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Lignan derivatives from the liverwort *Bazzania trilobata*

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Dedicated to Professor Sprecher on the occasion of his 80th birthday

Abstract

Eight lignan derivatives trilobatin D–K, as well as jamesopyrone were isolated from the liverwort *Bazzania trilobata*. Their structures have been elucidated based on extensive NMR spectral evidence.

Keywords: *Bazzania trilobata*; Hepaticae; Liverwort; Structure elucidation; Lignans

1. Introduction

Bryophytes are known to be a rich source of phenolic constituents (Asakawa, 1995; Becker, 2001). By far most of these compounds are flavonoids, bibenzyls and bisbibenzyls. In recent years a special group of the phenolic constituents, the lignans, were found to be present in liverworts. Only a few reports deal with this group of secondary metabolites (Cullmann et al., 1993, 1996, 1999; Cullmann and Becker, 1999; Tazaki et al., 1995; Martini et al., 1998).

Within the Hepaticae, the genus *Bazzania* with its several hundred species, mainly distributed in the tropics and subtropics, is a rich source of various types of secondary metabolites. *Bazzania trilobata* (L.) S.F. Gray represents one of the four European species that grow in dense, widespread pads on forest ground, boggy soil and trunks (Müller, 1954). Recently, we reported on the isolation and structural elucidation of six new lignans from *B. trilobata* (Martini et al., 1998). Continuing our studies on the secondary metabolites of liverworts, further investigation of the methanol extract from this liverwort has led to the isolation of the known jamesopyrone and eight new lignan derivatives (**1**–**8**).

2. Results and discussion

Air-dried plant material was first extracted with dichloromethane and then with methanol. The methanol extract was partitioned between *n*-butanol and ethyl acetate. The *n*-butanol-soluble fraction of the methanol extract was chromatographed on Sephadex LH-20 using methanol as the eluent. The resulting fractions were subjected to vacuum liquid chromatography (VLC) and further purified by HPLC to yield compounds **1**–**8**. The partial structures of the isolated lignan derivatives (Fig. 1) can be divided into aromatic subunits (epiphyllic acid, jamesopyrone and caffeic acid) and aliphatic C_7 or C_8 moieties (trilobatinoic acid A–D).

Compound **1** consists of two partial structures: epiphyllic acid (**Ea**, Fig. 1) and a C_7 moiety. The C_7 moiety consists of five oxygen bearing, aliphatic carbon atoms (C-3–C-7), one methylene group (C-2) and one carboxyl group (C-1). From the ^1H–^1H COSY, HSQC and HMBC data a chain of seven carbon atoms could be deduced, indicating a pentahydroxy-heptanoic acid moiety. Furthermore, a ^3J-correlation between H-3 and C-7, as well as H-7 and C-3, could be observed (Fig. 3). Therefore an ether linkage between C-3 and C-7 had to be assumed. The cyclic structure of the C_7 moiety was supported by the molecular ion peak $[M–H]^-$ of **1** at m/z 531.0 in the ESI-MS. A signal at m/z 549, necessary for an open-chain structure, could not be observed. Consequently the C_7 moiety is a trihydroxy-tetrahydropyranyl-acetic acid subunit, for which we want to introduce the trivial name trilobatinoic acid A (**TaA**,

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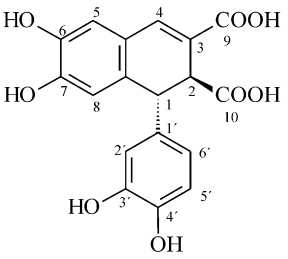
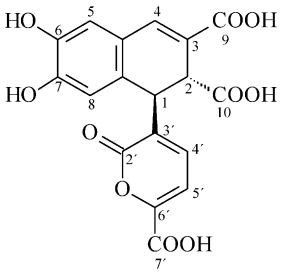


epiphyllic acid (**Ea**)



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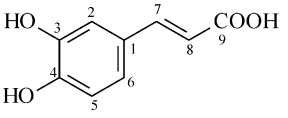

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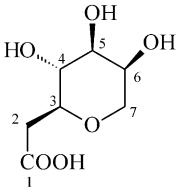
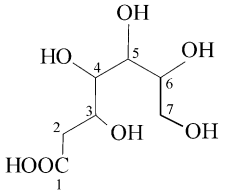


trilobatinoic acid A (**TaA**)



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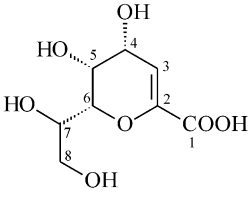
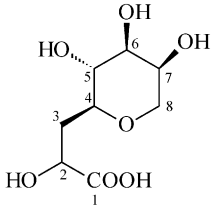


trilobatinoic acid C (**TaC**)



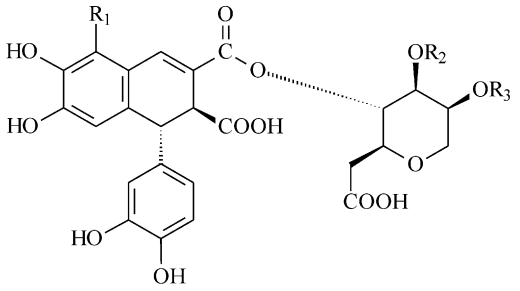
trilobatinoic acid D (**TaD**)

Fig. 1. Lignan subunits.

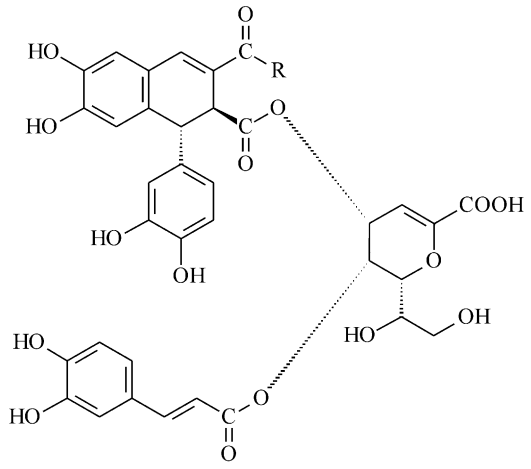
Fig. 1). The linkages of both subunits were obtained from the HMBC data. H-4 (δ_H 4.99, *t*, $J = 9.7$ Hz) of **TaA**, whose chemical shift is characteristic for an alcohol component of an ester, showed a correlation with the carboxylic C-9 (δ_C 168.5) of the lignan moiety (Fig. 3). Its coupling pattern revealed a sterically fixed trans-arrangement to its neighbours H-3 and H-5. The small coupling constant of H-5 (δ_H 3.77, *dd*, $J = 9.7$, 3.3 Hz) resulted from the equatorial position of H-6. Therefore the alignment of the substituents, with the exception of the hydroxyl group at C-6, was equatorial, establishing the relative configuration of **1** (Fig. 4). Because of the structural similarity with trilobatin A (Martini et al., 1998), compound **1** was named trilobatin D (Fig. 2).

The ^{1}H and ^{13}C NMR data of **2** were similar to those of **1**. Compound **2** is composed of three partial structures, two **Ea** moieties and one **TaA** moiety. The resonances for H-5 of both **Ea** subunits were absent and the multiplicity of their corresponding carbons was now singlet. The ESI-mass spectrum ($[M–H]^-$ *m*/*z* 887.7) of **2** was in agreement with the molecular formula $C_{43}H_{36}O_{21}$, which agrees with a structure of two C–C-linked epiphyllic acid moieties esterified with **TaA**. The key HMBC correlation, which gave the linkage between the **Ea** and the **TaA** moiety, was the ^3J-coupling from **TaA** H-4 (δ_H 4.89, *t*, $J = 9.3$ Hz) to C-9 (δ_C 168.6) of one **Ea** subunit and led to the structure of **2**, named trilobatin E. The symmetrical dimer with 5-5″ linked epiphyllic acids was already known from *B. trilobata* (Martini et al., 1998).

The structures of compounds **3** and **4** were very similar: Both consisted of the same three partial structures **Ea**, **TaA** and jamesopyrone (**Jp**, Fig. 1), previously known from the liverwort *Jamesoniella autumnalis* (Tazaki et al., 1995). The linkages of the subunits were



	R_1	R_2	R_3
1	H	H	H
2	^{5}Ea	H	H
3	H	H	^{9}Jp
4	H	^{9}Jp	H



R

5	^{4}TaB
6	^{5}TaD
7	^{4}TaC^{5} – ^{9}Caf

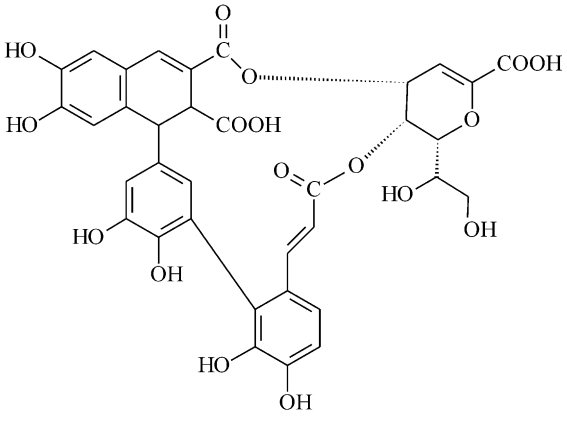


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Fig. 2. Structures of lignans **1**–**8**.

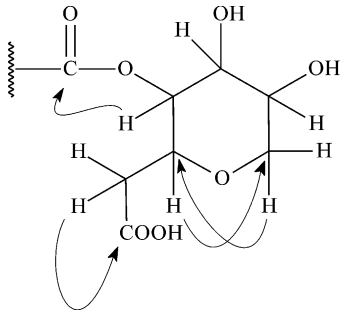

Fig. 3. Significant HMBC couplings of trilobatin D.

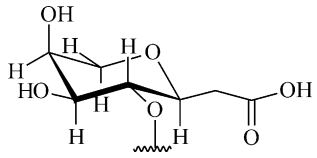

Fig. 4. Conformation of trilobationoic acid A (**TaC**).

obtained from HMBC data. In compound **3**, H-4 (δ_H 5.10, *t*, $J=9.5$ Hz) of **TaA** showed a correlation with C-9 (δ_C 168.3) of **Ea**, while H-6 (δ_H 5.20, *ddd*, $J=3.5$, 1.5, 1.0 Hz) of **TaA** showed a correlation with C-9 (δ_C 168.0) of **Jp**. Compound **3** was named trilobatin F, because of the structural similarity to trilobatin D **1** and E **2**. In compound **4** H-4 of **TaA** (δ_H 5.39, *t*, $J=9.7$ Hz) again showed a correlation with C-9 (δ_C 168.2) of **Ea**. But in contrast to **3**, H-5 of **TaA** (δ_H 4.80, *m*) showed a correlation with C-9 of **Jp** (δ_C 167.8). Compound **4** was named trilobatin G (Fig. 2).

Compound **5**, trilobatin H (Fig. 2), consists of four partial structures. The mass spectrum ($[M+K]^-$ *m*/*z* 952.3) was in agreement with a molecular formula of $C_{42}H_{42}O_{23}$. The ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) showed again an **Ea** moiety together with an aromatic ringsystem and a C_7 as well as a C_8 moiety. The aromatic moiety was characterized by a set of doublets at δ_H 6.42 (H-8) and δ_H 7.68 (H-7) with a coupling constant of 15.8 Hz, in agreement with the trans-double bond of a substituted cinnamoic acid. In addition, there were three aromatic protons (H-2, δ_H 7.12, *d*, $J=2.3$ Hz; H-5, δ_H 6.80, *d*, $J=8.2$ Hz; H-6, δ_H 7.12, *dd*, $J=8.2$, 2.3 Hz). The HSQC and the HMBC revealed the structure of a caffeic acid moiety (**Caf**, Fig. 1). The C_7 subunit consisted of a carboxyl group (C-1, δ_C 174.8), a methylene group (C-2, δ_C 38.3), an oxygen bearing methylene group (C-7, δ_C 70.9) and four oxygen bearing aliphatic signals (C-3, δ_C 76.8; C-4, δ_C 74.0; C-5, δ_C 73.4; C-6, δ_C 71.3). From the ^{1}H–^{1}H COSY, HSQC and HMBC data a chain of seven carbons could be deduced, indicating a 3,4,5,6,7-pentahydroxy-heptanoic acid moiety, for which we want to introduce the trivial name trilobatinoic acid B (**TaB**, Fig. 1). Cullmann et al. (1999) isolated a lignan containing a heptitol moiety, an unbranched chain of seven oxygen bearing carbon

atoms. Furthermore, there were eight additional carbon signals, a carboxyl group (C-1, δ_C 164.5), a trisubstituted double bond (C-2, δ_C 147.3 and C-3, δ_C 108.5) and five oxygen bearing aliphatic carbons (C-4, δ_C 68.2; C-5, δ_C 63.2; C-6, δ_C 77.1; C-7, δ_C 70.1; C-8, δ_C 63.9). The data matched those of the C_8 moiety of pelliatin (Cullmann et al., 1996), which we named trilobatinoic acid C (**TaC**, Fig. 1). The downfield shift of H-4 of **TaB** (δ_H 4.96, *t*, $J=9.5$ Hz) indicated an esterification. The HMBC clearly indicated a bond to C-9 of **Ea** (δ_C 168.0), explaining the upfield shift of this carbon atom. Key HMBC correlations included: H-4 of **TaC** (δ_H 5.71, *brs*) to C-10 of **Ea** (δ_C 173.2), H-5 of **TaC** (δ_H 5.80, *m*) to C-9 of **Caf** (δ_C 168.6). In addition the chemical shifts of H-4 and H-5 of **TaC** point to an acylation of the hydroxyl groups.

Compound **6**, trilobatin I (Fig. 2), contains four partial structures: **Ea**, **Caf**, **TaC** and one C_8 moiety, which was characterized by a carboxyl group (C-1, δ_C 177.7), a methylene group (C-3, δ_C 37.0) and six oxygen bearing aliphatic carbons (C-2, δ_C 68.3; C-4, δ_C 75.8; C-5, δ_C 74.6; C-6, δ_C 73.6; C-7, δ_C 70.9; C-8, δ_C 71.0). The data matched those of the C_8 moiety of trilobatin A (Martini et al., 1998), which we named trilobatinoic acid D (**TaD**, Fig. 1). Compound **6** gave a molecular ion peak $[M+Na]^-$ at *m*/*z* 949.2 in the ESI mass spectrum, which was in accordance with a molecular formula of $C_{43}H_{42}O_{23}$. Both carboxyls C-9 (δ_C 168.1) and C-10 (δ_C 173.2) of **Ea** showed the typical upfield shift of esterification when compared to the genuine epiphyllic acid (Cullmann et al., 1993); (C-9, δ_C 170.6; C-10, δ_C 176.5). Key HMBC correlations included: ^3J-couplings from H-4 of **TaC** (δ_H 5.68, *brs*) to C-10 of **Ea**, from H-5 of **TaC** (δ_H 5.77, *m*) to C-9 of **Caf** (δ_C 168.7) and the ^3J-coupling from H-5 of **TaD** (δ_H 4.94, *t*, $J=9.5$ Hz) to C-9 of **Ea** (δ_C 168.1).

The ^{1}H NMR spectrum of **7**, trilobatin J, contained the signals of an **Ea** moiety. Furthermore, there were signals for four other subunits, two **Caf** and two **TaC** moieties. Compound **7** gave in the negative ESI mass spectrum the molecular ion at *m*/*z* 1109.3 $[M+Na]^-$, in agreement with a molecular formula of $C_{52}H_{46}O_{26}$. The upfield shift in the carboxyl signals of **Ea** by 3.3 ppm for C-9 (δ_C 167.3) and 3.4 ppm for C-10 (δ_C 173.1) suggested that both carboxyl groups were esterified. The linkages of these two carboxyl groups were obtained from HMBC data. H-4 of one **TaC** (δ_H 5.73, *brs*) showed a correlation with the carboxylic carbon C-10 of **Ea**, and H-4 of the second **TaC** (δ_H 5.64, *brs*) showed a correlation with the carbon C-9 of **Ea**. In addition the chemical shifts of H-4 of both **TaC** moieties point to an acylation of the hydroxyl groups. H-5 of both **TaC** moities (δ_H 5.82, *brd* and δ_H 5.76, *brd*) also showed the characteristic chemical shifts of acylated alcohols. In the HMBC H-5 of both **TaC** moieties showed a correlation with C-9 of the **Caf** moieties.

The negative MALDI-TOF-MS spectra of compound **8** ($[M-H]^-$ *m*/*z* 718.989) was in accordance with a molecular formula of $C_{35}H_{28}O_{17}$. The ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) showed the presence of three substructures: **Ea**, **TaC** and **Caf**. H-2 of the **Caf** moiety was missing, revealing a substitution at position C-2. From the ^{1}H–^{1}H COSY, HSQC and the HMBC data, the structure of **8** could be assigned. The linkages of the subunits were obtained from the following data: H-4 of **TaC** (δ_H 5.61, *brs*) showed a correlation with carbon C-9 (δ_C 167.2) of **Ea**, and H-5 of **TaC** (δ_H 6.10, *brd*) showed a correlation with C-9 (δ_C 169.3) of **Caf**. The linkage between **Caf** and **Ea** could be assigned by the correlation between H-6′ of **Ea** (δ_H 5.53, *d*, $J=2.0$ Hz) and C-2 of **Caf** (δ_C 129.5). The macrocyclic lignan derivative **8** was named trilobatin K.

Once more, liverworts proved to be a source for new lignans. The key structure and parent compound of all lignans described in this study seems to be epiphyllic acid, derived from two caffeic acid moieties. Either oxidation, unsaturation and decarboxylation lead to give other basic structures, or the epiphyllic acid is unchanged and the lignans undergo esterification or glycosidation to form more complex structures. Various lignans are known to have anti-tumour, antimitotic and antiviral activity and to specifically inhibit certain enzymes (Mac Rae and Towers, 1984). Further work on biological activity of the lignan derivatives presented in the current here is in progress.

3. Experimental

3.1. Spectroscopy and spectrometry

The optical rotations were recorded on a Perkin Elmer model 241 polarimeter in MeOH. IR spectra were measured on a Perkin Elmer model 781 spectrometer with KBr pellets. UV spectra were recorded on a Shimadzu UV mini-1240 UV–*vis* spectrophotometer. LCMS data were recorded on a HP 59987 A electrospray MS, FABMS data were recorded on a Finnigan MAT 90 spectrometer and MALDI-TOF-MS data were recorded on a Bruker Reflex III spectrometer. ^{1}H and ^{13}C NMR data (including 2D spectra: DEPT, HSQC, HMBC and ^{1}H–^{1}H COSY spectra) were measured on a Bruker DRX-500 (^{1}H NMR: 500 MHz, ^{13}C NMR: 125 MHz). NMR Spectra were recorded in MeOH-d_4 relative to MeOH-d_4 at δ_H 3.30, δ_C 49.0. Silica gel 60 H 15 μm (Merck), silica gel 40–63 μm (Merck) and RP18-modified silica gel (LiChroprep RP 18, 25–40 μm, Merck) were used for vacuum liquid chromatography (VLC), respectively, while thin layer chromatography was performed on silica gel (Kieselgel 60 F_{254}, Merck) and reversed phase C18 (HPTLC-Fertigplatten RP18, F_{254}, Merck).

Table 1
^{1}H NMR spectral data for compounds **1**–**8** (CD_3OD)

H	**1**	**2**		**3**	**4**	**5**	**6**	**7**		**8**
Ea										
H-1	4.44 *d* (2.6)	4.54 *d* (2.6)	4.50 *d* (3.3)	4.45 *d* (2.7)	4.35 *d* (3.3)	4.42 *s*	4.37 *s*	4.34 *d* (2.4)		4.40 *brs*
H-2	3.86 *d* (2.6)	3.88 *d* (2.6)	3.88 *d* (3.3)	3.91 *d* (2.7)	3.71 *d* (3.3)	3.83 *s*	3.80 *s*	3.77 *d* (2.4)		3.63 *d* (1.1)
H-4	7.62 *s*	7.40 *s*	7.36 *s*	7.64 *s*	7.62 *s*	7.61 *s*	7.58 *s*	7.42 *s*		7.54 *s*
H-5	6.86 *s*	–	–	6.85 *s*	6.91 *s*	6.80 *s*	6.76 *s*	6.59 *s*		6.75 *s*
H-8	6.56 *s*	6.68 *s*	6.67 *brs*	6.55 *s*	6.63 *s*	6.63 *s*	6.59 *s*	6.56 *s*		6.44 *s*
H-2′	6.43 *d* (2.2)	6.62 *d* (2.0)	6.58 *d* (1.9)	6.41 *d* (2.0)	6.39 *d* (2.2)	6.31 *d* (2.3)	6.29 *d* (2.3)	6.33 *d* (1.8)		6.96 *d* (2.0)
H-5′	6.60 *d* (8.1)	6.71 *d* (8.4)	6.64 *d* (8.1)	6.59 *d* (8.3)	6.54 *d* (8.2)	6.47 *d* (8.3)	6.43 *d* (8.3)	6.46 *d* (8.7)		-
H-6′	6.37 *dd* (8.1, 2.2)	6.55 *dd* (8.4, 2.0)	6.41 *dd* (8.4, 2.0)	6.37 *dd* (8.3, 2.0)	6.34 *dd* (8.2, 2.2)	6.36 *dd* (8.3, 2.3)	6.32 *dd* (8.3, 2.3)	6.32 *dd* (8.7, 1.8)		5.53 *d* (2.0)
Jp										
H-1	–	–		4.74 *brd* (1.3)	4.71 *s*	–	–	–		–
H-2	–	–		4.07 *s*	3.91 *s*	–	–	–		–
H-4	–	–		7.66 *s*	7.62 *s*	–	–	–		–
H-5	–	–		6.64 *s*	6.52 *s*	–	–	–		–
H-8	–	–		6.55 *brs*	6.57 *s*	–	–	–		–
H-4′	–	–		6.60 *dd* (6.9, 1.3)	6.26 *d* (6.7)	–	–	–		–
H-5′	–	–		6.91 *d* (6.9)	6.03 *d* (6.7)	–	–	–		–
Caf										
H-2	–	–		–	–	7.12 *d* (2.3)	7.08 *d* (2.3)	7.07 *d* (1.9)	7.04 *d* (2.1)	-
H-5	–	–		–	–	6.80 *d* (8.2)	6.76 *d* (8.2)	6.77 *d* (8.1)	6.78 *d* (8.4)	6.77 *d* (8.0)
H-6	–	–		–	–	7.12 *dd* (8.2, 2.3)	6.96 *dd* (8.2, 2.3)	6.94 *dd* (8.1, 1.9)	6.93 *dd* (8.4, 2.1)	7.15 *d* (8.0)
H-7	–	–		–	–	7.68 *d* (15.8)	7.64 *d* (15.8)	7.65 *d* (15.9)	7.55 *d* (15.9)	7.13 *d* (16.0)
H-8	–	–		–	–	6.42 *d* (15.8)	6.39 *d* (15.8)	6.30 *d* (15.9)	6.35 *d* (15.9)	6.15 *d* (16.0)
TaA										
H-2	2.36 *dd* (15.7, 10.5)	2.29 *m*		2.34 *dd* (16.0, 9.5)	2.38 *dd* (15.8, 9.4)	–	–	–		–
	2.47 *brd* (15.7)			2.48 *dd* (16.0, 2.5)	2.48 *dd* (15.8, 2.7)	–	–	–		–
H-3	3.74 *dd* (9.6, 2.2)	3.55 *brd* (3.2)		3.85 *ddd* (9.5, 9.5, 2.5)	3.88 *m*	–	–	–		–
H-4	4.99 *t* (9.7)	4.89 *t* (9.3)		5.10 *t* (9.5)	5.39 *t* (9.7)	–	–	–		–
H-5	3.77 *dd* (9.7, 3.3)	3.59 *dd* (9.3, 3.2)		4.01 *dd* (9.5, 3.5)	4.80 *m*	–	–	–		–
H-6	3.89 *m*	3.86 *m*		5.20 *ddd* (3.5, 1.5, 1.0)	4.16 *brs*	–	–	–		–
H-7	3.60 *d* (12.0)	3.51 *brd* (12.6)		3.70 *dd* (12.5, 1.0)	3.69 *d* (11.5)	–	–	–		–
	3.87 *dd* (12.0, 2.5)	3.80 *dd* (12.6, 2.0)		3.97 *dd* (12.5, 1.5)	3.92 *d* (1.3)	–	–	–		–
TaB										
H-2	–	–		–	–	2.35 *dd* (16.0, 9.5)	–	–		–
	–	–		–	–	2.49 *dd* (16.0, 2.5)	–	–		–
H-3	–	–		–	–	3.72 *m*	–	–		–
H-4	–	–		–	–	4.96 *t* (9.5)	–	–		–
H-5	–	–		–	–	3.72 *m*	–	–		–
H-6	–	–		–	–	3.53 *d* (11.7)	–	–		–
H-7	–	–		–	–	3.83 *m* (16.0, 9.5)	–	–		–
TaC										
H-3	–	–		–	–	5.79 *d* (4.8)	5.78 *d* (4.8)	5.92 *brs*	5.75 *brs*	5.96 *brs*
H-4	–	–		–	–	5.71 *brs*	5.68 *brs*	5.73 *brs*	5.64 *brs*	5.61 *brs*
H-5	–	–		–	–	5.80 *m*	5.77 *m*	5.82 *brd* (3.6)	5.76 *brd* (10.5)	6.10 *brd* (5.3)
H-6	–	–		–	–	4.17 *brd* (8.7)	4.18 *brd* (8.7)	4.07 *brd* (8.9)	3.96 *brd* (8.7)	4.16 *d* (8.4)
H-7	–	–		–	–	3.75 *m*	3.71 *m*	3.72 *m*	3.70 *m*	3.75 *m*

(*continued on next page*)

Table 1 (*continued*)

H	1	2	3	4	5	6	7	8
H-8	–	–	–	–	3.75 *m*	3.73 *m*	3.75 *m*	3.85 *m*	3.80 *m*
					3.83 *m*	3.79 *m*			
TaD								
H-2	–	–	–	–	–	4.24 *m*	–	–
H-3	–	–	–	–	–	1.88 *m*	–	–
H-4	–	–	–	–	–	3.58 *m*	–	–
H-5	–	–	–	–	–	4.94 *t* (9.5)	–	–
H-6	–	–	–	–	–	3.68 *dd* (11.5, 3.5)	–	–
H-7	–	–	–	–	–	3.77 *m*	–	–
H-8	–	–	–	–	–	3.47 *brd* (15.0)	–	–
						3.80 *d* (5.0)		

3.2. Plant material

Bazzania trilobata (L.) S.F. Gray was collected in Rinnthal, Rheinland-Pfalz, Germany during June 1999. A voucher specimen is deposited at the Herbarium SAAR, Saarbrücken.

3.3. Extraction and isolation

The extraction scheme followed the standard procedure of our group (Adam et al., 1998). Air dried, powdered plant material (900 g) of *B. trilobata* was sequentially extracted with CH_2Cl_2 and MeOH. The MeOH extract was evapd. in vacuo and distributed between EtOAc and H_2O. The H_2O layer was evapd. in vacuo and distributed between H_2O and *n*-butanol. The butanol phase (15.3 g) was chromatographed on Sephadex LH-20 using MeOH as eluent to yield 2 frs. Fr. 1 (13.9 g) was chromatographed on RP-18 silica gel via VLC using an methanol–H_2O gradient (10% steps, 100 ml each) yielding 3 frs. 1.1–1.2. Further purification of fr. 1.1 by RP-18 HPLC (Phenomenex Aqua, 5 μm, 250×10 mm, mobile phase 5.0 ml/min H_2O–acetonitrile–HCOOH, 90:8:2) afforded trilobatin D **1** and E **2**. Compound **1** (21 mg) eluted with t_R 14 min and compound **2** (22 mg) with t_R 10 min. Fr. 1.2 yielded after HPLC separaration on RP-18 material (Phenomenex Aqua, 5 μm, 250×10 mm, mobile phase 5.0 ml/min H_2O–acetonitrile–HCOOH, 84:15:1) trilobatin H **5** (53 mg). Fraction 2 (185 mg) was chromatographed on RP-18 via HPLC (Phenomenex Aqua, 5 μm, 250×10 mm, mobile phase 5.0 ml/min H_2O–acetonitrile–HCOOH, 74:25:1) to afford jamesopyrone ($t_R = 2.5$ min), trilobatin G **4** ($t_R = 4.5$ min), trilobatin K **8** ($t_R = 5$ min), trilobatin F **3** ($t_R = 8$ min), trilobatin J **7** ($t_R = 13$ min) and one fraction containing 2 compounds. Further HPLC separation on RP-18 HPLC (Phenomenex Aqua, 5 μm, 250×10 mm, mobile phase 5.0 ml/min H_2O–acetonitrile–HCOOH, 60:39:1) gave 13 mg trilobatin I **6** ($t_R = 5$ min).

3.4. Trilobatin D (*1*)

$[\alpha]_D^{20}$ −146.7 (MeOH; *c* 0.23 g 100 ml^{-1}); IR ν (cm^{-1}): 3300, 1700, 1610, 1520, 1435, 1370, 1230, 1100, 1070; UV (MeOH) λ_{max} 253.2 nm, 343.2 nm; ^1H NMR Table 1. ^{13}C NMR Table 2; ESI-MS: $[M–H]^-$ *m*/*z* 531.0 (100), 487.1 (–CO_2,94).

3.5. Trilobatin E (*2*)

IR ν (cm^{-1}): 3300, 1670, 1590, 1435, 1370, 1220, 1100, 1070; UV (MeOH) λ_{max} 253.2 nm, 348.0 nm; ^1H NMR Table 1; ^{13}C NMR Table 2; ESI-MS: $[M–H]^-$ *m*/*z* 887.7 (100), 804.3 (12).

Table 2
^{13}C NMR spectral data for compounds **1**–**8** (CD_3OD)

C	1	2		3	4	5	6	7		8
Ea										
C-1	46.5	47.4	47.6	46.5	46.9	46.0	46.0	46.3		47.9
C-2	48.5	49.0	49.0	48.5	48.5	48.9	48.8	48.5		49.0
C-3	122.9	122.8	123.3	122.9	122.7	121.6	121.6	121.6		123.0
C-4	139.6	138.7	137.5	139.7	140.2	140.7	140.9	140.8		140.6
C-4a	124.9	124.2	124.1	124.9	125.3	124.7	124.7	124.6		124.2
C-5	117.2	117.0	117.0	117.2	117.1	117.3	117.3	117.2		117.0
C-6	145.5	144.1	144.3	145.6	145.7	145.6	145.5	145.5		145.5
C-7	149.1	149.6	149.3	149.3	149.4	149.3	149.7	149.3		149.5
C-8	117.3	117.0	116.7	117.3	117.3	117.5	117.5	117.4		117.1
C-8a	131.5	132.0	131.8	131.6	131.8	131.4	131.3	131.5		131.0
C-9	168.5	168.6	171.2	168.3	168.2	168.0	168.1	167.3		167.2
C-10	176.7	177.0	176.9	176.4	175.7	173.2	173.2	173.1		176.3
C-1′	136.4	136.3	136.5	136.4	136.4	136.4	136.4	136.4		136.4
C-2′	115.8	115.8	115.7	115.8	115.8	115.7	115.7	115.4		115.8
C-3′	145.9	146.1	146.1	146.0	146.0	144.8	144.8	145.9		146.8
C-4′	144.8	145.1	145.0	144.9	144.9	145.9	145.9	145.5		142.7
C-5′	116.3	116.6	116.5	116.2	116.3	116.3	116.3	116.4		124.2
C-6′	119.9	120.4	120.2	119.9	119.8	120.0	120.1	120.1		124.8
Jp										
C-1	–	–		41.7	41.2	–	–	–		–
C-2	–	–		43.5	43.4	–	–	–		–
C-3	–	–		123.2	123.3	–	–	–		–
C-4	–	–		139.3	140.1	–	–	–		–
C-4a	–	–		125.4	125.3	–	–	–		–
C-5	–	–		117.5	117.6	–	–	–		–
C-6	–	–		146.4	146.4	–	–	–		–
C-7	–	–		149.6	149.6	–	–	–		–
C-8	–	–		117.1	116.8	–	–	–		–
C-8a	–	–		128.0	128.2	–	–	–		–
C-9	–	–		168.0	167.8	–	–	–		–
C-10	–	–		175.0	175.4	–	–	–		–
C-2′	–	–		162.6	163.0	–	–	–		–
C-3′	–	–		134.6	134.1	–	–	–		–
C-4′	–	–		140.7	140.7	–	–	–		–
C-5′	–	–		111.1	111.1	–	–	–		–
C-6′	–	–		150.7	150.7	–	–	–		–
C-7′	–	–		162.5	162.9	–	–	–		–
Caf										
C-1	–	–		–	–	127.8	127.8	127.9	127.8	127.3
C-2	–	–		–	–	115.5	115.5	115.5	115.5	129.5
C-3	–	–		–		146.7	146.7	146.7	146.7	148.9
C-4	–	–		–	–	149.7	149.3	149.7	149.7	143.5
C-5	–	–		–	–	116.6	116.6	116.7	116.7	115.7
C-6	–	–		–	–	123.3	123.5	123.4	123.4	120.1
C-7	–	–		–	–	148.3	148.4	148.2	148.1	149.2
C-8	–	–		–	–	114.7	114.6	114.6	114.8	113.2
C-9	–	–		–	–	168.6	168.7	168.4	168.4	169.3
TaA										
C-1	175.2	174.9		174.7	174.4	–	–	–		–
C-2	38.4	38.0		38.3	38.2	–	–	–		–
C-3	76.9	76.5		77.0	76.7	–	–	–		–
C-4	73.9	74.3		73.9	70.8	–	–	–		–
C-5	73.2	73,3		71.8	77.2	–	–	–		–
C-6	70.9	71.2		74.0	68.2	–	–	–		–
C-7	71.4	70.7		69.1	71.3	–	–	–		–
TaB										
C-1	–	–		–	–	174.8	–	–		–
C-2	–	–		–	–	38.3	–	–		–
C-3	–	–		–	–	76.8	–	–		–
C-4	–	–		–	–	74.0	–	–		–

(*continued on next page*)

Table 2 (*continued*)

C	1	2	3	4	5	6	7		8
C-5	–	–	–	–	73.4	–	–		–
C-6	–	–	–	–	71.3	–	–		–
C-7	–	–	–	–	70.9	–	–		–
TaC									
C-1	–	–	–	–	164.5	165.1	165.3	165.4	167.2
C-2	–	–	–	–	147.3	147.3	146.7	146.7	148.0
C-3	–	–	–	–	108.5	108.5	108.5	108.7	108.5
C-4	–	–	–	–	68.2	68.2	68.2	67.9	67.0
C-5	–	–	–	–	63.2	63.2	63.2	63.4	62.8
C-6	–	–	–	–	77.1	77.1	77.3	77.3	77.0
C-7	–	–	–	–	70.1	70.1	70.2	70.2	70.0
C-8	–	–	–	–	63.9	63.9	64.1	64.1	64.1
TaD									
C-1	–	–	–	–	–	177.7	–		–
C-2	–	–	–	–	–	68.3	–		–
C-3	–	–	–	–	–	37.0	–		–
C-4	–	–	–	–	–	75.8	–		–
C-5	–	–	–	–	–	74.6	–		–
C-6	–	–	–	–	–	73.6	–		–
C-7	–	–	–	–	–	70.9	–		–
C-8	–	–	–	–	–	71.0	–		–

3.6. Trilobatin F (**3**)

$[\alpha]_D^{20}$ −75.3 (MeOH; *c* 0.27 g 100 ml^{-1}); IR ν (cm^{-1}): 3300, 1700, 1590, 1520, 1450, 1370, 1230, 1200; UV (MeOH) λ_{max} (log ε) 253.5 nm (4.14), 309.5 nm (4.32); ^{1}H NMR Table 1; ^{13}C NMR Table 2; ESI-MS: $[M+Na]^-$ *m*/*z* 925.0 (36.7), 907.0 (–H_2O, 12.0), 875.5 (6.6), 584.9 (100), 555.0 (48).

3.7. Trilobatin G (**4**)

$[\alpha]_D^{20}$ −20.0 (MeOH; *c* 0.11 g 100 ml^{-1}); IR ν cm^{-1}: 3300, 1700, 1620, 1580, 1510, 1440, 1360, 1230, 1200; UV λ_{max} 253.5, 312.5 nm; ^{1}H NMR Table 1; ^{13}C NMR Table 2; FABMS: $[M+H]^+$ *m*/*z* 903.5 (100), 825.7 (–C_6H_6).

3.8. Trilobatin H (**5**)

$[\alpha]_D^{20}$ −164.6 (MeOH; *c* 0.33 g 100 ml^{-1}); IR ν (cm^{-1}): 3300, 2230, 1720, 1640, 1520, 1440, 1370, 1240, 1030, 1000, 825, 765; UV (MeOH) λ_{max} (log ε) 250.0 nm (3.39) 334.0 nm (3.28); ^{1}H NMR Table 1; ^{13}C NMR Table 2; ESI-MS: $[M+K]^-$ *m*/*z* 952.3 (5), 935.3 (–OH, 31), 919.5 (–H_2O–CH_3, 100).

3.9. Trilobatin I (**6**)

$[\alpha]_D^{20}$−84.0 (MeOH; *c* 0.07 g 100 ml^{-1}). IR ν (cm^{-1}): 3300, 2920, 1710, 1590, 1510, 1440, 1360, 1250, 1100; UV (MeOH) λ_{max} (log ε) 251.0 nm (3.25) 332.0 nm (3.25); ^{1}H NMR Table 1; ^{13}C NMR Table 2; ESI-MS: $[M+K]^-$ *m*/*z* 966.2 (100) $[M+Na]^-$ 949.2 (85.2).

3.10. Trilobatin J (**7**)

$[\alpha]_D^{20}$ −164.6 (MeOH; *c* 0.33 g 100 ml^{-1}); IR ν cm^{-1}: 3300, 2920, 2850, 1590, 1370, 1230, 1200; UV (MeOH) λ_{max} (log ε) nm 248.0 (3.35) 330.0 (3.21); ^{1}H NMR Table 1; ^{13}C NMR Table 2; ESI-MS: $[M+Na]^-$ *m*/*z* 1109.3 (100), 1017.3 (–C_7H_8, 6.6).

3.11. Trilobatin K (**8**)

$[\alpha]_D^{20}$ −12.3 (MeOH; *c* 0.33 g 100 ml^{-1}); IR ν cm^{-1}: 3300, 2910, 1700, 1590, 1450, 1310, 1230, 1200, 1060; UV λ_{max} (log ε) 254.5 nm (4.56) 313.5 nm (4.37); ^{1}H NMR Table 1, ^{13}C NMR Table 2; MALDI-TOF-MS: $[M–H]^-$ *m*/*z* 718.989 (9), 673.021 (–C_2H_6O, 100).

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