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Phytochemistry 66 (2005) 1094–1099
PHYTOCHEMISTRY
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Sacculatane diterpenoids from axenic cultures of the liverwort *Fossombronia wondraczekii*

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Received 10 December 2004; received in revised form 2 March 2005
Available online 25 April 2005

Dedicated to Dr. W. Schild on the occasion of his retirement

Abstract

Five new sacculatane diterpenoids, 17,18-epoxy-7-sacculaten-12,11-olide, 7,17-sacculatadien-11,12-olide, 11β,12-epoxy-7,17-sacculatadien-11α-ol, 1β-acetoxy-11β,12-epoxy-7,17-sacculatadien-11α-ol and 1β,15ξ-diacetoxy-11,12-epoxy-8(12),9(11),17-sacculatatriene along with sacculatal and sacculatanolide have been isolated from axenic cultures of the liverwort *Fossombronia wondraczekii* and their structures assigned on the basis of their spectroscopical properties.

Keywords: *Fossombronia wondraczekii*; Hepaticae; Sacculatanes; Axenic culture

1. Introduction

Recent investigations on the lipophilic compounds of liverworts resulted in the isolation of numerous new natural products with a high range of biological activity (Zinsmeister et al., 1991; Asakawa, 1982, 1995, 2001). Because of the difficulty of collecting sufficient pure plant material for chemical studies many liverwort species have not yet been investigated. One solution to this problem is to grow liverworts in axenic culture (Becker, 1994). In this paper, we report the isolation and characterisation of seven sacculatane diterpenoids from the lipophilic extract of axenic cultures of the liverwort *Fossombronia wondraczekii*. *Fossombronia* is one of the most isolated genera of the Hepaticae and consists of about 50 thalloid liverwort species (Schuster, 1992). Only two of these species, *Fossombronia pusilla* and the rare Arctic *Fossombronia alaskana*, have been phytochemically investigated so far. Their lipophilic extracts afforded hopane triterpenoids (Grammes et al., 1994), sacculatane diterpenoids with antibacterial activity (Sauerwein and Becker, 1990) and *epi*-neoverrucosane and *epi*-homoverrucosane diterpenoids (Grammes et al., 1997). The biosynthesis of three diterpenoids and a triterpenoid in *F. alaskana* has also been studied (Hertewich et al., 2001).

2. Results and discussion

A combination of size exclusion chromatography, vacuum liquid chromatography and HPLC of the dichloromethane extract of *F. wondraczekii*, cultured in vitro, led to the isolation of five new sacculatane derivatives, 17,18-epoxy-7-sacculaten-12,11-olide (**3**), 7,17-sacculatadien-11,12-olide (**4**), 11β,12-epoxy-7,17-sacculatadien-11α-ol (**5**), 1β-acetoxy-11β,12-epoxy-7,17-sacculatadien-11α-ol (**6**) and 1β,15ξ-diacetoxy-11,12-epoxy-8(12),9(11),17-sacculatatriene (**7**) together with the known sacculatal (**1**) (Asakawa, 1980) and sacculatanolide (**2**) (Hashimoto et al., 1995). Several of the assignments in the published NMR data of **1** are incorrect.

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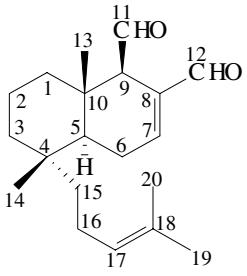
0031-9422/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2005.03.001

The corrected assignments of **1** and the spectral data of **2**, which have not previously been reported, are given in Table 1 and in Section 3. The negative optical rotation of sacculatal **1** ($[\alpha]_D^{20}$ − 14.2 ($CHCl_3$; *c* 1.0)) showed that it had the same absolute stereochemistry as reported by Asakawa et al. (1977). Within this series of sacculatane diterpenoids it seems very unlikely that the absolute stereochemistry of the other compounds will differ from that of **1**.



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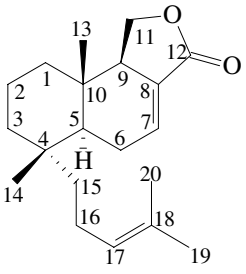

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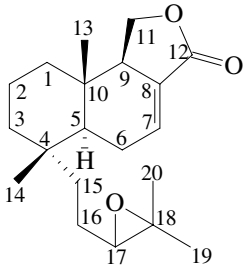

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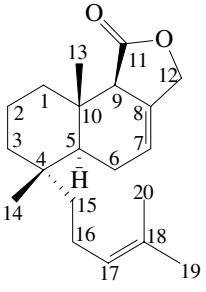

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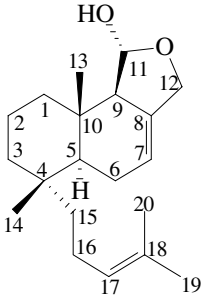

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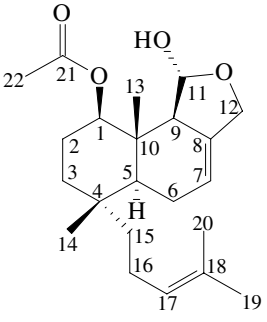

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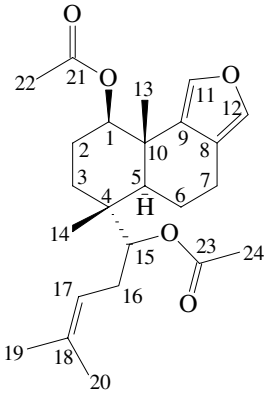

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Table 1
^{13}C NMR spectral data for compounds **1**–**7** in $CDCl_3$

C	1	2	3	4	5	6	7
1	39.3	39.3	39.3	38.3	39.6	81.2	80.5
2	17.8	18.1	18.0	17.9	18.2	24.0	23.6
3	37.3	37.6	37.7	37.7	37.8	37.7	30.7
4	35.5	35.2	35.0	35.4	35.3	35.0	39.2
5	46.9	47.5	47.8	47.2	47.5	46.8	45.0
6	25.0	24.7	24.9*	23.0	23.4	22.9	18.4
7	154.1	136.4	136.2	121.1	117.1	116.8	20.6
8	138.3	127.2	127.3	129.8	136.4	136.5	119.8
9	60.4	50.9	50.9	53.8	61.8	61.0	133.4
10	36.9	34.2	34.3	34.4	33.3	35.4	38.7
11	201.8	67.2	67.2	175.3	99.3	99.8	136.0
12	193.1	170.3	170.2	69.8	69.0	68.4	136.7
13	15.7	13.9	13.9	14.4	14.5	9.8	20.3
14	20.6	20.1	19.9	20.3	20.3	20.5	17.8
15	44.1	44.1	40.3	44.0	44.1	43.6	78.1
16	21.7	21.7	24.8*	21.7	21.7	21.7	28.0
17	124.5	124.6	64.6	124.8	125.0	124.6	120.6
18	131.5	131.4	58.4	131.2	131.1	131.4	134.0
19	25.7	25.7	25.6*	25.7	25.7	25.7	25.7
20	17.6	17.6	17.6	17.6	17.6	17.6	17.7
21						170.8	170.5
22						21.5	21.7
23							170.4
24							21.0

* Assignments interchangeable.

The EI mass spectrum of **2** gave a peak at *m/z* 302 $[M]^+$ consistent with a molecular formula of $C_{20}H_{30}O_2$, which requires six double bond equivalents. Its IR spectrum showed characteristic absorptions for a carbonyl (ν_{max} 1700 cm^{-1}) and a hydroxyl group (ν_{max} 3380 cm^{-1}). The ^1H and ^{13}C NMR spectra were very similar to those of **1** and revealed the presence of two trisubstituted double bonds (δ 6.84, *dd*, *J* = 3.5, 7.0 Hz, H-7; δ 136.4, C-7; δ 127.2, C-8 and δ 5.04, *t*, *J* = 7.0 Hz, H-17; δ 131.4, C-18; δ 124.6, C-17), two vinyl methyls (1.65, Me-19; 1.57, Me-20) and two aliphatic methyl groups (0.92, Me-14; 0.82, Me-13). However, the spectra lacked signals for the two aldehyde groups at C-11 and C-12 and, instead, showed the signals of a γ-lactone involving an oxymethylene group (δ 4.35, *t*, *J* = 9.0 Hz, H-11a; δ 4.02, *t*, *J* = 9.0 Hz, H-11b; δ 67.2, C-11) and a carbonyl group (δ 170.3, C-12). The protons of this oxymethylene group showed $^3J_{CH}$ correlations to the quaternary carbon C-10 in the HMBC spectrum and correlations to H-9 in the COSY spectrum which led to the placement of the γ-lactone moiety. The presence of the α,β-unsaturated carbonyl group was confirmed by the downfield shift of the olefinic proton H-7 (δ 6.84). Due to overlap of their proton signals the methylenes at δ 18.1 and 24.7 could not be assigned unambiguously by analysis of the 2D NMR spectra. However, comparison of the chemical shifts with the corresponding drimane sesquiterpenoid cinnamolide, where C-2 appeared at δ 18.3 and C-6 at δ 25.0 (Ayer and Trifonov, 1992), led to their assignment as C-2 and C-6,

respectively. The relative stereochemistry was deduced from the NOESY spectrum which showed correlations between H-11a and H-9, H-11b and the angular methyl group which in turn correlated with Me-14 and between H-5 and H-9. Hence compound **2** was identified as 7,17-sacculatadien-12,11-olide or sacculatanolide.

The signals of compound **3** in the ^{1}H and ^{13}C NMR spectrum were very close to those observed for **2**. The only difference was the lack of a double bond in the side chain. This was replaced by a trisubstituted epoxide ring (δ 2.64, *t*, $J = 6.0$ Hz, H-17; δ 64.6, C-17; 58.4, C-18). The upfield shifts of Me-19 and Me-20 (δ 1.24 and 1.28, respectively), relative to **2**, and the molecular ion peak at *m/z* 318 $[M]^+$ ($C_{20}H_{30}O_3$) in the EI mass spectrum were consistent with the presence of an epoxide. Therefore compound **3** was established as 17,18-epoxy-7-sacculaten-12,11-olide. It was unstable and decomposed before full spectroscopic data could be obtained.

Compound **4** (*m/z* 302 $[M]^+$, $C_{20}H_{30}O_2$) was an isomer of sacculatanolide **2** that differed in the orientation of the γ-lactone moiety. The ^{1}H and ^{13}C spectroscopic data revealed the characteristic resonances of a sacculatane derivative with two tertiary and two vinyl methyls, a prenyl side chain, a trisubstituted double bond (δ 5.66, *brs*, H-7; δ 121.1, C-7; δ 129.8, C-8) and a γ-lactone involving an oxymethylene group (δ 4.63, *brd*, $J =$ 11.8 Hz, H-12a; δ 4.58, *brd*, $J = 11.8$ Hz, H-12b; δ 69.8, C-12) and a carbonyl group (δ 175.3, C-11). However, it was clear from the chemical shifts of the trisubstituted double bond [H-7 (δ 5.66) and C-7 (δ 121.1)] that it was no longer in conjugation with the lactone carbonyl group and hence the orientation of the lactone ring of **4** was different. Allylic coupling from H-7 to 2H-12 in the COSY spectrum and $^{3}J_{CH}$ couplings from C-7 to 2H-12 in the HMBC spectrum supported this and led to 7,17-sacculatadien-11,12-olide for compound **4**.

Compound **5**, a yellow oil, had the molecular formula $C_{20}H_{32}O_2$ (EIMS, *m/z* 304 $[M]^+$), indicating five double bond equivalents. The ^{1}H and ^{13}C NMR spectroscopic properties revealed the presence of two tertiary methyls (δ 0.89, *s*, Me-14; 0.82, *s*, Me-13), two vinyl methyls (δ 1.65, *s*, Me-19; δ 1.57, *s*, Me-20) and two trisubstituted double bonds (δ 136.4, C-8; δ 117.1, C-7; δ 5.48, *brs*, H-7 and δ 131.1, C-18; δ 125.0, C-17; δ 5.04, *t*, $J = 7.0$ Hz, H-17), consistent with a sacculatane skeleton. In addition they showed peaks for a cyclic hemiacetal (δ 99.3, C-11; δ 5.26, *brs*, H-11) containing an oxymethylene group (δ 69.0, C-12; δ 4.46 and 4.15, both *brd*, $J = 11.5$ Hz, H-12a and H-12b, respectively). Correlations in the COSY, HSQC and HMBC spectra confirmed the structure and the orientation of this hemiacetal moiety. The HMBC correlations between the oxymethylene proton H-12b and the olefinic carbons C-7 ($^{3}J_{CH}$) and C-8 ($^{2}J_{CH}$), its $^{3}J_{CH}$ correlation to C-9, and its $^{3}J_{CH}$ correlation across oxygen to C-11 (thus establishing the lactol ring) were particularly useful. In agreement with this the double bond proton H-7 showed allylic couplings to both oxymethylene protons in the COSY spectrum. A NOE from Me-13 to H-11 defined the configuration of the 11-OH as α. Therefore compound **5** is 11,12-epoxy-7,17-sacculatadien-11α-ol. The 1-hydroxy derivative of **5** was reported from the liverwort *Pellia endiviifolia* (Hashimoto et al., 1995).

Compound **6**, which was obtained as a yellow oil, $C_{22}H_{34}O_4$, *m/z* 362 ($[M]^+$), was the acetoxy derivative of **5**. The ^{1}H and ^{13}C NMR spectra showed the characteristic signals for the isoprene side chain (Table 1 and Section 3) and for the hemiacetal between C-11 (δ 99.8, C-11; δ 5.42, H-11) and C-12 (δ 68.4, C-12; δ 4.37 and 4.10, both *d*, $J = 11.3$ Hz, H-12a and H-12b). The orientation of the lactol ring was confirmed by the correlations between H-7 and H-12a and between H-11 and H-9 in the COSY spectrum and by the correlations of H-12b to both olefinic carbons ($^{3}J_{CH}$ to C-7, $^{2}J_{CH}$ to C-8) in the HMBC spectrum. In contrast to **5**, the NMR spectra contained the resonances of an additional oxygenated methine (δ 4.59, *dd*, $J = 4.0$, 9.0 Hz, H-1; δ 81.2, C-1) associated with an acetate function. Correlations in the HMBC spectrum from this methine proton to C-2 and C-10 and from the oxygenated carbon to 3H-13 established the position of the acetate at C-1 while its equatorial nature followed from the couplings of H-1. The NOESY spectrum revealed couplings between the axial proton H-1 and H-9 which in turn was coupled with H-5 and couplings between H-11 and Me-13 and supported the relative stereochemistry. Hence compound **6** was identified as 1β-acetoxy-11,12-epoxy-7,17-sacculatadien-11α-ol.

The CI mass spectrum of compound **7** (*m/z* = 403 $[M + H]^+$) was in agreement with a molecular formula $C_{24}H_{34}O_5$ and hence eight double bond equivalents. The ^{13}C NMR spectrum had resonances for six methyls, five methylenes, six methines and seven quaternary carbons and, together with the ^{1}H NMR spectrum, revealed the presence of two acetates (δ 21.0, 21.7, 170.4 and 170.5) and a prenyl side chain. In addition, there were signals for a 3,4-disubstituted furan ring (δ 136.7, C-12; 136.0, C-11; 133.4, C-9 and 119.8, C-8; δ 7.05, *d*, $J = 1.5$ Hz, H-11 and 7.01, *d*, $J = 1.5$ Hz, H-12). The placement of this moiety was based on the couplings observed in the HMBC and in the COSY spectra. C-8 showed $^{3}J_{CH}$ correlations to H-11 and H-6 and $^{2}J_{CH}$ correlations to H-12 and 2H-7, whereas C-9 showed $^{3}J_{CH}$ correlations to Me-13 and to the furan protons H-11 ($^{2}J_{CH}$) and H-12 ($^{3}J_{CH}$). Especially important for the assignment of C-11 and C-12 was the long-range coupling between H-12 and 2H-7 in the COSY spectrum. One of the acetates

was attached to C-1 as indicated by the correlations of the oxygenated methine at δ 4.68 (H-1) to C-2, C-13 and C-10 and by the correlations between C-1 and Me-13 and H-2b. The position of the second acetate functionality was readily deduced from the correlations of the remaining oxygenated methine at δ 4.93 (H-15) with carbons of the prenyl side chain (C-16, C-17 and C-23), of the decalin ring system (C-3, C-4 and C-5) and with the methyl at δ 0.96 (Me-14). The couplings of H-15 and 2H-16 in the COSY spectrum furthermore supported the placement of the second acetate at C-15. The NOESY spectrum revealed effects between Me-13 and Me-14 and between H-1 and H-5. Thus the structure of compound **7** was assigned as 1β,15ξ-diacetoxy-11,12-epoxy-8(12),9(11),17-sacculatatriene.

Sacculatanes are rare diterpenoids and their occurrence seems to be restricted to the Hepaticae. Sacculatal **1**, which has a pungent taste and exhibits piscicidal, cytotoxic and antimicrobial activity (Hashimoto et al., 1995; Asakawa, 2001), is the most significant chemical marker of the Metzgeriales *Pellia endiviifolia*, *Pallavicinia levieri* and *Riccardia robata* var. *yakushimensis* (Asakawa, 2004). It was also reported from *F. alaskana* (Hertewich et al., 2001).

3. Experimental

3.1. Spectroscopy and spectrometry

Optical rotations were measured in $CHCl_3$. NMR spectra were recorded in $CDCl_3$ on a BRUKER DRX 500 spectrometer (^{1}H NMR: 500 MHz, ^{13}C NMR: 125 MHz) relative to $CDCl_3$ at δ 7.24, δ_C 77.0. ^{13}C multiplicities were determined using the DEPT pulse sequence. 2D NMR spectra were recorded as ^{1}H, ^{1}H COSY, HSQC and HMBC experiments. The IR spectra were recorded on a Carl Zeiss IMR-16 spectrophotometer. The mass spectra (70 eV) were recorded in the positive EI and CI mode on a Finnigan MAT-90 instrument.

3.2. Plant material

Fossombronia wondraczekii was collected in August 1987 near Eind Saar. Voucher specimens were deposited at the Fachrichtung 8.7, Pharmakognosie und Analytische Phytochemie, Universität des Saarlandes, Saarbrücken. An axenic culture of *F. wondraczekii* was induced from the surface-sterilized gametophyte of field material. The cultures were grown in 200 ml Erlenmeyer flasks with 80 ml of Gamborg B5 medium (Gamborg et al., 1968), solidified with 0.9% agar and supplemented with 1% sucrose. The flasks were kept under constant illumination (2000 lx) at 22 ± 1.5 °C.

3.3. Extraction and isolation

The dried plant material (480 g) was powdered and extracted with CH_2Cl_2. After removal of the CH_2Cl_2 the crude extract was chromatographed by CC on Sephadex LH-20 (150 × 3.5 cm i.d.) with MeOH: CH_2Cl_2 50:50 as eluent, to give three fractions (I, II and III). Fraction II (9.3 g) was separated by VLC (silica gel 15 m, 60 × 35 mm i.d., *n*-hexane-EtOAc gradient) and gave seven fractions (A–G). Further separation of fraction C (8–20% EtOAc) by HPLC on diol modified silica gel (LiChrospher Diol, 5 m, 4 × 250 mm; *n*-hexane:EtOAc 92.5:7.5) gave **4** (155 mg). HPLC of fraction D (20–25% EtOAc) on silica gel (LiChrospher Si 60, 5 m, 4 × 250 mm; *n*-hexane:EtOAc 90:10) gave **1** (95 mg) and **3** (14 mg). HPLC of fraction E (25–30% EtOAc) on diol modified silica gel (LiChrospher Diol, 5 m, 4 × 250 mm; *n*-hexane:EtOAc 88:12) lead to the isolation of **5** (85 mg) and **2** (62 mg). Separation of fraction F (30–50% EtOAc) by HPLC on silica gel (LiChrospher Si 60, 5 m, 4 × 250 mm; *n*-hexane:EtOAc 58:42) yielded **6** (88 mg) and **7** (48 mg).

3.4. 7,17-Sacculatadien-11,12-dial (sacculatal) (*1*)

$[\alpha]_D^{20}$ − 14.2 ($CHCl_3$; *c* 1.0); ^{1}H NMR ($CDCl_3$): δ_H 9.52 (*d*, *J* = 4.5 Hz, H-11), 9.44 (*s*, H-12), 7.09 (*dt*, *J* = 5.0, 2.0 Hz, H-7), 5.03 (*brt*, *J* = 7.0 Hz, H-17), 2.81 (*brs*, H-9), 2.45 (*ddt*, *J* = 20.0, 2.0, 5.0 Hz, H-6a), 2.27 (*dddd*, *J* = 20.0, 12.0, 4.0, 2.0 Hz, H-6b), 1.85 (*m*, 2x H-16), 1.83 (*m*, H-1a), 1.65 (*s*, Me-19), 1.57 (*s*, Me-20), 1.52 (*m*, 2x H-2), 1.45 (*m*, H-3a), 1.35 (*m*, H-1b), 1.31 (*m*, H-5), 1.27 (*m*, H-3b), 1.24 (*m*, H-15a), 1.17 (*m*, H-15b), 0.95 (*s*, Me-13), 0.94 (*s*, Me-14); ^{13}C NMR: Table 1.

3.5. 7,17-Sacculatadien-12,11-olide (sacculatanolide) (*2*)

$[\alpha]_D^{20}$ − 20.3 ($CHCl_3$; *c* 1.0); EIMS: *m/z* (rel. int.) = 302 (18) $[M]^+$, 217 (73), 205 (8), 189 (8), 177 (16), 163 (51), 149 (32), 123 (45), 109 (100), 81 (43), 69 (71), 55 (39); IR ν_{max}^{KBr}: cm^1: 3380, 2920, 1700, 1480, 1370, 1200, 1020, 880; ^{1}H NMR ($CDCl_3$): δ_H 6.84 (*dd*, *J* = 3.5, 7.0 Hz, H-7), 5.04 (*t*, *J* = 7.0 Hz, H-17), 4.35 (*t*, *J* = 9.0 Hz, H-11a), 4.02 (*t*, *J* = 9.0 Hz, H-11b), 2.81 (*m*, H-9), 2.34 (*m*, H-6a), 2.07 (*m*, H-6b), 1.85 (*m*, 2x H-16), 1.65 (*s*, Me-19), 1.57 (*s*, Me-20), 1.56 (*m*, H-1a), 1.51 (*m*, H-2a), 1.47 (*m*, H-3a), 1.43 (*m*, H-5), 1.29 (*m*, H-3b), 1.20 (*m*, 2x H-15), 1.17 (*m*, H-1b), 0.92 (*s*, Me-14), 0.82 (*s*, Me-13); ^{13}C NMR: Table 1.

3.6. 17,18-Epoxy-7-sacculaten-12,11-olide (*3*)

EIMS: *m/z* (rel. int.) = 318 (2) $[M]^+$, 275 (17), 249 (21), 219 (36), 122 (43), 109 (100), 91 (43), 81 (40), 71

(40), 55 (31); ^{1}H NMR ($CDCl_3$): δ_H 6.84 (*dd*, *J* = 4.0, 8.0 Hz, H-7), 4.35 (*t*, *J* = 9.0 Hz, H-11a), 4.02 (*t*, *J* = 9.0 Hz, H-11b), 2.64 (*t*, *J* = 6.0 Hz, H-17), 1.28 (*s*, Me-20), 1.24 (*s*, Me-19), 0.91 (*s*, Me-14), 0.81 (*s*, Me-13); ^{13}C NMR: Table 1.

3.7. 7,17-Sacculatadien-11,12-olide (**4**)

$[\alpha]_D^{20}$ − 15.7 ($CHCl_3$; *c* 2.5); EIMS: *m/z* (rel. int.) = 302 (6) $[M]^+$, 217 (100), 163 (17), 149 (22), 119 (30), 109 (32), 91 (29), 81 (24), 69 (53), 55 (29); HREIMS: *m/z* = 302.2240 $[M]^+$ (Calcd. for $C_{20}H_{30}O_2$: 302.2246); IR ν_{max}^{KBr}: cm^{-1}: 2850, 1750, 1680, 1430, 1350, 1260, 1200, 1110, 1000; ^{1}H NMR ($CDCl_3$): δ_H 5.66 (*brs*, H-7), 5.04 (*t*, *J* = 7.0 Hz, H-17), 4.63 (*brd*, *J* = 12.0 Hz, H-12a), 4.58 (*brd*, *J* = 12.0 Hz, H-12b), 2.76 (*brs*, H-9), 2.48 (*brd*, *J* = 13.5 Hz, H-1a), 2.12 (*brd*, *J* = 18.0 Hz, H-6a), 1.95 (*m*, H-6b), 1.85 (*m*, 2x H-16), 1.65 (*s*, Me-19), 1.57 (*s*, Me-20), 1.54 (*m*, 2x H-2), 1.44 (*m*, H-3a), 1.41 (*m*, H-5), 1.30 (*m*, H-3b), 1.19 (*m*, H-1b), 1.18 (*m*, 2x H-15), 0.89 (*s*, Me-14), 0.88 (*s*, Me-13); ^{13}C NMR: Table 1.

3.8. 11β,12-Epoxy-7,17-sacculatadien-11α-ol (**5**)

$[\alpha]_D^{20}$ − 6.3 ($CHCl_3$; *c* 2.5); EIMS: *m/z* (rel. int.) = 304 (8) $[M]^+$, 203 (18), 175 (20), 135 (25), 123 (29), 109 (37), 95 (51), 81 (62), 69 (100), 55 (62); IR ν_{max}^{KBr}: cm^{-1}: 3390, 2900, 1710, 1435, 1375, 1250, 1000, 810, 715; ^{1}H NMR ($CDCl_3$): δ_H 5.48 (*brs*, H-7), 5.26 (*brs*, H-11), 5.04 (*t*, *J* = 7.0 Hz, H-17), 4.46 (*brd*, *J* = 11.5 Hz, H-12a), 4.15 (*brd*, *J* = 11.5 Hz, H-12b), 2.75 (*brs*, OH-11) 2.19 (*brs*, H-9), 2.07 (*brd*, *J* = 18.5 Hz, H-6a), 1.87 (*m*, H-6b), 1.86 (*m*, 2x H-16), 1.76 (*brd*, *J* = 13.5 Hz, H-1a), 1.65 (*s*, Me-19), 1.58 (*m*, H-2a), 1.57 (*s*, Me-20), 1.49 (*m*, H-2b), 1.42 (*m*, H-3a), 1.35 (*dd*, *J* = 5.5, 11.5 Hz, H-5), 1.28 (*m*, H-3b), 1.22 (*m*, H-1b), 1.18 (*m*, H-15), 0.89 (*s*, Me-14), 0.82 (*s*, Me-13); ^{13}C NMR: Table 1.

3.9. 1β-Acetoxy-11β,12-epoxy-7,17-sacculatadien-11α-ol (**6**)

$[\alpha]_D^{20}$ − 8.7 ($CHCl_3$; *c* 1.0); EIMS: *m/z* (rel. int.) = 362 (2) $[M]^+$, 235 (65), 215 (79), 175 (24), 151 (78), 119 (30), 105 (36), 91 (51), 69 (100), 55 (72); IR ν_{max}^{KBr}: cm^{-1}: 3400, 2900, 1750, 1450, 1390, 1210, 1050, 1000, 950; ^{1}H NMR ($CDCl_3$): δ_H 5.48 (*brs*, H-7), 5.42 (*d*, *J* = 3.5 Hz, H-11), 5.02 (*t*, *J* = 7.0 Hz, H-17), 4.59 (*dd*, *J* = 4.0, 9.0 Hz, H-1), 4.37 (*d*, *J* = 11.5 Hz, H-12a), 4.10 (*d*, *J* = 11.5 Hz, H-12b), 2.95 (*brs*, OH-11), 2.25 (*brs*, H-9), 2.08 (*m*, H-6a), 2.02 (*s*, Me-22), 1.96 (*m*, H-6b), 1.83 (*m*, 2x H-16), 1.73 (*m*, H-2a), 1.64 (*s*, Me-19), 1.62 (*m*, H-2b), 1.56 (*s*, Me-20), 1.53 (*m*, H-3a), 1.43 (*m*, H-3b), 1.42 (*m*, H-5), 1.17 (*m*, H-15), 0.92 (*s*, Me-13), 0.91 (*s*, Me-14); ^{13}C NMR: Table 1.

3.10. 1β,15ξ-Diacetoxy-11,12-epoxy-8(12),9(11),17-sacculatatriene (**7**)

$[\alpha_D^{20}]$ − 46.5 ($CHCl_3$; *c* 2.5); CIMS: *m/z* (rel. int.) = 403 (26) $[M + H]^+$, 342 (47), 283 (100), 261 (7), 231 (3), 213 (5), 201 (9), 187 (5), 96 (12); IR ν_{max}^{KBr}: cm^{-1}: 3450, 2950, 1790, 1500, 1410, 1200, 1090, 980; ^{1}H NMR ($CDCl_3$): δ_H 7.05 (*d*, *J* = 1.5 Hz, H-11), 7.01 (*d*, *J* = 1.5 Hz, H-12), 5.01 (*t*, *J* = 6.0 Hz, H-17), 4.93 (*dd*, *J* = 3.5, 9.5 Hz, H-15), 4.68 (*dd*, *J* = 4.0, 11.0 Hz, H-1), 2.66 (*m*, H-7a), 2.25 (*dd*, *J* = 5.0, 16.0 Hz, H-7b), 2.19 (*m*, 2x H-16), 2.14 (*s*, Me-22), 1.96 (*s*, Me-24), 1.91 (*m*, H-2a), 1.89 (*m*, H-6a), 1.79 (*m*, H-3a), 1.74 (*m*, H-2b), 1.64 (*s*, Me-19), 1.60 (*m*, H-6b), 1.56 (*s*, Me-20), 1.54 (*dd*, *J* = 5.0, 13.0 Hz, H-5), 1.53 (*m*, H-3b), 1.33 (*s*, Me-13), 0.96 (*s*, Me-14); ^{13}C NMR: Table 1.

Acknowledgements

We are grateful to the "Saarländische Landesgraduiertenförderung" for providing financial support to U.M.H.

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