PERGAMON

Phytochemistry 51 (1999) 643–649

PHYTOCHEMISTRY

cis-Clerodandes from axenic cultures of the liverwort *Scapania nemorea*[1]

Wolfgang Geis, Birgit Buschauer, Hans Becker*

FR 12.3, Pharmakognosie und Analytische Phytochemie, Universität des Saarlandes, D-66041 Saarbrücken, Germany

Received 11 December 1998; received in revised form 20 January 1999; accepted 29 January 1999

Abstract

Seven *cis*-clerodanes, (−)-*cis*-cleroda-3,13-dien-16-hydroxy-15,18-dioic acid-15,16-olide, (−)-*cis*-cleroda-1,3,13-trien-15,18-dioic acid-15,16-olide, (−)-*cis*-cleroda-1,3,13-trien-16-hydroxy-15,18-dioic acid-15,16-olide, (−)-*cis*-cleroda-1,3,13-trien-15-hydroxy-16,18-dioic acid-16,15-olide, (−)-15,16-epoxy-*cis*-cleroda-3,13(16),14-trien-12-hydroxy-18-oic acid-18,6α-olide, (−)-*cis*-cleroda-3,13-dien-12,16-dihydroxy-15,18-dioic acid-15,16:18,6α-diolide and (−)-*cis*-cleroda-3,13-dien-12,15-dihydroxy-16,17,18-trioic acid-16,15:18,6α-diolide along with the 5-*epi*-nidoresedic acid have been isolated from axenic cultures of the liverwort *Scapania nemorea*. Furthermore, the enantiomers of the known 5-*epi*-hardwickiic acid and (+)-15,16-epoxy-*cis*-*ent*-cleroda-3,13(16),14-trien-12-hydroxy-18-oic acid-18,6α-olide besides the already reported (−)-strictic acid could be identified. The structures were elucidated by extensive 1D and 2D NMR techniques.

Keywords: *Scapania nemorea*; Scapaniaceae; Jungermanniales; Liverwort; Hepaticae; Axenic culture; *cis*-Clerodanes

1. Introduction

Bryophytes, especially liverworts are a rich source of terpenoid and phenolic compounds (Zinsmeister, Becker, & Eicher, 1991; Asakawa, 1995). Since it is often difficult to obtain sufficient and appropriate plant material from their natural habitat, axenic cultures of liverworts are a most promising alternative (Becker, 1994).

As part of our program to screen axenic cultures of liverworts (Tazaki, Adam, & Becker, 1995; Adam & Becker, 1994; Kunz & Becker, 1995), we have investigated *Scapania nemorea* L. Grolle, which is widespread throughout the northern hemisphere. In previous studies, the bibenzyls lunularic acid and lunularin (Gorham, 1977), and the sesquiterpenoids anastreptene (Andersen, Ohta, Moore, & Tseng, 1978) and longifolene (Svenson & Bendz, 1972) have been reported from this species. The present report describes the isolation and structure elucidation of seven *cis*-clerodanes.

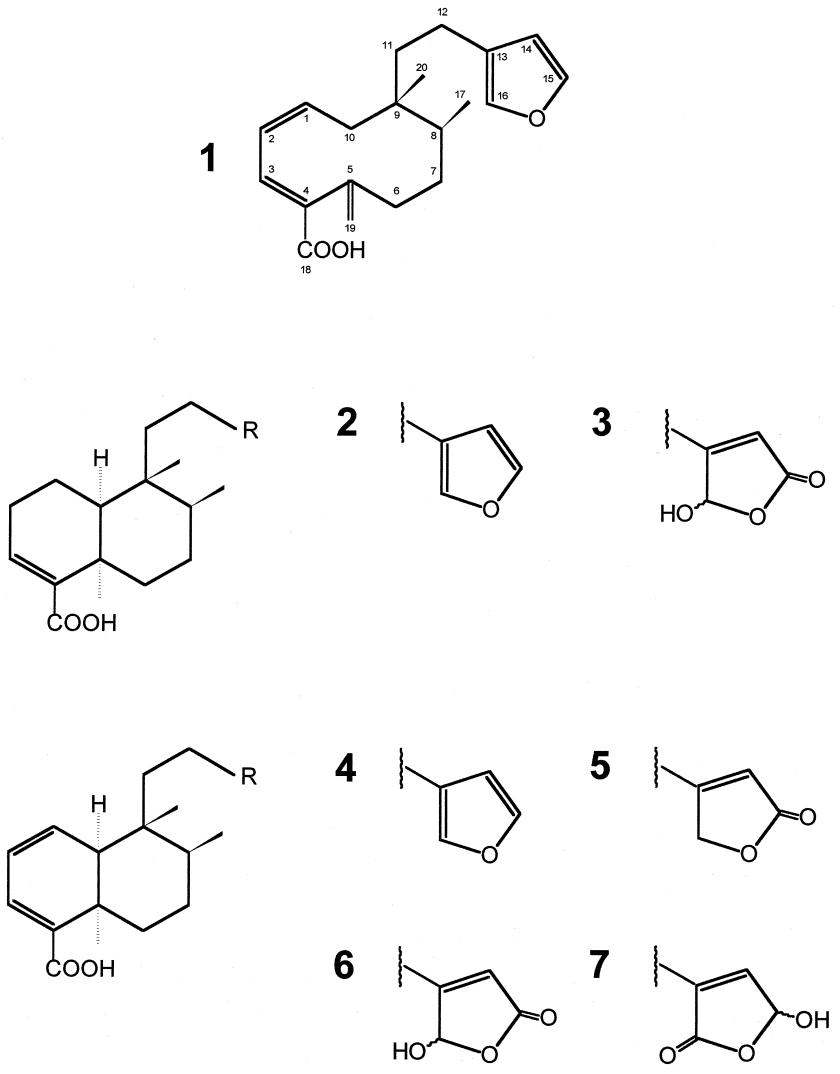
2. Results and discussion

A combination of column chromatography (Sephadex LH-20), vacuum liquid chromatography and HPLC of the diethyl ether extract and the ethyl acetate soluble phase of the methanolic extract of in vitro cultured *S. nemorea* afforded the seven new *cis*-clerodanes, (−)-*cis*-cleroda-3,13-dien-16-hydroxy-15,18-dioic acid-15,16-olide (**3**), (−)-*cis*-cleroda-1,3,13-trien-15,18-dioic acid-15,16-olide (**5**), (−)-*cis*-cleroda-1,3,13-trien-16-hydroxy-15,18-dioic acid-15,16-olide (**6**), (−)-*cis*-cleroda-1,3,13-trien-15-hydroxy-16,18-dioic acid-16,15-olide (**7**), (−)-15,16-epoxy-*cis*-cleroda-3,13(16),14-trien-12-hydroxy-18-oic acid-18,6α-olide (**9**), (−)-*cis*-cleroda-3,13-dien-12,16-dihydroxy-15,18-dioic acid-15,16:18,6α-diolide (**10**) and (−)-*cis*-cleroda-3,13-dien-12,15-dihydroxy-16,17,18-trioic acid-16,15:18,6α-diolide (**11**) along with the epimer of the

* Corresponding author.

[1] Publication Nr. 133 in the series of "Arbeitskreis Chemie und Biologie der Moose". For part 132, see Cullmann & Becker, in press.

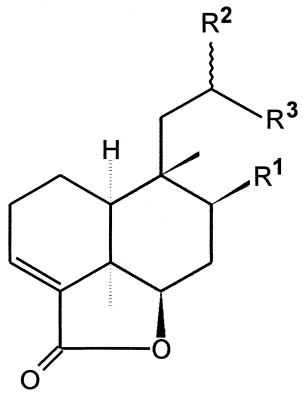
0031-9422/99/$ - see front matter
PII: S0031-9422(99)00069-2



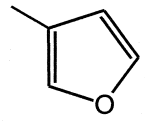
already known nidoresedic acid (Pandey et al., 1984), the 5-*epi*-nidoresedic acid (**4**). Furthermore, two enantiomers of already reported *cis*-clerodanes, (−)-*epi* hardwickiic acid (**2**) (Zdero, Bohlmann, & King, 1991) and (−)-15,16-epoxy-*cis*-cleroda-3,13(16),14-trien-18-oic acid-18,6α-olide (**8**) (Mc Crindle & Nakamura, 1976), together with strictic acid (**1**) (Muhammad, El-Faraly, Mossa, & Ramadan, 1992), a secoclerodane which shows structural similarities to schistochilic acid A from the liverwort *Schistolochia nobilis* (Tori, Masuya, & Asakawa, 1993), were also isolated.

The spectroscopic data of **2** and **8**, together with optical rotation, led to the conclusion that they are the enantiomers of the already described 5-*epi*-hardwickiic acid (Zdero et al., 1991), and (+)-15,16-epoxy-*cis*-cleroda-3,13(16),14-trien-18-oic acid-18,6α-olide (Mc Crindle & Nakamura, 1976), respectively.

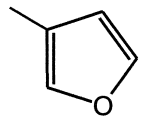
Compound **3**. Colorless oil, was assigned the molecular formula $C_{20}H_{28}O_5$ (EIMS, m/z 348 $[M]^+$). The ^1H NMR spectrum displayed the signals of two singlet methyl groups (δ_H 0.82 and δ_H 1.23), one doublet methyl group (δ_H 0.76, $J=6.8$ Hz) and two olefinic protons (δ_H 5.85, s and δ_H 6.79, t, $J=3.8$ Hz). Furthermore, a singlet at δ_H 6.08 could be assigned to a low field shifted acetalic proton, since a correlation from this proton to a methine carbon atom at δ_C 98.5 (d) was observed in the HSQC spectrum. The DEPT spectra revealed the existence of three methyls, six methylenes, six methines and five quaternary carbons. The ^{13}C NMR spectrum was similar to that of **2** and mainly differed by the absence of the signals of the furane moiety. Instead, signals for a conjugated carboxyl group (δ_C 170.5), a double bond (δ_C 169.6, s and δ_C 117.5, d) and an acetalic carbon (δ_C 98.5, d) appeared. This suggested the replacement of the furane moiety in **2** by a hydroxylated α,β-unsaturated γ-lactone structure, which was proved by ^1H–^1H COSY, ^1H–^{13}C COSY and HMBC spectra. The NOESY experiment confirmed the suggested *cis*-configuration of the decalin moiety, since a correlation between H-19 and H-10



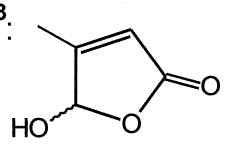
8 R^1: CH_3 R^2: H R^3:



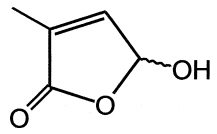
9 R^1: CH_3 R^2: OH R^3:



10 R^1: CH_3 R^2: OH R^3:



11 R^1: COOH R^2: OH R^3:



could be observed (Fig. 1). As there were a cross-peak between H-20 and H-17, and further correlations of H-8 with H-6 and H-10, respectively, both methyl groups had to be in the β-position. Therefore, the structure of **3** was established as (−)-*cis*-cleroda-3,13-dien-16-hydroxy-15,18-dioic acid-15,16-olide.

Compound **4**. The molecular formula, $C_{20}H_{26}O_3$, of **4** was obtained from the EI mass spectrum (*m*/*z* 314 $[M]^+$). The ^1H NMR spectrum, again, was similar to that of **2**, but showed two additional signals of olefinic protons (δ_H 6.06, dd, $J=9.5$ Hz, 6.2 Hz and δ_H 6.22, dd, $J=9.5$ Hz, 5.4 Hz). The fact, that the signal for H-3 (δ_H 6.87) had changed from a triplet in **2** to a doublet in **4** with a coupling constant of $J=5.4$ Hz, indicated the presence of an additional double bond in position C-1, which was proved by the ^1H–^1H COSY. The ^1H and ^{13}C NMR assignments with ^1H–^1H COSY, ^1H–^{13}C COSY, DEPT and NOESY experiments revealed the complete structure of **4** as 5-*epi*-nidoresedic acid, the epimer of the *trans*-clerodan, nidoresedic acid, which has been described from the higher plant *Grangea maderaspatena* (Pandey et al., 1984).

Compound **5** had the molecular formula $C_{20}H_{26}O_4$ (EIMS, *m*/*z* 330 $[M]^+$). The ^1H and ^{13}C NMR spectra displayed the characteristic signals of the decalin moiety of **4** (Table 1). Additionally, the ^1H NMR data revealed an olefinic proton (δ_H 5.80, s) and two isochronic ester protons (δ_H 4.73, s). This suggested an cardenolide structure for the side chain of **5**. One and two dimensional NMR data confirmed the structure of **5** as (−)-*cis*-cleroda-1,3,13-trien-15,18-dioic acid-15,16-olide.

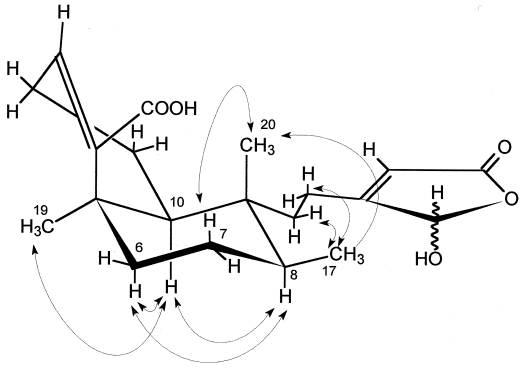


Fig. 1. Significant NOESY couplings of compound **3**.

Compound **6**. The ^{13}C NMR data of **6**, $C_{20}H_{26}O_5$ (EIMS, m/z 346 $[M]^+$), revealed the existence of the decalin moiety of compounds **4** and **5**, respectively (Table 2), together with the signals of the cardenolide moiety of compound **3**. The ^1H and ^{13}C NMR assignments by ^1H–^1H COSY, ^1H–^{13}C COSY, DEPT experiments indicated that the olefinic protons H-1α, H-1β and the acetalic proton H-16 appear as an unresolved multiplet in the ^1H NMR spectrum and confirmed the structure of the clerodane derivative **6** as (−)-*cis*-cleroda-1,3,13-trien-16-hydroxy-15,18-dioic acid-15,16-olide.

Compound **7**. The molecular formula, $C_{20}H_{26}O_5$, was obtained from the EI mass spectrum (EIMS, m/z 346 $[M]^+$). The ^{13}C NMR spectrum showed signals due to the unsaturated decalin moiety of **6** Table 2, an acetalic carbon (δ_C 96.5, d), a carboxyl carbon (δ_C 171.3), together with a trisubstituted double bond (δ_C 139.0, s and δ_C 142.6, d). The ^1H NMR data were similar to that of **6**, but showed a significant lowfield shift of H-14 (δ_H 6.81). This low field shift together with the ^{13}C NMR data suggested the existence of an isomeric lactone derived from **6**, what could be proved by comparison of ^1H NMR data from related lactone derivatives (Jaensch et al., 1990). The ^1H–^1H COSY, ^1H–^{13}C COSY data confirmed the structure of **7** as (−)-*cis*-cleroda-1,3,13-trien-15-hydroxy-16,18-dioic acid-16,15-olide.

Compound **9**. The EI-mass spectrometry $[M]^+$ ion at m/z 330 was in agreement with the molecular formula $C_{20}H_{26}O_4$. The ^1H NMR spectrum displayed signals assignable to an unsaturated decalin system as in **3** (δ_H 6.83, t, $J=3.6$ Hz (H-3), δ_H 0.79, d, $J=6.8$ Hz (H-17), δ_H 1.25, s (H19) and δ_H 0.68, s (H-20)). Furthermore, the signals of a furane moiety (δ_H 6.39, brs (H-14), δ_H 7.36, t, $J=0.8$ Hz (H-16) and δ_H 7.37, brs (H-15)) could be observed. In addition the ^1H NMR spectrum showed signals at δ_H 4.36 (dd, $J=10.8$ Hz, 7.0 Hz) and δ_H 4.81 (d, $J=7.7$ Hz), assignable to an ester proton and a secondary hydroxyl group, since correlation from these protons to carbon signals at δ_C 85.4 (d) and δ_C 63.4 (d), respectively, could be detected from the HSQC-spectrum. In the ^1H–^1H COSY experiment the ester proton showed vicinal coupling to both methylene protons of C-7. H-7α and H-7β showed coupling to the proton of methine C-8, which showed a correlation to the doublet methyl group C-18. This suggested a γ-lactone structure between C-18 and C-6,

Table 1
^1H NMR spectral data and coupling constants (in Hz in parenthesis) of compounds **3**–**11**. n.r. means not resolved due to overlapping of signals

H	3	4	5	6	7	9	10	11
H-1α	1.77 nr	6.06 dd (9.5, 6.2)	5.90 dd (9.5, 6.3)	6.02 nr	6.10 nr	2.06 nr	1.90 nr	1.87 nr
H-1β	1.73 nr					1.95 nr	1.87 nr	1.85 nr
H-2α	2.29 nr	6.22 dd (9.5, 5.4)	6.18 dd (9.5, 5,4)	6.23 dd (9.5, 5.4)	6.21 dd (9.5, 5.4)	2.27 nr	2.01 nr	2.25 nr
H-2β	2.35 nr					2.36 nr	2.33 m	2.31 nr
H-3	6.79 t (3.8)	6.87 d (5.4)	6.71 d (5.4)	6.90 d (5.4)	6.87 d (5.4)	6.83 t (3.6)	6.81 t (3.60)	6.76 t (3.6)
H-6α	1.09 nr	1.06 nr	1.07 nr	1.06 nr	1.05 nr	4.36 dd (10.8, 7.0)	4.34 dd (10.8, 6.9)	4.32 dd (10.7, 7.1)
H-6β	2.72 br d (13.6)	2.86 br d (13.8)	2.80 br d (13.8)	2.88 br d (13.8)	2.85 br d (13.5)			
H-7α	1.15 nr	1.16 nr	1.23 nr	1.20 nr	1.18 nr	1.26 nr	1.17 nr	1.17 nr
H-7β	1.39 nr	1.32 nr	1.37 nr	1.35 nr	1.36 nr	1.88 m	1.90 nr	2.63 nr
H-8	1.47 nr	1.50 nr	1.42 nr	1.43 nr	1.48 nr	1.71 nr	1.98 nr	2.09 br d (13.0)
H-10	1.54 nr	2.02 d (6.2)	1.84 d (6.3)	1.94 d (6.2)	1.97 d (6.1)	1.50 d (5.4)	1.60 nr	1.52 d (6.3)
H-11α	1.57 nr	1.47 nr	1.52 nr	1.52 nr	1.50 nr	2.02 nr	2.35 nr	2.43 m
H-11β	1.30 nr	1.36 nr	1.40 nr	1.34 nr	1.32 nr	1.55 dd (15.6,1.6)	1.88 nr	1.41 nr
H-12α	2.35 nr	2.38 nr	2.36 t (8.5)	2.34 nr	2.28 t (7.8)	4.81 d (7.7)	4.64 d (9.8)	5.09 br d (11.3)
H-12β	2.35 nr	2.38 nr	2.36 t (8.5)	2.34 nr	2.28 t (7.8)			
H-14	5.85 s	6.22 t (0.8)	5.80 s	5.85 s	6.81 s	6.39 br s	6.00 nr	7.02 s
H-15	–	7.30 t (1.6)	–	–	6.06 s	7.37 br s	–	6.00 s
H-16α	6.08 s	7.19 s	4,73 s	6.01 nr	–	7.36 t (0.8)	6.03 nr	–
H-16β			4,73 s					
H-17	0.76 d (6.8)	0.77 d (6.8)	0.71 d (6.8)	0.78 d (6.8)	0.76 d (6.8)	0.79 d (6.8)	0.90 d (6.42)	–
H-19	1.23 s	1.05 s	1.01 s	1.08 s	1.08 s	1.25 s	1.20 s	1.20 s
H-20	0.82 s	0.64 s	0.66 s	0.71 s	0.71 s	0.68 s	0.68 s	0.68 s

Table 2
^{13}C NMR spectral data of compounds **3**–**11**

C	3	4	5	6	7	9	10	11
C-1	16.9 t	134.3 d	131.8 d	133.4 d	133.9 d	17.9 t	17.6 t	16.9 t
C-2	24.3 t	125.0 d	125.4 d	125.5 d	125.2 d	24.8 t	24.6 t	23.7 t
C-3	141.9 d	135.7 d	133.4 d	135.7 d	135.7 d	136.2 d	136.3 d	136.8 d
C-4	137.3 d	135.7 s	136.8 s	135.5 s	135.6 s	133.9 s	133.5 s	132.4 s
C-5	40.3 s	41.1 s	40.6 s	40.8 s	40.9 s	39.7 s	39.6 s	38.6 s
C-6	34.8 t	37.3 t	34.5 t	34.7 t	34.7 t	85.4 d	85.0 d	84.2 d
C-7	28.5 t	28.8 d	28.4 t	28.7 t	28.8 t	35.2 t	35.0 t	26.7 t
C-8	37.9 d	36.2 d	36.1 d	36.3 d	36.3 d	32.5 d	32.0 d	45.7 d
C-9	36.4 s	36.9 d	36.8 s	36.9 s	37.0 s	39.8 s	39.8 s	36.2 s
C-10	45.5 d	48.5 d	48.0 d	48.3 d	48.3 d	41.6 d	41.5 d	46.4 d
C-11	36.8 t	34.8 t	33.9 t	33.7 t	34.4 t	45.6 t	42.4 t	41.5 t
C-12	21.2 t	18.2 t	22.3 t	21.5 t	19.2 t	63.4 d	64.8 d	71.7 d
C-13	169.6 s	125.5 s	169.7 s	169.2 s	139.0 s	131.0 s	169.9 s	146.1 s
C-14	117.5 d	110.9 d	114.8 d	117.6 d	142.6 d	108.2 d	117.3 d	146.0 d
C-15	170.4 s	142.8 d	174.5 s	170.4 s	96.5 d	143.7 d	172.0 s	97.6 d
C-16	98.5 d	138.5 d	73.2 t	98.5 d	171.3 s	138.3 d	97.1 d	169.1 s
C-17	15.9 q	16.1 s	15.7 q	15.9 q	15.8 q	15.7 q	16.3 q	170.3 s
C-18	171.4 s	172.2 s	171.3 s	171.1 s	171.7 s	170.0 s	169.9 s	169.5 s
C-19	33.4 q	29.6 q	29.4 q	29.5 q	29.6 q	30.7 q	30.5 q	29.9 q
C-20	17.9 q	16.0 q	15.8 q	16.1 q	16.0 q	16.5 q	15.4 q	14.8 q

which could be proved by a long range coupling between H-6 and H-3 in the ^{1}H–^{1}H COSY experiment. Based on ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY, and NOESY data, the hydroxyl group was assigned to C-12. Therefore, **9** is (−)-15,16-epoxy-*cis*-cleroda-3,13(16),14-trien-12-hydroxy-18-oic acid-18,6α-olide. The 12-oxo-compound of **9**, linguifolide, could be isolated from the liverwort *Demotarisia linguifolia* (Nagashima & Asakawa, 1990).

Compound **10** has the molecular formula $C_{20}H_{26}O_6$ (EIMS, *m*/*z* 362 $[M]^+$). The ^{13}C NMR spectrum of **10** was similar to that of **9** and revealed the decalin structure, including the γ-lactone moiety between C-18 and C-6, but lacked the signals assignable to the furane skeleton Table 2. However, the ^{13}C NMR spectrum displayed signals similar to those from the γ-lactone moiety of **3** (δ_C 169.9, s (C-13), δ_C 117.3, d (C-14), δ_C 172.0, s (C-15), and δ_C 97.1, d (C-16)). ^{1}H NMR, ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY, and HMBC spectra revealed the structure of **10** as (−)-*cis*-cleroda-3,13-dien-12,16-dihydroxy-15,18-dioic acid-15,16:18,6α-diolide.

Compound **11**. The molecular formula, $C_{20}H_{24}O_8$, was obtained from EI mass spectrum (EIMS, *m*/*z* 392 $[M]^+$). The ^{1}H NMR spectrum displayed signals due to the decalin/γ-lactone moiety of **10** and signals assignable to the hydroxylated γ-lactone structure of **3**, but lacked the doublet methyl group, typical for all described compounds **1**–**10**. Instead, the ^{13}C NMR revealed a further carboxyl carbon (δ_C 169.5). This suggested that the methyl group C-17 was functionalized to a carboxylic carbon. This was proved by further ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY and HMBC experiments. Thus, compound **11** is (−)-*cis*-cleroda-3,13-dien-12,15-dihydroxy-16,17,18-trioic acid-16,15:18,6α-diolide.

Clerodanes are diterpenoids with an enormous stereochemical and functional variability, distributed both in higher plants and in liverworts. In former investigations we had also isolated clerodanes from axenic cultures of the liverwort *Jamesionella autumnalis* (Blechschmidt & Becker, 1994; Tazaki, Zapp, & Becker, 1995; Tazaki, Kensuke, & Becker, 1998). In conclusion, within Hepaticae clerodane-typ diterpenoids are mainly reported from the order Jungermanniales (Asakawa, 1995).

3. Experimental

Solvents used for spectral measurements: $CDCl_3$ for NMR (^{1}H NMR: 400 MHz; ^{13}C NMR: 100 MHz for 1D, 500 and 125 MHz for 2D techniques, respectively. Chemical shifts are given in δ values (ppm) from TMS), $CHCl_3$ for optical rotation.

3.1. Plant material

S. nemorosa (L.) Dumortier was collected in June 1987 near Abrechviller (Vosges Mountains, France) and near Schönmünzach (Schwarzwald, Germany) and identified by Professor Mues (Universität des Saarlandes, Germany). Voucher specimens were deposited at the Pharmakognosie und Analytische Phytochemie der Universität des Saarlandes, Saarbrücken. An axenic culture of *S. nemorosa* was

established from the surface-sterilized spores of field material. The cultures were grown in 250-ml Erlenmeyer flasks with 70 ml solid modified B5 medium (pH 6.0) (Gamborg, Miller, & Ojima, 1968), containing 20 g l^{-1} sucrose. The flasks were kept under constant illumination (2000 lux) at 22°.

3.2. Extraction and isolation

The extraction scheme followed the standard procedures of our group (Zapp, Burkhardt, & Becker, 1994; Cullmann, Becker, Pandolfi, Roeckner, & Eicher, 1997). The plant material (500 g) was powdered and extracted with Et_2O to yield the nonpolar compounds, followed by MeOH. The ether extract (22.0 g) was subjected to a VLC on silica gel in a hexane–ethyl acetate gradient to yield four main frs. Fr. 1, containing hydrocarbons, was not further analyzed. As frs. 2–4 contained higher amounts of chlorophyll and other by-products, they were prepurified by CC on Sephadex LH20 (150 × 2.5 cm) with MeOH:CH_2Cl_2 (50:50) as mobile phase. Fr. 2, after CC, was purified by HPLC on silica gel LiChrospher Si 60 5 μm, 4 × 250 mm (*n*-hexane:*t*-BME (6:94)) to yield compound **2** (23.0 mg), **4** (20.0 mg) and **1** (27.0 mg). Fr. 3 was subjected to HPLC on diol LiChrospher 5 μm, 4 × 250 mm (*n*-hexane:EtOAc (83:17)) to give **8** (7 mg). Fr. 4 was purified by HPLC on silica gel LiChrospher Si 60 5 μm, 4 × 250 mm (*n*-hexane:EtOAC (75:25)) to yield compound **9** (3.0 mg).

The methanolic extract was concd in vacuo and partitioned between EtOAc and H_2O. The concd. organic layer (21.0 g) was chromatographed by CC on Sephadex LH20 (150 × 2.5 cm i.d.) with MeOH:CH_2Cl_2 (80:20) as mobile phase to yield three fractions (fr. I–III). Fr.II was separated by VLC (silica gel 15 μm, 60 mm × 35 i.d., in a *n*-hexane–EtOAC gradient) and gave the four main fractions II-1 (8–30% EtOAC), II-2 (30–40% EtOAC), II-3 (40–50% EtOAC) and II-4 (50–70% EtOAC). Fr. II-1 was purified by HPLC on silica gel LiChrospher Si 60 5 μm, 4 × 250 mm (*n*-hexane:EtOAc (55:45)) to yield compound **5** (14.4 mg). Fr. II-2 was subjected to HPLC on silica gel LiChrospher 5 μm, 4 × 250 mm (*n*-hexane:EtOAc (50:50)) to give **7** (3.7 mg). Fr. II-3 was further purified by HPLC on silica gel LiChrospher Si 60 5 μm, 4 × 250 mm (*n*-hexane:EtOAc (48:52)) for **6** (5.7 mg) and **3** (14.9 mg). The last fraction, fr. II-4, was chromatographed by HPLC on CN LiChrospher 5 μm, 4 × 250 mm (*n*-hexane:EtOAc (40:60)) to yield **10** (21.0 mg) and **11** (0.9 mg).

3.3. (−)-cis-cleroda-3,13-dien-16-hydroxy-15,18-dioic acid-15,16-olide (*3*)

$[\alpha]_D^{20}$ −190.6° ($c = 0.09$); EIMS *m/z* (rel. Int.): 348 $[M]^+$ (2), 315 (1), 297 (2), 286 (2), 202 (10), 163 (20), 137 (63), 91 (100), 41 (52); IR ν_{max}^{KBr} cm^{-1}: 3350, 2950, 2860, 1740, 1680, 1280, 790; ^1H NMR: Table 1; ^{13}C NMR: Table 2.

3.4. (−)-5-epi-nidoresedic acid (*4*)

$[\alpha]_D^{20}$ −425.0° ($c = 0.49$); EIMS *m/z* (rel. Int.): 314 $[M]^+$ (8), 299 (3), 269 (3), 219 (5), 163 (18), 149 (50), 95 (44), 81 (100), 55 (30), 41 (44); IR ν_{max}^{KBr} cm^{-1}: 3500, 3420, 2960, 1800, 1680, 1380, 1080, 920, 870, 810, 790; ^1H NMR: Table 1; ^{13}C NMR: Table 2.

3.5. (−)-cis-cleroda-1,3,13-trien-15,18-dioic acid-15,16-olide (*5*)

$[\alpha]_D^{20}$ −203.8° ($c = 0.08$); EIMS *m/z* (rel. Int.): 330 $[M]^+$ (1), 315 (2), 221 (30), 149 (20), 119 (24), 95 (35), 77 (22), 43 (100); IR ν_{max}^{KBr} cm^{-1}: 3350, 2930, 2860, 1680, 1570, 1420, 1240, 1050, 870, 780; ^1H NMR: Table 1; ^{13}C NMR: Table 2.

3.6. (−)-cis-cleroda-1,3,13-trien-16-hydroxy-15,18-dioic acid-15,16-olide (*6*)

$[\alpha]_D^{20}$ −161.7° ($c = 0.06$); EIMS *m/z* (rel. Int.): 346 $[M]^+$ (2), 299 (2), 249 (2), 202 (10), 163 (20), 137 (62), 91 (100), 41 (60); IR ν_{max}^{KBr} cm^{-1}: 3350, 2960, 2930, 2870, 1730, 1670, 1450, 1270, 790; ^1H NMR: Table 1; ^{13}C NMR: Table 2.

3.7. (−)-cis-cleroda-1,3,13-trien-15-hydroxy-16,18-dioic acid-16,15-olide (*7*)

$[\alpha]_D^{20}$ −201.8° ($c = 0.11$); EIMS *m/z* (rel. Int.): 346 $[M]^+$ (2), 286 (2), 164 (10), 137 (60), 91 (100), 41 (55); IR ν_{max}^{KBr} cm^{-1}: 3480, 2980, 2920, 2880, 1750, 1680, 1270, 1200, 1000, 920, 760; ^1H NMR: Table 1; ^{13}C NMR: Table 2.

3.8. (−)-15,16-epoxy-cis-cleroda-3,13(16),14-trien-12-hydroxy-18-oic acid-18,6α-olide (*9*)

$[\alpha]_D^{20}$ −10.4° ($c = 0.66$); EIMS *m/z* (rel. Int.): 330 $[M]^+$ (2), 299 (1) 219 (5), 149 (96), 95 (48), 81 (95), 55 (90), 41 (98); IR ν_{max}^{KBr} cm^{-1}: 3480, 2980, 2920, 2880, 1750, 1680, 1270, 1200, 1000, 920, 760; ^1H NMR: Table 1; ^{13}C NMR: Table 2.

3.9. (−)-cis-cleroda-3,13-dien-12,16-dihydroxy-15,18-dioic acid-15,16:18,6α-diolide (*10*)

$[\alpha]_D^{20}$ −221.1° ($c = 0.20$); EIMS *m/z* (rel. Int.): 362 $[M]^+$ (1), 295 (3), 196 (8), 85 (100), 69 (49), 47 (70); IR ν_{max}^{KBr} cm^{-1}: 3390, 2980, 2920, 1740, 1450, 1020, 980, 760; ^1H NMR: Table 1; ^{13}C NMR: Table 2.

*3.10. (−)-cis-cleroda-3,13-dien-12,15-dihydroxy-16,17,18-trioic acid-16,15:18,6α-diolide (**11**)*

$[\alpha]_D^{20}$ −199.7° ($c=0.09$); EIMS m/z (rel. Int.): 392 $[M]^+$ (1), 348 (1), 295 (3), 196 (4), 105 (20), 85 (100), 69 (50), 57 (80), 47 (74); IR ν_{max}^{KBr} cm^{-1}: 3390, 2980, 2920, 1740, 1450, 1020, 980, 760; ^1H NMR: Table 1; ^{13}C NMR: Table 2.

Acknowledgements

The authors thank Professor R. Mues, Saarbrücken, for identification of the plant material and Dr. J. Zapp for NMR measurements. The authors are especially very grateful to Dr. F. Cullmann for fruitful discussions.

References

Adam, K.-P., & Becker, H. (1994). *Phytochemistry*, *35*, 139.

Andersen, N. H., Ohta, Y., Moore, A., & Tseng, C. W. (1978). *Tetrahedron*, *34*, 41.

Asakawa, Y. (1995). In W. Herz, G. W. Kirby, R. E. Moore, W. Steglich, & Ch. Tamm, *Progress in the chemistry of organic natural products (* (pp. 1–562). Wien: Springer.

Becker, H. (1994). *J. Hattori Bot. Lab.*, *76*, 283.

Blechschmidt, M., & Becker, H. (1994). *Journal of Natural Products*, *37*, 491.

Cullmann, F., & Becker, H. (in press). *Zeitschrift fuer Naturforschung C*.

Cullmann, F., Becker, H., Pandolfi, E., Roeckner, E., & Eicher, T. (1997). *Phytochemistry*, *45*, 1235.

Gamborg, O. L., Miller, R. A., & Ojima, K. (1968). *Experimental Cell Research*, *50*, 151.

Gorham, J. (1977). *Phytochemistry*, *16*, 249.

Jaensch, M., Jakupovic, I., Sanchez, H., & Dominguez, X. A. (1990). *Phytochemistry*, *29*, 3587.

Kunz, S., & Becker, H. (1995). *Zeitschrift fuer Naturforschung*, *50c*, 235.

Mc Crindle, R., & Nakamura, E. (1976). *Journal of the Chemical Society, Perkin Transection I*, 1590.

Muhammad, F. S., El-Faraly, F. S., Mossa, J. S., & Ramadan, A. (1992). *Phytochemistry*, *31*, 4245.

Nagashima, F., & Asakawa, Y. (1990). *Phytochemistry*, *29*, 3229.

Pandey, U., Singal, A. K., Barua, N. C., Sharma, R. P., Watanabe, K., Kulanthaivel, P., & Herz, W. (1984). *Phytochemistry*, *23*, 391.

Svenson, L., & Bendz, G. (1972). *Phytochemistry*, *11*, 1172.

Tazaki, H., Adam, K.-P., & Becker, H. (1995). *Phytochemistry*, *40*, 1671.

Tazaki, H., Kensuke, N., & Becker, H. (1998). *Phytochemistry*, *48*, 681.

Tazaki, H., Zapp, J., & Becker, H. (1995). *Phytochemistry*, *39*, 859.

Tori, M., Masuya, T., & Asakawa, Y. (1993). *Phytochemistry*, *32*, 1229.

Zapp, J., Burkhardt, G., & Becker, H. (1994). *Phytochemistry*, *37*, 787.

Zdero, C., Bohlmann, F., & King, R. M. (1991). *Phytochemistry*, *30*, 2991.

Zinsmeister, H. D., Becker, H., & Eicher, Th. (1991). *Angew. Chemie (Intern. ed. Engl.)*, *30*, 130.