICR-MS: *m*/*z* 377.22897 ($[M+Na]^+$, calc. for $C_{20}H_{34}NaO_5^+$ 377.22984).

4.3.8. 4,5-Di-O-acetyl hygrophorone A[14] (4)

4,5-*trans*-4-Acetoxy-5-hydroxy-5-(1-acetoxypentadecyl)-2-cyclopenten-1-one: colorless oil; ν_{max}^{film} cm^{-1} 3447 (br, w), 2950 (m), 2923 (s), 2852 (s), 1744 (s), 1727 (s), 1645 (vw), 1594 (vw), 1465 (m), 1437 (w), 1371 (m), 1330 (w), 1234 (s), 1119 (w), 1102 (m), 1041 (m), 910 (w), 826 (vw), 753 (vw), 713 (vw); ^{1}H NMR (500 MHz, $CDCl_3$) 7.462 *dd* (6.2/2.1) H-3, 6.441 *dd* (6.2/1.8) H-2, 5.721 *dd* (2.1/1.8) H-4, 5.017 *m* H-6, 3.247 *s* 5-OH, 2.136 *s* 4-OAc, 1.996 *s* 6-OAc, 1.7 *m* H-7, 1.20- 1.33 *m* H-8–H-19, 0.888 *t* (7.0) H-20; ^{13}C NMR (125 MHz, $CDCl_3$) 202.1 C-1, 170.7 4-OAc, 170.0 6-OAc, 156.8 C-3, 134.5 C-2, 81.6 C-5, 79.9 C-4, 74.1 C-6, 28.8 C-7, 21.1 4-OAc, 20.9 6-OAc, 32.0, 29.80, 29.79, 29.77, 29.76, 29.74, 29.69, 29.58, 29.51, 29.47, 26.1, 22.8 C-8–C-19, 14.3 C-20 ESI-FT-ICR-MS: *m*/*z* 447.27221 ($[M+Na]^+$, calc. for $C_{24}H_{40}NaO_6$ 447.27171).

4.3.9. 4-O-Acetyl hygrophorone A[14] (5)

4,5-*trans*-4-Acetoxy-5-hydroxy-5-(1-hydroxypentadecyl)-2-cyclopenten-1-one: colorless oil; ^{1}H NMR (500 MHz, $CDCl_3$) 7.561 *dd* (6.1/2.2) H-3, 6.437 *dd* (6.1/1.6) H-2, 5.801 *dd* (2.2/1.6) H-4, 3.725 *dt* (9.6/3.0) H-6, 3.185 *s* 5-OH, 2.39 *br* 6-OH, 2.171 *s* 4-OAc, 1.7 *m* H-7, 1.20-1.33 *m* H-8–H-19, 0.880 *t* (7.0) H-20; ESI-FT-ICR-MS: *m*/*z* 405.26152 ($[M+Na]^+$, calc. for $C_{22}H_{38}NaO_5^+$ 405.26115).

4.3.10. 6-O-Acetyl hygrophorone A[14] (6)

4,5-*trans*-4,5-Dihydroxy-5-(1-acetoxypentadecyl)-2-cyclopenten-1-one: colorless oil; ^{1}H NMR (500 MHz, $CDCl_3$) 7.524 *dd* (6.1/2.0) H-3, 6.316 *dd* (6.1/1.8) H-2, 5.145 *dt* (10.4/2.9) H-6, 4.835 *ddd* (6.7/2.0/1.8) H-4, 3.012 *d* (6.7) 4-OH, 2.958 *s* 5-OH, 2.045 *s* 6-OAc, 1.7 *m* H-7, 1.20–1.33 *m* H-8–H-19, 0.880 *t* (7.0) H-20; ESI-FT-ICR-MS: *m*/*z* 405.26152 ($[M+Na]^+$, calc. for $C_{22}H_{38}NaO_5^+$ 405.26115).

4.3.11. Hygrophorone A[12] (7)

4,5-*trans*-4,5-Dihydroxy-5-(1-hydroxytridecyl)-2-cyclopenten-1-one: ^{1}H NMR (500 MHz, $CDCl_3$) see Table 1; ^{13}C NMR (125 MHz, $CDCl_3$) see Table 2; ESI-FT-ICR-MS (positive ion mode): *m*/*z* 335.21881 ($[M+Na]^+$, calc. for $C_{18}H_{32}NaO_4^+$ 335.21928).

4.3.12. 4,5,6-Tri-O-acetyl hygrophorone A[12] (8)

4,5-*trans*-4,5-Diacetoxy-5-(1-hydroxytridecyl)-2-cyclopenten-1-one: colorless oil; ν_{max}^{film} cm^{-1} 2955 (m), 2925 (s), 2854 (s), 1746 (s), 1731 (s), 1597 (vw), 1465 (w), 1434 (w), 1372 (m), 1355 (w), 1249 (s), 1225 (s), 1185 (w), 1155 (w), 1108 (w), 1073 (w), 1032 (m), 980 (w), 922 (w), 905 (w), 825 (w), 794 (w), 757 (w); ^{1}H NMR (500 MHz, $CDCl_3$) see Table 1; ^{13}C NMR (125 MHz, $CDCl_3$) see Table 2; ESI-FT-ICR-MS: *m*/*z* 461.25013 ($[M+Na]^+$, calc. for $C_{24}H_{38}NaO_7^+$ 461.25097).

4.3.13. Hygrophorone B[14] (9)

4,5-*cis*-4,5-Dihydroxy-5-(1-hydroxypentadecyl)-2-cyclopenten-1-one: white amorphous solid; $[\alpha]_D^{23}$ +10.5° (MeOH; *c* 0.640); ν_{max}^{film} cm^{-1} 3509 (m), 3445 (m), 3403 (m), 3372 (m), 2954 (m), 2916 (s), 2871 (m), 2849 (s), 1715 (s), 1696 (s), 1676 (w), 1653 (w), 1595 (w), 1470 (m), 1399 (w), 1355 (w), 1243 (w), 1214 (w), 1115 (m),

1102 (vw), 1078 (m), 1011 (w), 983 (w), 946 (w), 919 (vw), 900 (vw), 843 (m), 791 (w), 720 (w), 687 (w); ^{1}H NMR (500 MHz, $CDCl_3$) see Table 1; ^{13}C NMR (125 MHz, $CDCl_3$) see Table 2; ESI-FT-ICR-MS: *m/z* 363.24825 ($[M+Na]^+$, calc. for $C_{20}H_{36}NaO_4^+$ 363.25058).

4.3.14. Hygrophorone B[16] (10)

4,5-*cis*-4,5-Dihydroxy-5-(1-hydroxyheptadecyl)-2-cyclopenten-1-one: white amorphous solid; ^{1}H NMR (500 MHz, $CDCl_3$) 7.644 *dd* (6.0/2.3) H-3, 6.301 *dd* (6.0/1.3) H-2, 4.727 *dd* (2.3/1.3) H-4, 3.777 *br* d (10.1) H-6, 3.718 *br s* 6-OH, 3.073 *br s* 4-OH, 2.196 *br s* 5-OH, 1.7 *m* H-7, 1.2–1.4 *m* H-8–H-21, 0.880 *t* (6.9) H-22; ESI-FT-ICR-MS: *m/z* 391.28259 ($[M+Na]^+$, calc. for $C_{22}H_{44}NaO_4^+$ 391.28188).

4.3.15. 4-O-Acetyl hygrophorone B[14] (11)

4,5-*cis*-4-Acetoxy-5-hydroxy-5-(1-hydroxypentadecyl)-2-cyclopenten-1-one: colorless oil; ^{1}H NMR (500 MHz, $CDCl_3$) see Table 1; ESI-FT-ICR-MS: *m/z* 405.26155 ($[M+Na]^+$, calc. for $C_{22}H_{38}NaO_5^+$ 405.26115).

4.3.16. 6-O-Acetyl hygrophorone B[14] (12)

4,5-*cis*-4,5-Dihydroxy-5-(1-acetoxypentadecyl)-2-cyclopenten-1-one: colorless oil; ^{1}H NMR (500 MHz, $CDCl_3$) see Table 1; ^{13}C NMR (125 MHz, $CDCl_3$) see Table 2; ESI-FT-ICR-MS: *m/z* 405.26155 ($[M+Na]^+$, calc. for $C_{22}H_{38}NaO_5^+$ 405.26115).

4.3.17. 4,6-Di-O-acetyl hygrophorone B[14] (13)

4,5-*cis*-4-Acetoxy-5-hydroxy-5-(1-acetoxypentadecyl)-2-cyclopenten-1-one: colorless oil; ^{1}H NMR (500 MHz, $CDCl_3$) see Table 1; ^{13}C NMR (125 MHz, $CDCl_3$) see Table 2; ESI-FT-ICR-MS: *m/z* 447.27131 ($[M+Na]^+$, calc. for $C_{24}H_{40}NaO_6^+$ 447.27171).

4.3.18. 4,5,6-Tri-O-acetyl hygrophorone B[14] (14)

4,5-*cis*-4,5-Diacetoxy-(1-acetoxypentadecyl)-2-cyclopenten-1-one: colorless oil; ν_{max}^{film} cm^{-1} 5956 (m), 2922 (s), 2850 (s), 1760 (s), 1601 (w), 1468 (m), 1434 (m), 1371 (m), 1258 (s), 1122 (w), 1102 (w), 1047 (m), 1027 (m), 961 (w), 814 (w), 758 (w), 720 (w); ^{1}H NMR (500 MHz, $CDCl_3$) see Table 1; ^{13}C NMR (125 MHz, $CDCl_3$) see Table 2; ESI-FT-ICR-MS: *m/z* 489.28213 ($[M+Na]^+$, calc. for $C_{26}H_{42}NaO_7^+$ 489.28227).

4.3.19. 4-O-Acetyl hygrophorone C[12] (17)

cis-4-Acetoxy-5-hydroxy-5-tridecanoyl-2-cyclopenten-1-one: white solid; ν_{max}^{film} cm^{-1} 3360 (br, m), 3092 (vw), 3020 (vw), 2950 (m), 2918 (s), 2871 (m), 2850 (s), 1746 (s), 1725 (s), 1689 (s), 1593 (w), 1463 (m), 1404 (w), 1373 (m), 1344 (m), 1315 (w), 1272 (w), 1260 (w), 1227 (m), 1198 (m), 1154 (m), 1112 (w), 1093 (m), 1066 (m), 1046 (w), 955 (vw), 930 (vw), 915 (w), 900 (w), 882 (vw), 845 (w), 826 (vw), 795 (w), 756 (m), 746 (m), 729 (w), 717 (m); ^{1}H NMR (500 MHz, $CDCl_3$) see Table 1; ^{13}C NMR (125 MHz, $CDCl_3$) see Table 2; ESI-FT-ICR-MS (negative ion mode): *m/z* 387.19523 ($[M+Cl]^-$, calc. for $C_{20}H_{32}O_5{}^{35}Cl^-$ 387.19438).

4.3.20. Hygrophorone C[12] (18)

cis-4,5-Dihydroxy-5-tridecanoyl-2-cyclopenten-1-one: white solid; ν_{max}^{film} cm^{-1} 3416 (br, w), 2954 (m), 2922 (s), 2852 (s), 1728 (m), 1711 (m), 1465 (w), 1438 (w), 1399 (w), 1377 (w), 1341 (w), 1256 (w), 1226 (w), 1199 (w), 1173 (w), 1159 (w), 1111 (w), 1078 (w), 1018 (w), 758 (w), 720 (w); ^{1}H NMR (500 MHz, $CDCl_3$) see Table 1; ^{13}C NMR (125 MHz, $CDCl_3$) see Table 2; 70eV-EIMS of the methylboronate, *m/z* (rel. int.,%): 334 ($[M^+]$, 4), 197 (58), 151 (12), 138 (51), 137 (100), 123 (11), 109 (21), 96 (37), 95 (44), 85 (26), 71 (46), 57 (67); ESI-FT-ICR-MS (negative ion mode): *m/z* 345.18679 $[M+Cl]^-$, calc. for $C_{18}H_{30}{}^{35}ClO_4^-$ 345.18381).

4.3.21. 4-O-Acetyl hygrophorone D[12] (19)

trans-4-Acetoxy-5-hydroxy-5-tridecanoyl-2-cyclopenten-1-one: color oil; $[\alpha]_D^{23}$ +111.7° (MeOH; *c* 0.470); ν_{max}^{film} cm^{-1} 3441 (br, m), 3081 (w), 2954 (m), 2923 (s), 2872 (m), 2853 (s), 1757 (s), 1746 (s), 1739 (s), 1731 (s), 1713 (s), 1591 (w), 1466 (m), 1401 (w), 1372 (m), 1349 (m), 1283 (w), 1224 (s), 1183 (w), 1143 (m), 1131 (m), 1093 (m), 1035 (m), 981 (w), 960 (w), 897 w), 821 (w), 758 (w), 722 (w); ^{1}H NMR (500 MHz, $CDCl_3$) see Table 1; ^{13}C NMR (125 MHz, $CDCl_3$) see Table 2; ESI-FT-ICR-MS: *m/z* 375.21436 ($[M+Na]^+$, calc. for $C_{20}H_{32}NaO_5^+$ 375.21419).

4.3.22. Hygrophorone D[12] (20)

trans-4,5-Dihydroxy-5-tridecanoyl-2-cyclopenten-1-one: colorless oil; ν_{max}^{film} cm^{-1} 3416 (br, m), 2952 (m), 2924 (s), 2853 (s), 1729 (s), 1712 (s), 1629 (w), 1464 (m), 1438 (w), 1406 (m), 1376 (m), 1236 (m), 1180 (m), 1149 (m), 1125 (m), 1080 (w), 1047 (w), 977 (w), 822 (vw), 722 (vw); ^{1}H NMR (500 MHz, $CDCl_3$) see Table 1; ^{13}C NMR (125 MHz, $CDCl_3$) see Table 2; ESI-FT-ICR-MS: *m/z* 333.20417 ($[M+Na]^+$, calc. for $C_{18}H_{30}NaO_4^+$ 333.20363).

4.3.23. 4-O-Acetyl hygrophorone D[14] (21)

trans-4-Acetoxy-5-hydroxy-5-pentadecanoyl-2-cyclopenten-1-one: colorless oil; $[\alpha]_D^{23}$ +98.7° (MeOH; *c* 0.475); ν_{max}^{film} cm^{-1} 3446 (br, m), 3080 (w), 2955 (m), 2923 (s), 2853 (s), 1734 (s), 1711 (s), 1591 (w), 1465 (m), 1400 (w), 1371 (m), 1326 (w), 1282 (w), 1225 (s), 1185 (w), 1131 (m), 1097 (m), 1086 (m), 1035 (m), 981 (w), 897 (w), 822 (w), 762 (vw); 721 (vw); ^{1}H NMR (500 MHz, $CDCl_3$) 7.700 *dd* (6.2/2.1) H-3, 6.591 *dd* (6.2/1.7) H-2, 5.826 *dd* (2.1/1.7) H-4, 4.613 *s* 5-OH, 2.590 *ddd* (17.9/7.2/7.1) H-7A, 2.319 *ddd* (17.9/7.5/6.7) H-7B, 2.088 *s* 4-OAc, 1.619 *m* H-8, 1.20–1.33 *m* H-9–H-19, 0.878 *t* (6.8) H-20; ^{13}C NMR (125 MHz, $CDCl_3$) 202.7 C-6, 198.6 C-1, 169.5 4-OAc, 158.2 C-3, 136.0 C-2, 88.0 C-5, 80.4 C-4,

39.0 C-7, 32.0, 29.78, 29.76, 29.74, 29.73, 29.47, 29.51, 29.45, 29.44, 29.13, 23.3, 22.8: C-8–C-19, 20.8 4-OAc, 14.3 C-20; ESI-FT-ICR-MS: *m/z* 403.24474 ($[M+Na]^+$, calc. for $C_{22}H_{37}NaO_5^+$ 403.24604).

*4.3.24. 1,4-Di-O-acetyl hygrophorone E[12] (**22**)*

1-(2,5-Diacetoxy-1-hydroxy-cyclopent-3-enyl)-tridecan-1-one: colorless oil; $[\alpha]_D^{23}$ +80.3° (MeOH; *c* 0.395); ν_{max}^{film} cm^{-1} 3449 (br, m), 3073 (w), 2954 (m), 2924 (s), 2853 (s), 1744 (s), 1718 (s), 1465 (w), 1435 (w), 1372 (m), 1319 (vw), 1300 (vw), 1225 (s), 1154 (vw), 1114 vw), 1024 (m), 967 (w), 915 (w), 831 (vw), 786 (vw), 721 (vw); ^1H NMR (500 MHz, $CDCl_3$) see Table 1; ^{13}C NMR (125 MHz, $CDCl_3$) see Table 2; ESI-FT-ICR-MS: *m/z* 419.24159 ($[M+Na]^+$, calc. for $C_{22}H_{36}NaO_6^+$ 419.24041).

*4.3.25. 1,4-Di-O-acetyl hygrophorone E[10] (**23**)*

1-(2,5-Diacetoxy-1-hydroxy-cyclopent-3-enyl)-undecan-1-one: colorless oil; ^1H NMR (500 MHz, $CDCl_3$) 6.190 *ddd* (6.1/1.9/1.1) H-3, 6.159 *ddd* (6.1/2.1/1.1) H-2, 5.724 *ddd* (2.0/1.9/1.1) H-4, 5.707 *ddd* (2.1/2.0/1.1) H-1, 4.003 *br s* 5-OH, 2.659 *ddd* (17.8/9.0/6.0) H-7A, 2.589 *ddd* (17.8/8.8/6.1) H-7B, 2.108 *s* 1-OAc, 2.018 *s* 4-OAc, 1.20–1.33 *m* H-8–H-15, 0.881 *t* (7.0) H-16; ESI-FT-ICR-MS: *m/z* 391.21037 ($[M+Na]^+$, calc. for $C_{20}H_{32}NaO_6^+$ 391.20911).

*4.3.26. 1,4-Di-O-acetyl hygrophorone E[14] (**24**)*

1-(2,5-Diacetoxy-1-hydroxy-cyclopent-3-enyl)-pentadecan-1-one: colorless oil; ^1H NMR (500 MHz, $CDCl_3$) 6.190 *ddd* (6.1/1.9/1.1) H-3, 6.159 *ddd* (6.1/2.1/1.1) H-2, 5.724 *ddd* (2.0/1.9/1.1) H-4, 5.707 *ddd* (2.1/2.0/1.1) H-1, 4.003 *br s* 5-OH, 2.659 *ddd* (17.8/9.0/6.0) H-7A, 2.589 *ddd* (17.8/8.8/6.1) H-7B, 2.108 *s* 1-OAc, 2.018 *s* 4-OAc, 1.20–1.33 *m* H-8–H-19, 0.881 *t* (7.0) H-20; ESI-FT-ICR-MS: *m/z* 447.27530 ($[M+Na]^+$, calc for $C_{24}H_{40}NaO_6^+$ 447.27171).

*4.3.27. 1-O-Acetyl hygrophorone E[12] (**25**)*

1-(2-Acetoxy-1,5-dihydroxy-cyclopent-3-enyl)-tridecan-1-one: colorless oil; ^1H NMR (500 MHz, $CDCl_3$) see Table 1; ESI-FT-ICR-MS: *m/z* 377.23029 ($[M+Na]^+$, calc for $C_{20}H_{34}NaO_5^+$ 377.22984).

*4.3.28. 1-O-Acetyl hygrophorone E[10] (**26**)*

1-(2-Acetoxy-1,5-dihydroxy-cyclopent-3-enyl)-undecan-1-one: colorless oil; ^1H NMR (500 MHz, $CDCl_3$) see 6.192 *ddd* (6.0/2.0/1.0) H-3, 6.058 *ddd* (6.0/2.2/1.2) H-2, 5.659 *ddd* (2.2/1.5/1.0) H-1, 4.932 *ddd* (2.0/1.4/1.4) H-4, 2.698 *ddd* (17.7/8.2/6.7) 7A, 2.541 *ddd* (17.7/8.2/6.6) 7B, 2.018 *s* 1-OAc, 1.20–1.33 *m* H-8–H-15, 0.881 *t* (7.0) H-16; ESI-FT-ICR-MS: *m/z* 349.20050 ($[M+Na]^+$, calc for $C_{18}H_{30}NaO_5^+$ 349.19854).

*4.3.29. Hygrophorone F[12] (**27**)*

(5*E*)-5-(2-Hydroxytetradexylidene)-furan-2(5*H*)-one: white amorphous solid; ν_{max}^{film} cm^{-1} 3487 (m), 3396 (br, m), 3269 (br, m), 3133 (w), 3100 (w), 3074 (w), 2953 (m), 2918 (s), 2850 (s), 1785 (m), 1751 (s), 1718 (w), 1669 (w), 1554 (w), 1466 (w), 1372 (vw), 1297 (vw), 1237 (w), 1195 (vw), 1122 (w), 1077 (w), 1064 (w), 1035 (w), 1019 (w), 1008 (vw), 920 (w), 908 (w), 894 (w), 840 (vw), 816 (w), 757 (w), 711 (w); ^1H NMR (500 MHz, $CDCl_3$) see Table 1; ^{13}C NMR (125 MHz, $CDCl_3$) see Table 2; ESI-FT-ICR-MS: *m/z* 317.20921 ($[M+Na]^+$, calc. for $C_{18}H_{30}NaO_3^+$ 317.20872).

*4.3.30. Hygrophorone G[12] (**28**)*

(5*Z*)-5-(2-Hydroxytetradexylidene)-furan-2(5*H*)-one: colorless oil; ^1H NMR (500 MHz, $CDCl_3$) see Table 1.

*4.3.31. 2,3-Dihydroxy-2-(1-hydroxytridecyl)-4-methoxy-cyclopentanone (**31**)*

^1H NMR (500 MHz, $CDCl_3$): 4.073 *ddd* (8.8/5.3/4.5) H-4, 2.956 *ddd* (19.4/8.8/0.7) 5A, 2.360 *ddd* (19.4/4.5/0.7) 5B, 3.435 *s* 4-OMe, 4.224 *dd* (5.3/0.7) H-3, 3.916 *ddd* (10.2/4.3/1.6) H-6; ^{13}C NMR (125 MHz, $CDCl_3$): 214.6 (C-1), 83.0 (C-2), 82.6 (C-3), 80.5 (C-4), 73.2 (C-6), 57.6 (4-OMe), 41.9 (C-5), 31-22 (C-7–C17), 14.4 (C-18); ESI-FT-ICR-MS: *m/z* 367.24501 ($[M+Na]^+$, calc for $C_{19}H_{36}NaO_5^+$ 367.24549).

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