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Sesquiterpenoids and norsesquiterpenoids from three liverworts

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Abstract—Six new sesquiterpenoids and two new norsesquiterpenoids were isolated from the essential oils of three liverworts. The isolated compounds include (+)-eudesma-4,11-dien-8α-ol from the liverwort *Diplophyllum albicans*, (−)-4β,5β-diacetoxygymnomitr-3(15)-ene, (+)-5β-acetoxygymnomitr-3(15)-ene, (−)-15-acetoxygymnomitr-3-ene, (−)-3β,15β-epoxy-4β-acetoxygymnomitrane, and (−)-3α,15α-epoxy-4β-acetoxygymnomitrane from *Marsupella emarginata*, and (+)-1,2,3,6-tetrahydro-1,4-dimethylazulene and (−)-2,3,3a,4,5,6-hexahydro-1,4-dimethylazulen-4-ol from *Barbilophozia floerkei*. These compounds were isolated by a combination of different chromatographic techniques, and their structures were determined by extensive spectroscopic studies (MS, ^{1}H, ^{13}C, and 2D NMR) and chemical transformations using enantioselective GC.

1. Introduction

Liverworts are rich sources of mono-, sesqui-, and di-terpenoids and/or lipophilic aromatic compounds. Several of these compounds have been reported to have biological activities.[1,2] In continuation of our work on phytochemical analysis of the liverworts,[3–5] we herein report our results on the investigation of the constituents of the essential oils of *Diplophyllum albicans*, and *Barbilophozia floerkei* collected from the Harz mountains near Altenau, and *Marsupella emarginata* collected from Saarland, Germany.

Previous investigations[6–8] of *D. albicans* revealed the presence of *ent*-eudesmane-type sesquiterpenoids, and it has been known to produce pungent substances, which showed inhibitory activity towards the germination and root elongation of rice husks.[9] Additionally, diplophyllin isolated from the essential oil of this liverwort had been reported to show significant activity against human epidermoid carcinoma.[7]

Barbilophozia species are known to produce rare skeletal sesqui- and diterpenoids[10–14] such as (−)-maalioxide, barbilycopodin, barbifusicoccins A and B, and stigmasterol.

The chemical composition of the liverwort *M. emarginata* has been studied by several groups with a number of interesting compounds being reported.[3,15–17] The longipinane-type sesquiterpenes are the major constituents isolated from *M. emarginata* subsp. *tubulosa*.[15] In addition, eremophila-9,11-dien-8α-o1[16] and gymnomitrane-type sesquiterpenoids[18] have also been characterized from *M. emarginata*.

2. Results and discussion

2.1. Composition of the essential oil of *D. albicans*

GC–MS investigations of the hydrodistillation product of *D. albicans* revealed a complex mixture of sesquiterpenoids. The identification of the sesquiterpene hydrocarbons and oxygenated sesquiterpenes was carried out by comparison of their mass spectra and gas chromatographic retention indices with a spectral library established under identical experimental conditions.[19,20] The hydrocarbon fraction consists of bicycloelemene, maali-1,3-diene, anastreptene, β-elemene, tritomarene, β-barbatene, β-acoradiene, δ-selinene, bicyclogermacrene, α-selinene, β-bazzanene, aromadendra-4,10(14)-diene, aromadendra-4,9-diene, and aromadendra-1(10),4-diene. The oxygenated fraction consists of 4-dehydro-viridiflorol, globulol, 3α-acetoxy-bicyclogermacrene, diplophyllin, *ent*-diplophyllolide, *ent*-dihydrodiplophyllin, and traces of other unidentified constituents.

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✠ Deceased 19, November 2004. His scientific achievements keep him among us.

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2.1.1. Structure elucidation of (+)-eudesma-4,11-dien-8α-ol 1. Compound **1** (0.3%) exhibits a molecular ion at *m/z* 220 corresponding to the molecular formula of $C_{15}H_{24}O$. The ^{13}C NMR spectrum revealed the presence of 15 carbon resonances. ^{1}H NMR and HMQC spectroscopic analyses indicated a total of 23 protons directly attached to the carbon skeleton. The ^{1}H NMR spectrum showed signals of three singlets for methyl groups at δ 1.49 (3H, s, H-14), 1.53 (3H, s, H-12), and 1.58 (3H, s, H-15). The signal at δ 3.86 (1H, d, $J = 2.2$ Hz) was assigned to the oxygenated methine proton H-8. Additional structural information was obtained from the ^{13}C NMR spectrum of **1** (Table 1). All the information from 2D ^{1}H–^{1}H COSY, HMQC, and HMBC (Fig. 1) analyses was consistent with the structure assigned for **1**. Its relative configuration was derived from the NOESY spectrum (Fig. 2), which indicates the spatial interactions of protons H-7 and H-8. The absolute configuration of **1** was determined by comparison of its hydrogenation products with that of the hydrogenation products of authentic (+)-α-selinene **2** by capillary GC with different cyclodextrin derived stationary phases. The assigned absolute configuration (7*S*,8*S*,10*S*) is consistent with the co-occurrence of *ent*-(+)-α-selinene **2** and *ent*-(−)-selina-4,11-diene in the hydrocarbon fraction of the liverwort sample.

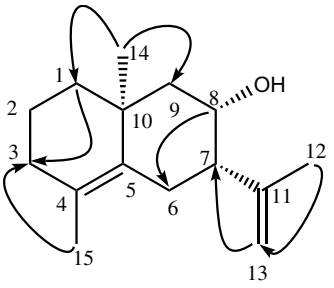


Figure 1. Long-range ^{1}H–^{13}C-1 couplings for **1**.

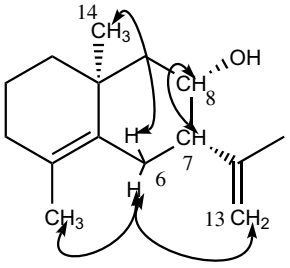


Figure 2. NOE correlations for **1**.

2.2. Composition of the essential oil of *B. floerkei*

GC–MS investigations of the hydrodistillation product of *B. floerkei* revealed a complex mixture of mono-, sesqui-, and diterpenoids. The compounds identified include α-pinene, camphene, β-pinene, limonene, trisnoranastreptene **3** (33.0%), anastreptene, tritomarene, *trans*-α-bergamotene, allo-aromadendra-4(15),10(14)diene, bicyclogermacrene, β-bisabolene, 1,4-dimethylazulene **4**, and 3α-acetoxybicyclogermacrene by comparison with our spectral library.[19,20] The major hydrodistillation product, (+)-trisnoranastreptene **3** (33.0%),[21] has been reported to have ichthyotoxic properties.[22]

2.2.1. Structure elucidation of (−)-2,3,3a,4,5,6-hexahydro-1,4-dimethylazulen-4-ol 5. Compound **5**, a new trinor-sesquiterpene alcohol, occurred as a trace component of the essential oil. Compound **5** exhibits a molecular ion signal at *m/z* 178 corresponding to the molecular formula $C_{12}H_{18}O$. The ^{1}H NMR spectrum (C_6D_6) of **5** showed sig-

Table 1. 1D NMR data of compounds **1**, **5**, and **6** (in C_6D_6)[a]

Atom. no.	1		5		6	
	δC	δH	δC	δH	δC	δH
1a	41.4	1.25–1.31 m	139.1	—	42.6	2.84–2.90 m
1b	—	1.47–1.55 m	—	—	—	—
2a	19.3	1.47–1.55 m	37.3	2.04–2.13 m	32.0	1.28–1.35 m
2b	—	1.64–1.68 m	—	2.14–2.25 m	—	1.97–2.03 m
3a	33.6	1.82–1.95 m	24.8	1.71–1.82 m	34.2	2.45–2.51 m
3b	—	—	—	1.84–1.91 m	—	2.52–2.58 m
4	124.8	—	74.7	—	133.8	—
5	135.7	—	44.4	1.71–1.82 m	115.2	5.05 t (6.6)
6a	24.6	2.35–2.41 m	25.9	1.95–2.03 m	28.2	2.04–2.10 m
6b	—	2.41–2.49 m	—	2.14–2.25 m	—	2.35–2.40 m
7	50.7	1.82–1.95 m	128.7	5.51–5.55 m	119.2	5.36 dd (6.6, 16.1)
8	67.5	3.86 d (2.2)	124.8	6.26 d (11.7)	125.3	6.10 d (9.5)
9a	48.1	1.25–1.31 m	133.7	—	146.1	—
9b	—	2.01 dd (2.5, 14.2)	—	—	—	—
10	34.8	—	60.3	2.93 br t, (6.3)	142.6	—
11	147.7	—	14.6	1.57 br s	21.0	1.12 d (6.9)
12	22.8	1.53 s	20.7	1.00 s	20.5	1.79 s
13a	111.6	4.79 s	—		—	
13b	—	4.83 s				
14	28.2	1.49 s	—		—	
15	19.6	1.58 s	—	—		

[a]All assignments were confirmed by HMBC and HMQC.

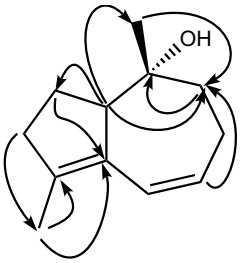


Figure 3. Long-range ^{1}H–^{13}C couplings of **5**.

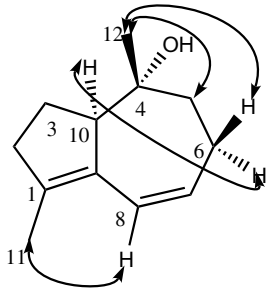


Figure 4. NOE correlations of **5**.

nals of two singlets for methyl groups at δ 1.00 (3H, s, H-12) and 1.57 (3H, s, H-11), respectively. The deshielded signals at δ 5.51 (1H, m) and 6.26 (1H, d, $J = 11.7$ Hz) were assigned to the vinylic protons at H-7, and H-8, respectively. The carbon signal at δ 74.7 (s) was assigned to the tertiary hydroxyl group. All these informations from ^{13}C NMR (Table 1), as well as from ^{1}H–^{1}H COSY, HMQC, and HMBC (Fig. 3) led to structure **5** for this compound. Its relative configuration resulted from the NOESY spectrum (Fig. 4), and shows spatial interactions of proton H-10 with H-6a, H-12 with H-6b, and the methyl protons H-11 with H-8.

2.2.2. Structure elucidation of (+)-1,2,3,6-tetrahydro-1,4-dimethylazulene 6. Compound **6** exhibits a molecular ion signal at *m*/*z* 160 corresponding to the molecular formula of $C_{12}H_{16}$. The ^{1}H NMR spectrum (C_6D_6) showed signals of one doublet and one singlet for methyl groups at δ 1.12 (3H, d, H-11, $J = 6.9$ Hz) and 1.79 (3H, s, H-12), respectively. The methyl singlet at δ 1.79 indicated that this methyl group is attached to a double bond. The deshielded signals at δ 5.05 (1H, t, $J = 6.6$ Hz), 5.36 (1H, dd, $J = 6.6$, 16.1 Hz), and 6.10 (1H, d, $J = 9.5$ Hz) were assigned to vinylic protons H-5, H-7, and H-8, respectively. In the ^{1}H–^{1}H COSY spectrum, proton H-5 exhibits a long-range ^{4}J-coupling with H-7. Additional structural information was obtained from the ^{13}C NMR spectrum of **6** (Table 1). Information from the ^{13}C data, in addition to HMBC and HMQC of **6** indicated the presence of five degrees of unsaturation hence, a bicyclic molecule was concluded. All these informations from 2D ^{1}H–^{1}H COSY, HMQC, and HMBC (Fig. 5) analyses were consistent with structure **6**. We adopted the azulene numbering system in the nomenclature of these two new compounds **5** and **6**.

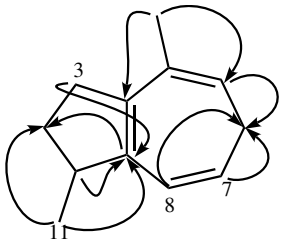


Figure 5. Long-range ^{1}H–^{13}C couplings of **6**.

In addition, since traces of six unknown compounds with *m*/*z* 160 were detected in the essential oil of *B. floerkei*, the major component, trisnoranastreptene **3** (33.0%) with a similar mass spectrum comparable to the trace components was subjected to acid transformation using Amberlyst® 15 to determine if all the compounds with *m*/*z* 160 were related. The transformation of **3** afforded compounds **5** and **6**, and three unidentified compounds with the molecular mass at *m*/*z* 160. These unidentified compounds could not be characterized because they decomposed almost immediately during isolation through prep-GC and silica-based prep-TLC. The mass spectral of two of these three compounds gave a base peak of 131 and the third with a base peak of 117. Hence, compounds **5** and **6** could be natural or artefacts of the isolation procedures.

2.3. Composition of the essential oil of *M. emarginata*

GC–MS investigations of the hydrodistillation product of *M. emarginata* revealed a complex mixture of sesquiterpenoids. All the known constituents such as α-barbatene, β-barbatene, β-bazzanene, β-acoradiene, α-chamigrene, β-chamigrene, gymnomitr-3(15),4-diene, gymnomitr-3(15)-en-4β-ol, gymnomitrane-4-one, 15-*nor*-3-gymnomitrone, and gymnomitr-3(15)-en-4-one were identified with our spectral library.[19,20]

2.3.1. Structure elucidation of (−)-4β,5β-diacetoxygymnomitr-3(15)-ene 7. The GC–CI mass spectrum of **7** exhibits a molecular ion at *m*/*z* 338 [M^{+}+NH_4^{+}] corresponding to the molecular formula of $C_{19}H_{28}O_4$. The ^{1}H NMR spectrum (C_6D_6) showed five singlets for methyl groups at δ 0.74 (3H, s, H-13), 0.78 (3H, s, H-14), 0.86 (3H, s, H-12), 1.76 (3H, s, H-19), and 1.80 (3H, s, H-17). The olefinic carbon signals at δ 146.8 (s) and 107.9 (t) suggested an exomethylene double bond, which was confirmed by two signals in the ^{1}H NMR spectrum at δ 4.87 (1H, t, H-15a, $J = 2.2$ Hz) and 5.03 (1H, t, H-15b, $J = 2.2$ Hz). The signals at δ 5.48 (1H, d, $J = 4.1$ Hz) and 6.27–6.29 (1H, m) were assigned to the methine protons connected to the acetoxy groups at C-5 (δ 76.5) and C-4 (δ 69.6), respectively. Information from 2D ^{1}H–^{1}H COSY, HMQC, and HMBC spectra (Fig. 6) in addition to the ^{13}C NMR (Table 2) lead to structure **7**. Its relative configuration resulted from the NOE interactions of the two methine protons H-4 and H-5, which showed cross-peaks with H-8b and H-10b. On the other face of the molecule, interactions of protons H-12 and H-13, and H-12 to one of the bridge protons H-1 were observed (Fig. 7). The relative configuration of **7** at C-4 and C-5 was assigned 4β and 5β because its

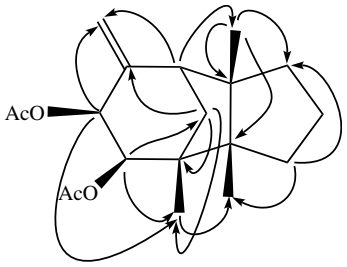


Figure 6. Long-range ^{1}H–^{13}C couplings for **7**.

Table 2. ^{13}C NMR (125.7 MHz, C_6D_6); δ (ppm) data of compounds **7**, **9**, **11**, **12**, **13**, **14**, and **16**[a]

Carbon	7	9	11	12	13	14	16
1	41.7	41.3	44.0	43.8	45.4	43.1	41.7
2	56.1	56.2	55.8	54.3	54.8	48.0	43.1
3	146.8	148.6	149.0	58.9	61.9	139.4	147.5
4	69.6	36.2	128.0	65.9	67.9	124.7	149.5
5	76.5	76.4	144.0	43.1	44.4	40.8	41.9
6	49.7	47.4	46.7	44.9	44.4	44.2	44.7
7	55.3	55.3	56.7	54.7	55.1	56.0	55.7
8	34.5	35.1	37.5	36.3	36.2	37.7	37.5
9	28.2	27.8	27.1	27.7	28.0	27.7	27.3
10	38.1	38.1	39.1	36.8	37.5	38.9	39.2
11	55.9	55.5	57.9	55.2	55.5	58.6	58.1
12	28.3	27.8	27.7	27.9	28.3	27.9	27.4
13	24.2	24.2	23.1	23.2	23.0	24.1	24.5
14	19.5	19.8	21.6	24.0	23.9	25.0	24.2
15	107.9	110.1	110.1	50.3	47.9	68.4	191.7
16	169.8	169.7		170.2	170.0	169.7	
17	20.3	21.2		20.5	20.4	20.8	
18	170.0						
19	20.7						

[a]All assignments were confirmed by HMBC and HMQC.

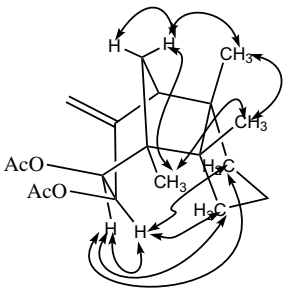


Figure 7. NOE correlations for **7**.

NOESY interactions were very similar to that of (−)-4β-acetoxygymnomitr-3(15)-ene **8**[3] of known absolute configuration, which was also isolated from the same essential oil.

2.3.2. Structure elucidation of (+)-5β-acetoxygymnomitr-3(15)-ene 9. Compound **9** exhibits a molecular ion signal at m/z 262 consistent with $C_{17}H_{26}O_2$. The ^{13}C NMR spectrum (Table 2) revealed the presence of 17 carbon resonances. ^{1}H NMR and HMQC demonstrated that 26 protons were directly attached to the carbon skeleton. The ^{1}H NMR spectrum (C_6D_6) showed signals of four singlets for the methyl groups at δ 0.79 (3H, s, H-13), 0.83 (3H, s, H-14), 0.92 (3H, s, H-15), and 1.71 (3H, s, H-17). In addition, **9** shared several spectral similarities with **7** and (−)-4β-acetoxygymnomitr-3(15)-ene **8**.[3] Thus, suggesting that **7** is a structural isomer of **8** with the acetoxy group at C-5. The difference in substitution pattern is reflected principally in the ^{1}H NMR signal shift of methyls (H-13 and H-14), in addition to the ^{13}C NMR signal for C-5 (δ 76.4) as compared to δ 70.4 (C-4) in **8**.[3] Information from 2D ^{1}H–^{1}H COSY, HMQC, and HMBC spectra in addition to the ^{13}C NMR (Table 2) suggested structure **9**. Its relative configuration from the NOESY spectrum could not be conclusively ascertained from the interactions of the methine protons H-5 with H-4b and the overlapped signals of protons H-8a, H-10a, H-9, and H-1a. The stereochemistry of **9** at C-5 was confirmed to be of β-orientation because its K_2CO_3/MeOH hydrolysis gave gymnomitr-3(15)-en-5β-ol **10**, an epimer of (−)-gymnomitr-3(15)-en-5α-ol[23] isolated from the liverwort *Cylindrocolea recurvifolia*. Purification of **10** by preparative GC gave the sesquiterpene hydrocarbon (−)-gymnomitr-3(15),4-diene **11**[24] as a dehydration product. The ^{13}C NMR data of **11** are reported for the first time (Table 2).

2.3.3. Structure elucidations of (−)-3β,15β-epoxy-4β-acetoxygymnomitrane 12 and (−)-3α,15α-epoxy-4β-acetoxygymnomitrane 13. Compound **12** the 3β,15β-epoxide of (−)-4β-acetoxygymnomitr-3(15)-ene **8**, and its diastereomer (3α,15α-epoxide) **13** were also isolated. Both **12** and **13** showed similar MS and NMR spectral data and exhibited a molecular ion signal at m/z 278 corresponding to the molecular formula of $C_{17}H_{26}O_3$. ^{1}H NMR and HMQC spectroscopic analyses indicated a total of 26 protons directly attached to the carbon skeleton of each. The ^{1}H NMR spectrum (C_6D_6) of **12** showed signals of four singlets for methyl groups at δ 0.72 (3H, s, H-13), 0.73 (3H, s, H-14), 0.82 (3H, s, H-12), and 1.68 (3H, s, H-17). The downfield signals at δ 2.27 (1H, d, $J = 5.0$ Hz) and 2.60 (1H, d, $J = 5.0$ Hz) were assigned to the epoxy-methylene at C-15. The signal of the proton at δ 5.85 (1H, dd, $J = 7.6$, 11.4 Hz), attached to C-4 (δ 65.9), was assigned to the acetoxy methine proton H-4. The ^{1}H NMR spectrum (C_6D_6) of **13** were very similar to that of compound **12**. The ^{13}C NMR data of **12** and **13** are given in Table 2. All the informations from the 2D ^{1}H–^{1}H COSY, HMQC, and HMBC analyses (Fig. 8) was consistent with the assigned structures **12** and **13**. The relative configuration of **13** was derived from the NOESY spectrum, which

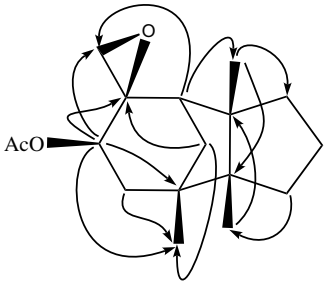


Figure 8. Long-range ^{1}H–^{13}C couplings for **12**.

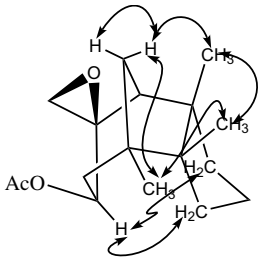
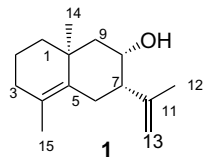


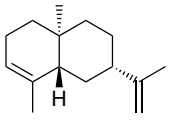
Figure 9. NOE correlations for **12**.

indicates the spatial interactions of proton H-4 with both H-8a and H-10b. The NOE interactions observed for proton H-4 of **13** are similar to that of the supposed precursor **8**,[3] thus suggesting a β-orientation for the acetate group at C-4. Compound **12** demonstrated similar NOE interactions (Fig. 9) but in this case H-4 interacted with the overlapped signals of protons H-8b, H-10b, and H-5b. Therefore, in order to established the stereochemistry at C-4 and that of the epoxy rings in **12** and **13**, the supposed precursor **8**[3] of known absolute configuration was oxidized with *m*-chloroperbenzoic acid (*m*-CPBA) in chloroform. The epoxidation of **8** gave a mixture of **12** and **13** in a ratio of 10:1, respectively, as compared to the ratio of their natural occurrence of 4:3. Therefore, the β-orientation of the acetate group at C-4 in compounds **12** and **13** was confirmed. The relative configurations of the *epoxy*-rings in **12** and **13** were assigned by comparison of the shielding–deshielding effect of the oxirane ring on the chemical shifts of other neighboring protons with respect to compound **8** by the analysis of the ^{1}H NMR spectrum (C_6D_6). In the major epoxide **12**, deshielding of the protons H-4, H-5a and the bridge protons, H-1a,b (at δ 1.80 and 1.89) might be due to the proximity of the electronegative oxygen of the epoxide ring. While in the minor epoxide **13**, although H-4, 8b, and 10b were slightly deshielded, the bridge protons H-1a,b (at δ 1.29 and 1.82) are shielded as compared to the bridge protons of **8** (at δ 1.40 and 1.92). Interestingly, **12** has been isolated from *M. emarginata* collected in Scotland (Wu, 2004, personal communication).

2.3.4. Structure elucidation of (−)-15-acetoxygymnomitr-3-ene 14. Compound **14** has been reported as a reaction product in the structural establishment of (+)-gymnomitren-15-ol **15**,[25] and only partial ^{1}H NMR data of **14** are reported in the literature.[25] The ^{1}H NMR spectrum (C_6D_6) of **14** showed signals of four singlets for methyl groups at δ 0.78 (3H, s, H-13), 0.83 (3H, s, H-14), 0.94 (3H, s, H-12), and 1.70 (3H, s, H-17). The deshielded signals at δ 4.51 (2H, d, $J = 8.8$ Hz) and 5.41 (1H, br s,) were assigned to protons H-15 and H-4, respectively. All these informations from the 2D ^{1}H–^{1}H COSY, HMQC, and HMBC analyses in addition to the ^{13}C NMR (Table 2) were consistent with the assigned structure **14**. Treatment of **14** with K_2CO_3 in methanol gave the de-acetylated compound (+)-**15**, which was also isolated and identified in the essential oil of *M. emarginata*. The spectral data of **15** were consistent with the literature data.[26] In addition, (+)-α-barbatenal **16**,[27] the oxidized form of **15** was isolated for the first time in a liverwort. The spectral data of (+)-**16** were identical with that of (−)-enantiomer[27] isolated from the roots of the higher plant *Joannesia princeps*. ^{13}C NMR data (C_6D_6) of (+)-**16** are given in Table 2.



1



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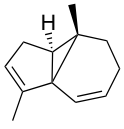

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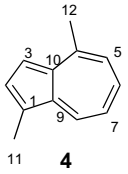

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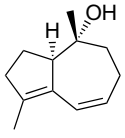

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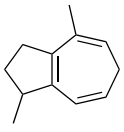
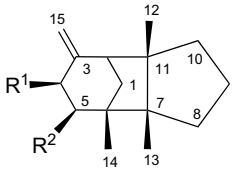
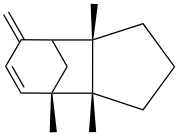


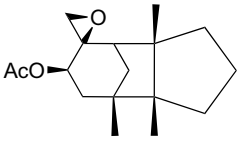
6



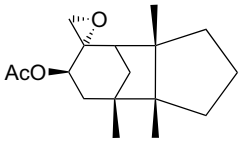
7 $R^1 = R^2 = OAc$
8 $R^1 = OAc$; $R^2 = H$
9 $R^2 = OAc$; $R^1 = H$
10 $R^2 = OH$; $R^1 = H$



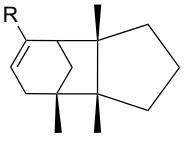
11



12



13



14 R = CH_2OAc
15 R = CH_2OH
16 R = CHO

3. Conclusion

In conclusion, compound **1** was isolated from the essential oil of *D. albicans*, while compounds **5** and **6** were isolated from the essential oil of *B. floerkei*, and compounds **7**, **9**, **12**, **13**, and **14** were isolated from the essential oil of *M. emarginata*.

4. Experimental

4.1. General experimental procedures

4.1.1. Gas chromatography. Carlo Erba HRGC 5300 Mega series instrument fitted with 25 m fused silica capillaries coated with polysiloxane CPSil-5 and polysiloxane CPSil-19 (Chrompack); Carlo Erba Fractovap 2150 or 4160 gas chromatographs with 25 m fused silica capillaries coated with octakis(2,6-di-*O*-methyl-3-*O*-pentyl)-γ-cyclodextrin,[28] heptakis(2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin[28] or heptakis(6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin[29] in OV 1701 (50%, w/w), split injection; split ratio approx. 1:30; FID; carrier gas 0.5 bar H_2; injector and detector temperatures were 200 and 250 °C, respectively.

4.1.2. Preparative GC. Modified Varian 1400 and 2800 instruments, equipped with stainless steel columns (1.85 m × 4.3 mm) with 10% polydimethylsiloxane SE-30 on Chromosorb W-HP or with 2.5% octakis(2,6-di-*O*-methyl-3-*O*-pentyl)-γ-cyclodextrin in OV-1701 (50%, w/w) on Chromosorb G-HP or with 6% heptakis(6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin in SE-52 (50%, w/w) on Chromosorb W-HP; FID; helium as carrier gas at a flow rate of 120 mL/min.; injector and detector temperatures were 200 and 250 °C, respectively.[30]

4.1.3. GC–MS. Electron impact (70 eV) and chemical ionization (NH_3) GC–MS were carried out on a Hewlett Packard HP 5890 gas chromatograph coupled with a VG Analytical 70-250S magnetic field mass spectrometer. All compounds were identified by comparison of their mass spectra and gas chromatographic retention indices with a spectral library established under identical experimental conditions.[19,20]

4.1.4. NMR spectroscopy. NMR measurements were carried out with a Bruker WM 500 (500 MHz) instrument in C_6D_6 using TMS as the internal standard. NMR-experiment acquisition for ^1H–^1H COSY was with 4096 (F2) and 256 (F1) data points. Delay was 1.1s. F2 and F1 were each processed as Qsine with a sinus shift of 0. HSQC was recorded as a phase sensitive experiment with TD = 4096 (F2) and TD = 128 (F1) data points and with a delay of 1/4* 145 Hz. Processing was carried out as an exponential multiplication in F2 with line broadening of 4.00 Hz and with QSINE in F1 with a sinus shift of 2. HMBC was recorded as a *J* filtered experiment with TD = 4096 (F2) and TD = 256 (F1). Delay was 0.065s. F2 and F1 were each processed as Qsine with a sinus shift of 4. NOESY was recorded as phase sensitive with TD = 4096 (F2) and TD = 400 (F1) data points, respectively. Mixing time was 600 ms. F2 and F1 were each processed as Qsine with a sinus shift of 2.

4.1.5. Polarimetry. Measurements were performed with a polarimeter 341 (Perkin–Elmer) at 589 nm at 20 °C. Due to very small amounts of isolated compounds only the direction of optical rotation is given to avoid inaccuracies.

4.1.6. Thin layer chromatography. Thin layer chromatography was effected using glass and aluminum plates coated with Silica 60 F_{254} (Merck). An ethanolic solution of sulfuric acid (10%) and anisaldehyde was used as spray reagent. The solvent system used was *n*-hexane/ethyl acetate (3:1, v/v). The R_f values were also determined in this solvent system.

4.1.7. Reactions. Hydrogenation reactions were performed by bubbling hydrogen gas through a stirred solution of ca. 1 mg of sample in 1 mL *n*-hexane and 0.5 mg Pd/C at room temperature for 1 h. The reaction mixture was filtered, and the reaction products analyzed by GC–MS and by GC on several capillary columns with modified cyclodextrin derivatives.

Deacetylation reactions were carried out by treating ca. 1 mg of samples in methanol (1 mL) with ca. 2 mg of K_2CO_3. The reaction mixture was stirred at room temperature for 12 h. After workup, the product was analyzed by GC and GC–MS.

Acidic transformations were carried out by treatment of ca. 0.3 mg of samples in hexane with a few granules of Amberlyst® 15 at room temperature for about 2 h. The reaction mixture was monitored by GC.

Epoxidation was performed with ca. 2 mg of sample in 5 mL chloroform and ca. 2 mg *m*-chloroperbenzoic acid (*m*-CPBA). After 1 h of stirring at room temperature the reaction mixture was filtered through a short column of florisil and the reaction products were analyzed by GC–MS and by GC on several capillary columns with modified cyclodextrin derivatives.

4.2. Origin of *Marsupella aquatica*

D. albicans plant material was collected in Altenau/Harz, Germany in June 2002, *B. floerkei* plant material was collected in Altenau, Germany in August 2002 and *M. emarginata* was collected in Saarland, Germany in March 2001.

4.3. Isolation of compounds

The hydrodistillate of liverworts samples was subjected to flash column chromatography (column packed dry with silica gel; elution with *n*-hexane to yield the hydrocarbon fraction and gradient elution with ethyl acetate in hexane to yield the oxygenated fractions). Oxygenated fractions were further subjected to prep-TLC and final isolations were carried out using GC Varian 1400 and 2800 on a packed column with SE-30- and/or SE-52-columns combined with at least one cyclodextrin phase column.

4.4. (+)-Eudesma-4,11-dien-8α-ol 1

Colourless oil; $RI_{CPSIL\ 5} = 1648$; sense of optical rotation (benzene): (+); ^1H NMR (500 MHz, C_6D_6) and ^{13}C NMR (125.7 MHz, C_6D_6 see Table 1); MS (EI, 70 eV) *m/z* (rel. int.): 220 [M^+] (28), 205 (10), 202 (21), 187 (60), 177 (22), 161 (100), 147 (28), 133 (29), 123 (36), 105 (68), 91 (59), 79 (42), 67 (20), 55 (36).

4.5. (−)-2,3,3a,4,5,6-Hexahydro-1,4-dimethylazulen-4-ol 5

Colourless oil; $RI_{CPSIL\ 5} = 1448$; sense of optical rotation (benzene): (δ); ^1H NMR (500 MHz, C_6D_6): and ^{13}C NMR (125.7 MHz, C_6D_6 see Table 1); MS (EI 70 eV): *m/z* (rel. int.): 178 [M^+] (12), 160 (43), 131 (73), 117 (48), 115 (50), 105 (57), 91 (100), 79 (59), 77 (70), 65 (35), 51 (53).

4.6. (+)-1,2,3,6-Tetrahydro-1,4-dimethylazulene 6

Colourless oil; $RI_{CPSIL\ 5} = 1244$; sense of optical rotation (benzene): (+); ^1H NMR (500 MHz, C_6D_6) and ^{13}C NMR (125.7 MHz, C_6D_6 see Table 1); MS (EI 70 eV), *m/z* (rel. int.): 160 [M^+] (22), 145 (100), 130 (23), 128 (29), 118 (39), 115 (36), 105 (12), 91 (28), 77 (22), 63 (22), 51 (31).

4.7. (−)-4β,5β-Diacetoxygymnomitr-3(15)-ene 7

Colourless oil; $RI_{CPSIL\ 5} = 1943$; sense of optical rotation (benzene): (−); ^1H NMR (500 MHz, C_6D_6): δ 0.74 (3H, s, H-13), 0.78 (3H, s, H-14), 0.86 (3H, s, H-12), 1.01 (1H, dd, H-8a, $J = 7.3$, 13.6 Hz), 1.21 (1H, dd, H-10a, $J = 6.9$, 12.3 Hz), 1.67–1.72 (2H, m, H-1a, H-9a), 1.76 (3H, s, H-19), 1.80 (3H, s, H-17), 1.92–2.04 (2H, m, H-10b, H-9b), 2.10 (1H, d, H-1b, $J = 12.0$ Hz), 2.25–2.32 (1H, m, H-8b, $J = 6.9$ Hz), 2.28 (1H, d, H-2, $J = 4.8$ Hz), 4.87 (1H, t, H-15a, $J = 2.2$ Hz), 5.03 (1H, t, H-15b, $J = 2.2$ Hz), 5.48 (1H, d, H-5, $J = 4.1$ Hz), 6.27–6.29 (1H, m, H-4); ^{13}C NMR (125.7 MHz, C_6D_6) see Table 2; MS (EI, 70 eV), *m/z* (rel. int.): 200 (7), 185 (4), 164 (10), 153 (11), 122 (52), 105 (10), 96 (21), 95 (32), 91 (17), 81 (22), 67 (8), 55 (11), 43 (100); CIMS *m/z* (rel. int.): 338 [M^++NH_4^+] (75), 261 (33), 236 (7), 219 (67), 201 (25), 185 (5), 164 (10), 153 (12), 122 (100), 106 (84), 96 (64), 95 (76), 91 (57), 81 (34).

4.8. (+)-5β-Acetoxygymnomitr-3(15)-ene 9

Colourless oil; $RI_{CPSIL\ 5} = 1755$; sense of optical rotation (benzene): (+); ^1H NMR (500 MHz, C_6D_6): δ 0.79 (3H, s, H-13), 0.83 (3H, s, H-14), 0.89–0.94 (1H, m, H-8a), 0.92 (3H, s, H-15), 1.12–1.18 (1H, m, H-10a), 1.64–1.69 (3H, m, H-9, H-10b), 1.70–1.75 (2H, m, H-1a, H-8b), 1.71 (3H, s, H-17), 2.05 (1H, d, H-1b, $J = 11.7$ Hz), 2.18 (1H, d, H-2, $J = 4.7$ Hz), 2.38 (1H, d, H-4a, $J = 18.0$ Hz), 2.73 (1H, ddd, H-4b, $J = 2.5$, 5.7, 18.0 Hz), 4.68 (1H, t, H-15a, $J = 2.2$ Hz), 4.74 (1H, t, H-15b, $J = 2.5$ Hz), 5.10 (1H, d, H-5, $J = 5.7$ Hz); ^{13}C NMR (125.7 MHz, C_6D_6) see Table 2; MS (EI, 70 eV), *m/z* (rel. int.): 262 [M^+] (2), 202 (10), 187 (6), 173 (2), 166 (2), 159 (4), 153 (6), 145 (4), 131 (6), 124 (6), 115 (4), 106 (100), 96 (36), 95 (54), 91 (94), 81 (38), 67 (15), 55 (24), 43 (74).

4.9. (−)-Gymnomitr-3(15)-en-5β-ol 10

Colourless needles; $RI_{CPSIL\ 5} = 1654$; sense of optical rotation (benzene): (−); ^1H NMR (500 MHz, C_6D_6): δ 0.83 (3H, s), 0.89–0.94 (1H, m), 0.93 (3H, s), 0.94 (3H, s), 1.16–1.21 (1H, m), 1.64 (1H, dd, $J = 4.7$, 12.0 Hz), 1.68–1.73 (4H, m), 1.96 (1H, d, $J = 12.0$ Hz), 2.06 (1H, d, $J = 17.0$ Hz), 2.16 (1H, d, $J = 4.7$ Hz), 2.68 (1H, ddd, $J = 2.5$, 5.4, 17.0 Hz), 3.38 (1H, s), 4.7 (1H, t, 2.5 Hz), 4.75 (1H, t, $J = 2.2$ Hz); ^1H NMR (500 MHz, $CDCl_3$): δ 0.94 (3H, s), 0.96 (3H, s), 1.05 (3H, s), 1.06–1.11 (1H, m), 1.19–1.22 (1H, m), 1.43 (1H, d, $J = 6.0$ Hz), 1.77 (2H, d, $J = 2.5$ Hz), 1.80–1.90 (3H, m), 2.18 (1H, br s), 2.27 (1H, d, $J = 17.0$ Hz), 2.90 (1H, ddd, $J = 2.8$, 5.4, 11.0 Hz), 3.64 (1H, t, $J = 5.4$ Hz), 4.66 (1H, t, $J = 2.2$ Hz), 4.68 (1H, t, $J = 2.5$ Hz); MS (EI, 70 eV), *m/z* (rel. int.): 202 (M^+−18), (5), 187 (2), 159 (2), 153 (4), 145 (2), 129 (4), 124 (10), 115 (8), 106 (88), 96 (30), 95 (39), 91 (100), 81 (38), 67 (12), 55 (18), 41 (32).

4.10. 5(−)-Gymnomitr-3(15)-4-diene 11

Colourless oil; $RI_{CPSIL\ 5} = 1408$; sense of optical rotation (benzene): (−); ^1H NMR (500 MHz, C_6D_6): δ 0.80 (3H, s, H-13), 0.87 (3H, s, H-14), 0.99 (3H, s, H-12), 1.02–1.08 (1H, m, H-8a), 1.17–1.23 (1H, m, H-10a), 1.50–1.56 (1H, m, H-9a), 1.58 (1H, d, H-1a, $J = 10.7$ Hz), 1.68–1.74 (1H, m, H-9b), 1.80–1.85 (2H, m, H-1b, H-8b), 1.86–1.92 (1H, m, H-10b), 2.29 (1H, d, H-2, $J = 4.4$ Hz), 4.68 (1H, br s, H-15a), 4.80 (1H, d, H-15b, $J = 2.5$ Hz), 5.55 (1H, d, H-5, $J = 8.8$ Hz), 5.97 (1H, d, H-4, $J = 9.5$ Hz); ^{13}C NMR (125.7 MHz, C_6D_6) see Table 2; MS (EI, 70 eV), *m/z* (rel. int.): 202 [M^+] (8), 106 (88), 91 (100), 81 (28), 65 (12), 53 (11), 41 (20).

4.11. (−)3β,15β-Epoxy-4β-acetoxygymnomitrane 12

Colourless oil; $RI_{CPSIL\ 5} = 1875$; sense of optical rotation (benzene): (−); ^1H NMR (500 MHz, C_6D_6): δ 0.72 (3H, s, H-13), 0.73 (3H, s, H-14), 0.82 (3H, s, H-12), 1.09 (1H, dd, H-8a, $J = 6.93$, 13.24 Hz), 1.18 (1H, dd, H-10a, $J = 7.56$, 14.81 Hz), 1.19 (1H, d, H-2, $J = 4.7$ Hz), 1.50–1.58 (1H, m, H-5a), 1.68 (3H, s, H-17), 1.68–1.80 (2H, m, H-1a, H-9a), 1.89 (1H, d, H-1b, $J = 11.4$ Hz), 1.88–1.92 (1H, m, H-9b), 2.04–2.18 (3H, m, H-8b, H-10b, H-5b), 2.27 (1H, d, H-15a, $J = 5.0$ Hz), 2.60 (1H, d, H-15b, $J = 5.0$ Hz), 5.85 (1H, dd, H-4, J = 7.6, 11.4 Hz); ^{13}C NMR (125.7 MHz, C_6D_6) see Table 2; MS (EI, 70 eV), *m/z* (rel. int.): 278 [M^+] (2), 236 (2), 218 (8), 203 (4), 188 (4), 175 (4), 162 (4), 147 (6), 133 (5), 122 (22), 107 (29), 96 (91), 95 (100), 91 (48), 81 (79), 79 (31), 77 (30), 67 (18), 60 (10), 55 (35), 43 (56).

4.12. (−)3α,15α-Epoxy-4β-acetoxygymnomitrane 13

Colourless oil; $RI_{CPSIL\ 5} = 1887$; sense of optical rotation (benzene): (−); ^1H NMR (500 MHz, C_6D_6): δ 0.73 (3H, s, H-13), 0.74 (3H, s, H-14), 0.87 (3H, s, H-12), 1.09 (1H, dd, H-8a, $J = 6.9$, 13.6 Hz), 1.16–1.21 (2H, m, H-5a, H-2), 1.29 (1H, d, H-1a, $J = 11.7$ Hz), 1.41 (1H, dd, H-10a, $J = 7.6$, 13.6 Hz), 1.60 (3H, s, H-17), 1.67–1.75 (1H, m, H-9a), 1.82 (1H, ddd, H-1b, $J = 3.2$, 5.0, 11.7 Hz), 1.91

(1H, p, H-9b, $J = 6.9$ Hz), 2.09 (1H, d, H-15a, $J = 5.7$ Hz), 2.17 (1H, dt, H-8b, $J = 6.3$, 12.9 Hz), 2.30 (1H, ddd, H-5b, $J = 3.2$, 7.6, 13.2 Hz), 2.65 (1H, dt, H-10b, $J = 6.9$, 13.9 Hz), 2.90 (1H, H-15b, d, $J = 5.7$ Hz), 5.95 (1H, dd, H-4, $J = 7.6$, 10.7 Hz); ^{13}C NMR (125.7 MHz, C_6D_6) see Table 2; MS (EI, 70 eV), *m*/*z* (rel. int.): 278 [M^+], 236 (2), 218 (12), 203 (5), 188 (4), 181 (2), 175 (5), 161 (5), 147 (8), 133 (8), 122 (28), 107 (35), 96 (100), 95 (94), 91 (44), 81 (79), 79 (42), 77 (31), 67 (18), 60 (13), 55 (34), 43 (53); MS (CI, NH_3 gas), *m*/*z* (rel. int.): 279 [M^++1] (12), 236 (25), 219 (100), 205 (17), 122 (7), 106 (17), 96 (37), 95 (52), 81 (22).

4.13. (−)-15-Acetoxygymnomitr-3-ene 14

Colourless oil; $RI_{CPSIL\ 5} = 1784$; sense of optical rotation (benzene): (−); ^{1}H NMR (500 MHz, C_6D_6): δ 0.78 (3H, s, H-13), 0.83 (3H, s, H-14), 0.94 (3H, s, H-12), 1.00 (1H, dd, H-8a, $J = 6.9$, 12.0 Hz), 1.14–1.20 (1H, m, H-10a), 1.42 (1H, d, H-1a, $J = 9.5$ Hz), 1.51–1.64 (3H, m, H-9, H-8b), 1.70 (3H, s, H-17), 1.78–1.87 (4H, m, H-2, H-1b, H-5a, H-10b), 2.10 (1H, br d, H-5b, $J = 18.9$ Hz), 4.51 (2H, d, H-15, $J = 8.8$ Hz), 5.41 (1H, br s, H-4); ^{13}C NMR (125.7 MHz, C_6D_6) see Table 2; MS (EI, 70 eV), *m*/*z* (rel. int.): 262 [M^+] (3), 220 (7), 202 (9), 187 (5), 159 (5), 136 (4), 131 (6), 121 (6), 106 (100), 95 (70), 91 (85), 81 (38), 67 (16), 55 (30), 43 (73).

4.14. (+)-Barbatenal 16

Colourless oil; $RI_{CPSIL\ 5} = 1659$; sense of optical rotation (benzene): (+); ^{1}H NMR (500 MHz, C_6D_6): δ 0.70 (3H, s, H-14), 0.72 (3H, s, H-13), 0.85–0.92 (1H, m, H-8a), 0.97 (3H, s, H-12), 1.05 (1H, d, H-1a, $J = 11.5$ Hz), 1.12–1.21 (2H, m, H-9a, H-10a), 1.32–1.45 (2H, m, H-8b, H-9b), 1.50–1.55 (1H, m, H-10b), 1.65 (1H, dd, H-5a, $J = 3.3$, 21.1 Hz), 1.71 (1H, dd, H-1b, $J = 4.6$, 11.5 Hz), 2.04 (1H, dd, H-5b, $J = 2.8$, 20.1 Hz), 2.78 (1H, d, H-2, $J = 4.3$ Hz), 5.87 (1H, H-4, br t, $J = 3.3$ Hz), 9.30 (1H, s, H-15); ^{13}C NMR (125.7 MHz, C_6D_6) see Table 2; MS (EI, 70 eV), *m*/*z* (rel. int.): 218 [M^+] (8), 200 (4), 189 (3), 175 (3), 161 (3), 147 (6), 133 (5), 124 (16), 122 (14), 107 (22), 96 (95), 95 (100), 81 (75), 79 (33), 77 (36), 67 (17), 55 (32), 41 (48).

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References

1. Asakawa, Y. Chemical constituents of the Bryophytes. In *Progress in the Chemistry of Organic Natural Products*; Herz, W., Kirby, G. W., Moore, R. E., Steglich, W., Tamm, C., Eds.; Springer: Vienna, New York, 1995; Vol. 65, pp 179–181.
2. (a) Zinsmeister, H. D.; Becker, H.; Eicher, T. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 130–147; (b) Asakawa, Y. *Pure Appl. Chem.* **2007**, *79*, 557–580.
3. Adio, A. M.; Paul, C.; König, W. A.; Muhle, H. *Phytochemistry* **2002**, *61*, 79–91.
4. Adio, A. M.; Paul, C.; Kloth, P.; König, W. A. *Phytochemistry* **2003**, *65*, 199–206.
5. Adio, A. M.; Paul, C.; Tesso, H.; Kloth, P.; König, W. A. *Tetrahedron: Asymmetry* **2004**, *15*, 1631–1635.
6. Benesova, V.; Samek, Z.; Vasickova, S. *Collect. Czech., Chem. Commun.* **1975**, *40*, 1966–1970.
7. Ohta, Y.; Andersen, N. H.; Liu, C.-B. *Tetrahedron* **1977**, *33*, 617–628.
8. Toyota, M.; Ooiso, Y.; Kusuyama, T.; Asakawa, Y. *Phytochemistry* **1994**, *35*, 1263–1265.
9. Asakawa, Y.; Toyota, M.; Takemoto, T.; Suire, C. *Phytochemistry* **1979**, *18*, 1007–1009.
10. Andersen, N. H.; Costin, C. R.; Kramer, C. M., Jr.; Ohta, Y.; Huneck, S. *Phytochemistry* **1973**, *12*, 2709–2716.
11. Huneck, S.; Forbes, C. A.; Connolly, J. D.; McLaren, M.; Rycroft, D. S. *Tetrahedron Lett.* **1982**, *23*, 3959–3962.
12. Huneck, S.; Baxter, G. A.; Forbes, C. A.; Connolly, J. D.; Harrison, L. J.; Phillips, W. R.; Rycroft, D. S.; Sim, G. A. *J. Chem. Soc., Perkin Trans. 1* **1986**, 809–814.
13. Nagashima, F.; Takaoka, S.; Huneck, S.; Asakawa, Y. *Phytochemistry* **1999**, *51*, 563–566.
14. Tori, M.; Nagai, T.; Asakawa, Y.; Huneck, S.; Ogawa, K. *Phytochemistry* **1993**, *34*, 181–190.
15. Matsuo, A.; Uto, S.; Sakuda, K.; Uchio, Y.; Nakayama, M.; Hayashi, S. *Chem. Lett.* **1979**, 73–76.
16. Harrison, L. J.; Becker, H.; Connolly, J. D.; Rycroft, D. S. *Phytochemistry* **1992**, *31*, 4027–4028.
17. Nagashima, F.; Ohi, Y.; Nagai, T.; Tori, M.; Asakawa, Y.; Huneck, S. *Phytochemistry* **1993**, *33*, 1445–1448.
18. Matsuo, A.; Nozaki, H.; Yano, K.; Uto, S.; Nakayama, M.; Huneck, S. *Phytochemistry* **1990**, *29*, 1921–1924.
19. Joulain, D.; König, W. A. *The Atlas of Spectral Data of Sesquiterpene Hydrocarbons*; E. B.: Hamburg, 1998.
20. Hochmuth, D. H.; Joulain, D.; König, W. A. *MassFinder 3.1, Software and Data*; University of Hamburg, 2004. www.massfinder.com.
21. Takeda, R.; Katoh, K. *Bull. Chem. Soc. Jpn.* **1983**, *56*, 1265–1266.
22. Izac, R. R.; Fenical, W.; Wright, J. M. *Tetrahedron Lett.* **1984**, *25*, 1325–1328.
23. Wu, C.-L.; Kao, T.-L. *J. Asian Nat. Prod. Res.* **2002**, *4*, 281–285.
24. Warmers, U.; König, W. A. *Phytochemistry* **1999**, *52*, 1501–1505.
25. Andersen, N. H.; Costin, C. R.; Kramer, C. M., Jr.; Ohta, Y.; Huneck, S. *Phytochemistry* **1973**, *12*, 2709–2716.
26. Buchanan, M. S.; Connolly, J. D.; Kadir, A. A.; Rycroft, D. S. *Phytochemistry* **1996**, *42*, 1641–1646.
27. Achenbach, H.; Benirschke, G. *Phytochemistry* **1997**, *45*, 149–157.
28. König, W. A.; Gehrcke, B.; Icheln, D.; Evers, P.; Dönnecke, J.; Wang, W. *J. High Res. Chromatogr.* **1992**, *15*, 367–372.
29. Dietrich, A.; Maas, B.; Messer, W.; Bruche, G.; Karll, V.; Kaunzinger, A.; Mosandl, A. *J. High Res. Chromatogr.* **1992**, *15*, 590–593.
30. Hardt, I. H.; König, W. A. *J. Chromatogr., A* **1994**, *666*, 611–615.