Pergamon

PII: S0031–9422(97)01076–5

Phytochemistry, Vol. 49, No. 4, pp. 1139–1146, 1998

Printed in Great Britain
0031–9422/98/$—see front matter

LIGNANS FROM THE LIVERWORT *BAZZANIA TRILOBATA*†

ULRIKE MARTINI, JOSEF ZAPP and HANS BECKER*

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

(*Received in revised form* 26 *October* 1997)

Key Word Index—*Bazzania trilobata*; Hepaticae; liverwort; lignans; structural elucidation.

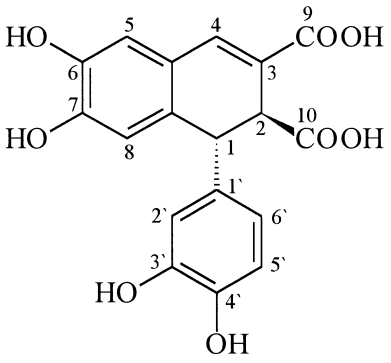
Abstract—Six new lignans, 5,5″-*bis* [2,3-dicarboxy-6,7-dihydroxy-l-(3′,4′-dihydroxyphenyl)-1,2-dihydronaphthalene], trilobatins A–C, trilobatin A-1″-methyl ester and bazzania acid, as well as 2,3-dicarboxy-6,7-dihydroxy-l-(3′,4′-dihydroxyphenyl)-1,2-dihydronaphthalene and 3-carboxy-6,7-dihydroxy-l-(3′,4′-dihydroxyphenyl)-naphthalene were isolated from the liverwort *Bazzania trilobata*. Their structures have been elucidated from extensive NMR spectral evidence and, in the case of trilobatin C, additionally by chemical derivatization. A possible biosynthetic pathway for bazzania acid is discussed. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

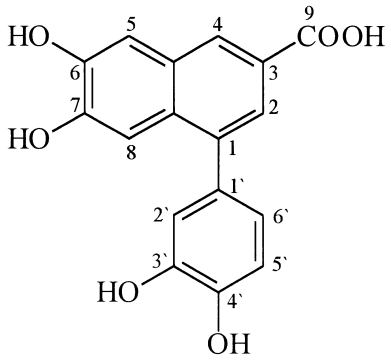
Recently we reported on the isolation and structural elucidation of 11 new *bis*bibenzyls from *Bazzania trilobata* [2]. Further investigation of the methanol extract from this liverwort has now led to the isolation of two known (**1** and **2**) and six new (**3**–**8**) lignans.

RESULTS AND DISCUSSION

All compounds were isolated from the ethyl acetate-soluble fraction of the methanol extract, obtained as previously reported [2]. 2,3-Dicarboxy-6,7-dihydroxy-l-(3′,4′-dihydroxyphenyl)-1,2-dihydronaphthalene (**1**) and



1



2

3-carboxy-6,7-dihydroxy-l-(3′,4′-dihydroxyphenyl)-naphthalene (**2**) could be identified from their NMR and mass spectral data [3, 4]. Both had been isolated previously from the liverwort, *Pellia epiphylla* [3, 4].

The negative FAB mass spectrum ($[M–H]^-$ *m*/*z* 713) of **3** was in agreement with the molecular formula $C_{36}H_{26}O_{16}$. The ^1H NMR spectrum (Table 1) displayed two aliphatic methine protons, three signals of a 3,4-dihydroxyphenyl group and two singlets. The ^{13}C NMR and DEPT spectra showed 18 signals, two of them belonging to carboxylic carbons, 14 aromatic or olefinic carbons and two aliphatic methine carbons. The ^1H and ^{13}C NMR data of **3** were similar to those of **1**, except that the resonance of H-5 was missing and the multiplicity of C-5 has changed from *d* to *s*. Therefore, compound **3** is the symmetrical dimer of **1** with 5-5″ linked subunits. HMBC and NOESY spectra confirmed the structure of **3** to be 5,5″-*bis* [2,3-

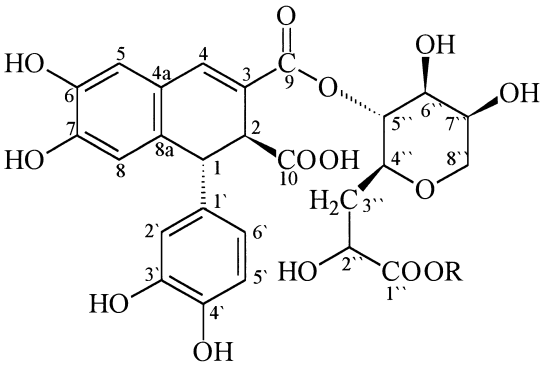
* Author to whom correspondence should be addressed.

† Part 121 in the series of "Arbeitskreis Chemie und Biologie der Moose". For part 120 see ref. [1].

Table 1. ^{1}H NMR spectral data for compound **3**

H	**3**
H-1, 1″	4.53 *br s*
H-2, 2″	3.85 *br s*
H-4, 4″	7.41 *s*
H-8, 8″	6.68 *s*
H-2′, 2‴	6.68 *d* (1.8)
H-5′, 5‴	6.64 *d* (8.1, 1.8)
H-6′, 6‴	6.43 *dd* (8.1, 1.8)

Coupling constants (*J* in Hz) in parentheses



	R
4	H
5	CH_3

phyto4397st4

dicarboxy-6,7-dihydroxy-1-(3′,4′-dihydroxyphenyl)-1,2-dihydronaphthalene.

Compound **4**, which we named trilobatin A, was isolated as a colourless oil. ^{1}H and ^{13}C NMR spectra (Tables 2 and 3) showed the presence of two partial structures. The first one was the phenyldihydronaphthalene moiety **1**. The second one was characterized by a carboxyl group (C-1″), a methylene group (C-3″) and six oxygen-bearing aliphatic carbons (C-2″, C-4″–C-8″). From the ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY and the HMBC data, a linear chain of eight carbon atoms could be deduced, indicating a 2,4,5,6,7,8-hexahydroxy octanoic acid moiety. Furthermore, a ^3J-correlation between H-4″ and C-8″, as well as H-8″ and C-4″, could be observed (Fig. 1). Therefore, an ether linkage between C-4″ and C-8″ had to be assumed. The cyclic structure was supported by the molecular ion peak $[M+H]^+$ at *m*/*z* 563 in the positive FAB mass spectrum. A signal at *m*/*z* 581 necessary for an open-chain structure could not be observed. Consequently, the C-8 moiety is a 2-hydroxy-3-(3′,4′,5′-trihydroxy-tetrahydropyran-2-yl)-propanoic acid subunit. The linkages of both subunits were obtained from HMBC results. H-5″ showed a correlation with the carboxylic carbon C-9 of the lignan moiety (Fig. 1). In addition, the chemical shift of H-5″ is characteristic of an alcohol component of an ester. The stereochemistry of the C-8 moiety could be explained on the three-dimensional model (Fig. 2). The chair is the preferred conformation of similar arranged carbohydrates of the pyranose type, wherein

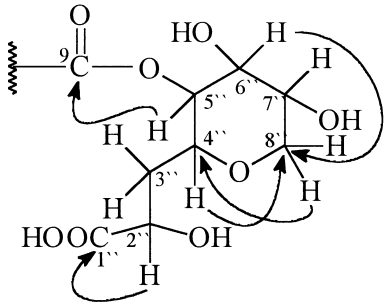


Fig. 1. Significant HMBC couplings of compound **4**.

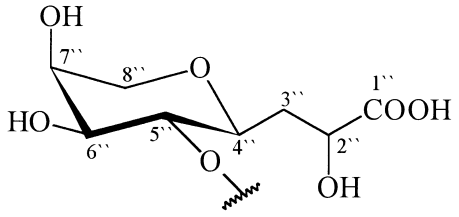


Fig. 2. Conformation of C-8 moiety of compound **4**.

the C-8 moiety could also be arranged. Proton H-5″ gave rise to a triplet with $J = 9.6$ Hz, revealing a sterically fixed *trans*-arrangement to the neighbours H-4″ and H-6″. The small coupling constant of H-6″ ($J = 3.4$ Hz) resulted from the equatorial position of H-7″. Therefore, the alignment of the substituents, with the exception of the hydroxyl group on C-7″, was equatorial, establishing the relative configuration of **4**.

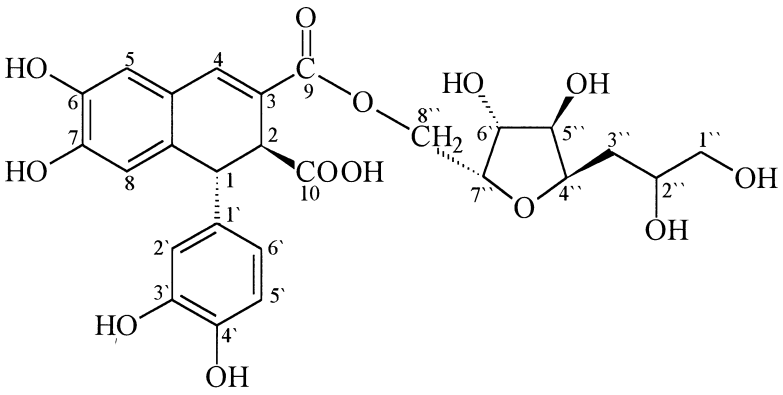
Compound **5** gave a positive FAB mass spectrum $[M+H]^+$ at *m/z* 577, in agreement with a molecular formula of $C_{27}H_{28}O_{14}$. The ^1H and ^{13}C NMR spectral (Tables 2 and 3) were very similar to those of compound **4**. The only difference was the presence of an additional methyl group (C-9″), indicating a methyl ester or ether. Because of the upfield shift of the carboxyl signal C-1″ by 1.4 ppm, which is in accordance with esterfication of a carboxyl function, [5] compound **5** should be trilobatin A-1″-methyl ester.

Compound **6** also showed two partial structures, one of which was again the phenyldihydronaphthalene **1**. The second moiety was composed of seven oxygen-bearing , aliphatic carbon atoms (C-1″; C-2″; C-4″–C-8″) and one methylene group (C-3″). The sequence of the carbon atoms could be deduced from the ^1H–^1H and ^1H–^{13}C COSY. With the exception of C-1″, that was reduced from a carboxyl group to a secondary alcohol, the succession of the carbon atoms correspond to the C-8 moiety of compound **4**. The position of the ether linkage could not be obtained from correlations of the HBMC spectrum, probably due to the electronegative substituents and a dihedral angle of ca 90° between the vicinal C,H atoms of

Table 2. ^1H NMR spectral data for compounds **4**–**6**

H	**4**	**5**	**6**
Lignan moiety			
H-1	4.39 *d* (2.8)	4.43 *d* (2.8)	4.42 *d* (2.6)
H-2	3.84 *d* (2.8)	3.87 *d* (2.8)	3.85 *d* (2.6)
H-4	7.57 *s*	7.61 *s*	7.65 *s*
H-5	6.80 *s*	6.83 *s*	6.83 *s*
H-8	6.51 *s*	6.54 *s*	6.54 *s*
H-2′	6.39 *d* (1.9)	6.41 *d* (2.1)	6.40 *d* (2.1)
H-5′	6.57 *d* (8.1)	6.60 *d* (8.1)	6.61 *d* (8.2)
H-6′	6.35 *dd* (8.1, 1.9)	6.37 *dd* (8.1, 2.1)	6.37 *dd* (8.2, 2.1)
C_8 moiety			
H-1″a	—	—	3.46 *dd* (11.3, 4.5)
H-1″b	—	—	3.51 *dd* (11.3, 4.5)
H-2″	4.28 *dd* (10.8, 2.3)	4.33 *dd* (10.5, 2.8)	3.80 *m*
H-3″	1.79 *m*	1.80 *m*	1.65 *m* and 1.83 *m*
H-4″	3.55 *d* (9.6)	3.52 *d* (9.6)	4.19 *m*
H-5″	4.93 *t* (9.6)	4.96 *t* (9.6)	3.94 *d* (11.6)
H-6″	3.70 *dd* (9.6, 3.4)	3.73 *dd* (9.6, 3.4)	3.97 *d* (11.6)
H-7″	3.84 *d* (3.4)	3.87 *d* (3.4)	3.88*m*
H-8″a	3.57 *d* (14.2)	3.59 *d* (12.4)	4.22*m*
H-8″b	3.90 *d* (14.2)	3.90 *d* (12.4)	—
OCH_3	—	3.68 *s*	—

Coupling constants (*J* in Hz) in parentheses; signals indicated as *m* were unresolved or overlapped multiplets



6

Table 3. ^{13}C NMR spectral data for compounds **4**–**7** and **7a** ($CDCl_3$), **8**

C	**4**	**5**	**6**	**7**	**7a**	**8**
C-1	46.6 *d*	46.7 *d*	46.8 *d*	46.6 *d*	45.2 *d*	55.2 *d*
C-2	48.6 *d*	48.8 *d*	48.6 *d*	48.6 *d*	46.8 *d*	48.5 *d*
C-3	123.1 *s*	123.1 *s*	123.0 *s*	123.3 *s*	122.5 *s*	138.1 *s*
C-4	139.5 *d*	139.6 *d*	139.9 *d*	139.4 *d*	137.3 *d*	129.1 *d*
C-4a	124.9 *s*	125.0 *s*	125.1 *s*	125.0 *s*	124.1 *s*	165.1 *s*
C-5	117.2* *d*	117.2* *d*	117.2* *d*	117.0 *d*	111.8* *d*	118.1 *d*
C-6	145.5 *s*	145.6 *s*	145.6 *s*	145.4 *s*	148.2 *s*	173.3 *s*
C-7	149.1 *s*	149.1 *s*	149.1 *s*	148.7 *s*	150.8 *s*	171.8 *s*
C-8	117.3* *d*	117.3* *d*	117.3* *d*	117.0 *d*	111.9* *d*	37.2 *t*
C-8a	131.5 *s*	131.6 *s*	131.5 *s*	130.8 *s*	130.2 *s*	86.9 *s*
C-9	168.4 *s*	168.4 *s*	168.6 *s*	170.5 *s*	166.8 *s*	167.8 *s*
C-10	176.4 *s*	176.4† *s*	176.3 *s*	176.1 *s*	172.7 *s*	175.2 *s*
C-1′	136.4 *s*	136.5 *s*	136.5 *s*	136.5 *s*	134.9 *s*	126.4 *s*
C-2′	115.8 *d*	115.8 *d*	115.8 *d*	115.4 *d*	113.1 *d*	118.6 *d*
C-3′	144.5 *s*	144.9 *s*	145.0 *s*	145.5 *s*	145.4 *s*	146.0 *s*
C-4′	146.0 *s*	146.0 *s*	146.0 *s*	146.5 *s*	147.6 *s*	146.6 *s*
C-5′	116.3 *d*	116.3 *d*	116.3 *d*	117.4 *d*	110.7 *d*	116.1 *d*
C-6′	119.9 *d*	120.0 *d*	119.9 *d*	122.9 *d*	121.5 *d*	122.6 *d*
C-1″	178.0 *s*	176.6† *s*	67.9 *t*	125.9 *s*	125.3 *s*	—
C-2″	67.7 *d*	68.2 *d*	70.8 *d*	118.0 *d*	112.4 *d*	—
C-3″	37.7 *t*	37.6 *t*	33.9 *t*	146.2 *s*	148.7 *s*	—
C-4″	75.9 *d*	75.9 *d*	80.3 *d*	148.5 *s*	150.3 *s*	—
C-5″	74.6 *d*	74.6 *d*	79.9 *d*	116.4 *d*	112.5 *d*	—
C-6″	73.6 *d*	73.6 *d*	80.8 *d*	125.0 *d*	124.7 *d*	—
C-7″	71.0 *d*	71.0 *d*	84.2 *d*	129.2 *d*	127.5 *d*	—
C-8″	71.3 *t*	71.3 *t*	66.2 *t*	139.4 *s*	137.8 *s*	—
C-9″	—	—	—	167.0 *s*	164.1 *s*	—
R═OCH_3	—	52.4 *q*	—	—	—	—
R^1	—	—	—	—	52.2* *q*	—
R^2	—	—	—	—	52.3* *q*	—
R^3	—	—	—	—	55.4 *q*	—
R^4, R^7	—	—	—	—	56.0† *q*	—
R^5, R^6	—	—	—	—	55.8† *q*	—
R^8	—	—	—	—	51.7 *q*	—

Assignments confirmed by ^1H–^{13}C COSY and HMBC.
*, †Values may be interchanged within the same column

the ether sequence, both minimizing the ^3J coupling constant [5]. Because an ether-bearing carbon should resonate at lower field than a free alcohol [5], we had to analyze the ^{13}C data of the C-8 moiety. The lowest shift was found for C-7″ at 84.2, which therefore should be part of the ether linkage. The second carbon atom of the ether could be only C-4″, C-2″ or C-1″, of which C-4″ showed the lowest shift. Therefore, the ether group connects C-7″ with C-4″, furnishing a tetrahydrofuran ring. The linkage of the subunits could be confirmed from the HMBC data, in which H-8″ showed a correlation with C-9. Additionally, a NOESY experiment was carried out to reveal the stereochemistry of the C-8 moiety (Fig. 3). H-4″ showed a correlation with H-5″, as well as H-6″ with H-7″. Cross-peaks between H-5″ and H-6″ could not be observed. If we arrange the tetrahydrofuran ring in the $^2T_3$ conformation, frequently shown for furanoses, the absence of a correlation between H-5″ and H-6″ could only be explained by a quasi-axial arrangement

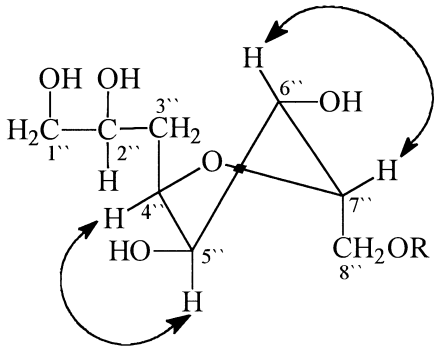


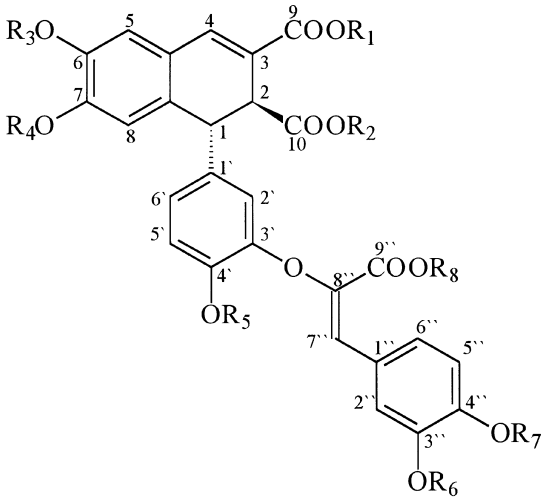
Fig. 3. Conformation and significant NOESY correlations of compound **6**.

of these protons. The coupling constant of H-5″ and H-6″, each $J = 11.6$ Hz, confirmed this assumption. Thus, the neighbours H-4″ and H-7″ should show a quasi equatorial position. Because of the structural

similarity with **4**, compound **6** was named trilobatin B.

Compound **7** showed in the negative FAB mass spectrum a $[M–H]^-$ at *m/z* 537, in agreement with a molecular formula $C_{27}H_{20}O_{12}$. The ^1H and ^{13}C NMR spectra (Tables 3 and 4) showed again the phenyldihydronaphthalene moiety. Furthermore, there are nine additional carbons, viz, a carboxyl group and eight partially oxygen-bearing, olefinic or aromatic carbons. The ^1H NMR spectrum showed the typical signal pattern of a 1,3,4-trisubstituted aromatic system and a singlet belonging to a trisubstituted double bond, conjugated to the aromatic ring. Since there are no further carbon atoms with a free valency, the fourth substituent of the double bond should be an oxygen atom. From the ^1H–^1H COSY, ^1H–^{13}C COSY and HMBC, as well as comparison with the published NMR data [6, 7], the structure of 8″-*O*-substituted caffeic acid was established. The configuration of the olefinic bond at C-7″-C-8″ was *Z*, based on comparison between the chemical shift of the olefinic proton H- 7″ with those of corresponding protons of known compounds [6] (*Z*-form: δ_H7.20, *E*-form: δ_H 6.88) with analogous partial structures. The connection of the 8″-*O*-substituted caffeic acid with the lignan moiety could result from an ester or an ether bond. Since there were no conclusive spectroscopic data supporting the linkage, chemical derivatization of **7** was necessary.

Methylation of **7** afforded the octamethyl derivative **7a** (positive FAB mass spectrum, $[M+H]^+$ *m/z* 649, in accordance with the molecular formula $C_{35}H_{36}O_{12}$). The ^{13}C spectrum of **7a** (Table 3) indicated the presence of five aromatic methoxyl groups and three carboxy methyl groups. Therefore, C-8″ of the caffeic acid moiety must be connected to one of the phenolic oxygens of the lignan subunit via an ether. Confirmation of the ether bond was given by the HMBC spectra of **7a**; the methoxyl protons of R^5, R^6 and R^7 showed correlations with carbon atoms C-4′, C-3 and C-4″. Furthermore, there were cross-peaks between the signals of the methyl groups R^3 and R^4 with the quaternary carbon atoms C-6 and C-7. Since no ^3J long-range correlations were observed between a

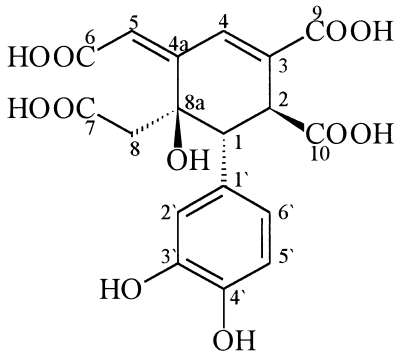


	R_1-R_8
7	H
7a	CH_3

Table 4. ^1H NMR spectral data for compounds **7** (CD_3OD) and **7a** ($CDCl_3$)

H	**7**	**7a**
Lignan moiety		
H-1	4.37 *d* (1.8)	4.47 *d* (2.9)
H-2	3.73 *d* (1.8)	3.72 *d* (2.9)
H-4	7.41 *s*	7.52 *s*
H-5	6.71 *s*	6.75 *s*
H-8	6.47 *s*	6.49 *s*
H-2′	6.43 *d* (1.4)	6.43 *d* (2.0)
H-5′	6.75 *d* (8.1)	6.78 *d* (8.4)
H-6′	6.59 *dd* (8.1, 1.4)	6.53 *dd* (8.4, 2.0)
8″-*O*-substituted caffeic acid moeity		
H-2″	7.23 *d* (1.6)	7.33 *d* (1.7)
H-5″	6.74 *d* (8.3)	6.77 *d* (8.4)
H-6″	7.01 *dd* (8.3, 1.6)	7.12 *dd* (8.4, 1.7)
H-7″	7.20 *s*	7.26 *s*
R═OCH_3		
R^1, R^8	—	3.67 *s*
R^2	—	3.54 *s*
R^3	—	3.68 *s*
R^4	—	3.70 *s*
R^5, R^6, R^7	—	3.84 *s*

Coupling constants (*J* in Hz) in parentheses



8

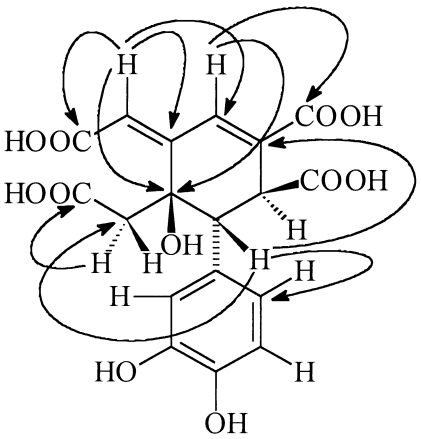


Fig. 4. Significant HMBC couplings of compound **8**.

methoxyl group and C-8″ or C-3′, only a linkage between these two carbons *via* an oxygen was possible, establishing the complete structure of **7**. Similar compounds are known from *Melissa officinalis*, melitric acid A [6], and *Salvia cavaleriei* [7], salvianolic acid, which show an ether linkage between the hydroxyl group at C-8″ of a 8″-*O*-substituted caffeic acid and the phenolic oxygen at C-4′ of a rosmarinic acid moiety.

Compound **8**, bazzania acid, was obtained as an oil. Its DCI mass spectrum ($[M]^+$ *m*/*z* 408) and NMR spectral data were in agreement with a molecular formula of $C_{18}H_{16}O_{11}$. The signals of the ^1H NMR spectrum (Table 5) integrated for nine protons. Five of these resonated in the region of conjugated double bonds or aromatic rings. The signal with the highest down-field shift gave rise to a doublet (H-4), followed by a higher order spin-system integrating for three protons (H-2′, H-5′ and H-6′) and a singlet (H-5). The aliphatic protons region showed resonances for four protons (H-1; H-2; 2 × H-8). The ^{13}C NMR spectrum (Table 3) showed 18 signals, four of them carboxylic carbons, ten due to aromatic or olefinic carbons and four to aliphatic carbons, including one quaternary, oxygen-bearing carbon. The ^1H–^1H COSY, ^1H–^{13}C COSY and HMBC (Fig. 4) revealed the complete constitution of **8** indicating that it is formally an oxidation product of **1**. The relative configuration was determined by a NOESY experiment and the coupling constants shown in the ^1H NMR spectrum. The magnitude of $J_{1,2}$ (11.3 Hz) revealed for H-1 and H-2 a quasi axial position, implying a quasi equatorial position of the carboxyl group C-10 and the aryl substituent of C-1. This part of the structure showed the same relative configuration as that found in **1**. The stereochemistry of the quaternary centre C-8a was deduced from the NOESY spectrum. H-8 showed a cross-peak with H-2 due to an axial-axial arrangement; however, there was no correlation with H-1. Thus, the hydroxyl function at C-8a had to be equatorial. The NOESY cross-peak between H-4 and H-5 confirmed the 4a*E*-geometry. On basis of the above evidence, the molecular structure and relative configuration of **8** was established.

A possible biosynthetic pathway for **8** is shown in Fig. 5. Oxidative cleavage of the ene-diol structure of the dihydronaphthalene system **1** by an intradioldioxygenase could lead to the formation of two carboxyl functions, whereby the geometry of the remaining double bonds are retained. The subsequent addition of H_2O, proceeding in Markownikow-orientation, would lead to the formation of bazzania acid.

Table 5. ^1H NMR spectral data for compound **8**

H	**8**
H-1	3.28 *d* (11.3)
H-2	4.12 *dd* (11.3, 2.2)
H-4	7.71 *d* (2.2)
H-5	6.22 *s*
H-8	2.80 *d* (5.6)
H-2′	6.81 *s* (*br*)
H-5′	6.77 *m*
H-6′	6.77 *m*

Coupling constants (*J* in Hz) in parentheses; signals indicated as *m* were unresolved or overlapped multiplets

EXPERIMENTAL

Solvents used for spectral measurements: MeOH-d_4, $CDCl_3$ (only for **7a**) [^1H NMR: 400 MHz, ^{13}C NMR 100 MHz for 1D spectra, 500 MHz and 125 MHz for 2D spectra]. Chemical shifts are given in δ relative to CH_3OD at δ_H 3.3 or CD_3OD at δ_C 49.0, respectively, to $CHCl_3$ at δ_H 7.24 or $CDCl_3$ at δ_C 77.0. MeOH (UV, rotation). IR: KBr.

Plant material

Bazzania trilobata (L.) S.F. Gray was collected in Weißkirchen, Saarland, Germany, during March 1994. The plant was identified by Prof. R. Mues at the Institute of Botanik der Universität des Saarlandes, Saarbrücken, Germany, and a herbarium specimen

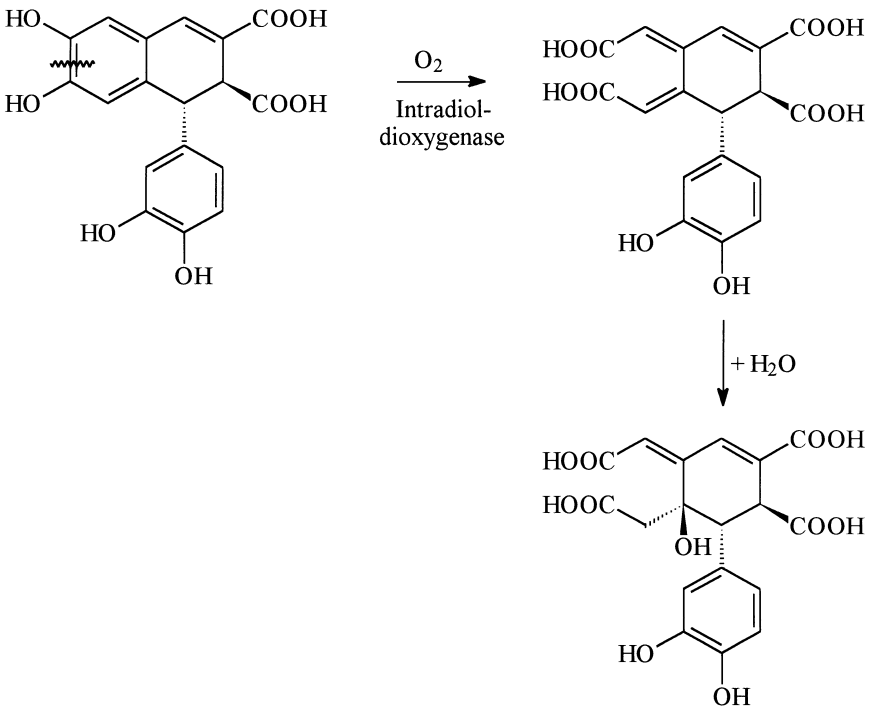


Fig. 5. Possible biosynthetic pathway of compound **8**.

with the voucher number 40 is deposited at Pharmakognosie und Analytische Phytochemie, Universität des Saarlandes, Saarbrücken, Germany.

Extraction and isolation

Air-dried, powdered plant material (930 g) [2] was first extracted with Et_2O and then with MeOH. The MeOH extract was evapd *in vacuo* and distributed between EtOAc and H_2O. The organic layer was evapd *in vacuo* and chromatographed on Sephadex LH-20 using MeOH–CH_2Cl_2 (4:1) to yield 5 frs. Fr. 2 was chromatographed on diol-modified silica gel via VLC using a hexane–EtOAc–MeOH gradient yielding 4 frs, 2.1–2.4. Further purification of fr. 2.3 by HPLC (LiChrospher Diol, 5 μm, *tert*. butylmethylether–MeOH, 49:1) afforded bazzania acid (**8**) (24 mg). Fr. 2.4 yielded after HPLC separation (LiChrospher Diol, 5 μm, EtOAc–MeOH, 49:1) and subsequent purification (LiChrospher Diol, 5 μm, EtOAc–MeOH, 97:3) trilobatin A (**4**) (79.5 mg), trilobatin A-1″-methyl ester (**5**) (27 mg) and trilobatin B (**6**) (12.5 mg). Fr. 3 was also chromatographed on diol-modified silica gel *via* VLC with a *n*-hexane–EtOAc–MeOH gradient to afford 2,3-dicarboxy-6,7-dihydroxy-l-(3′,4′-dihydroxyphenyl)-1,2-dihydronaphthalene (**1**) (408 mg) and fr. 3.1. Further HPLC separation of 3.1 (Spherisorb ODS 1, 5 μm, MeOH–H_2O, 13:17+1% HCO_2H) gave trilobatin C (**7**) (67 mg). Fr. 4 was chromatographed as described above via VLC and further separated using HPLC (LiChrospher Diol, 5 μm, *n*-hexane–EtOAc, 1:9) to yield 3-carboxy-6,7-dihydroxy-l-(3′,4′-dihydroxyphenyl)naphthalene (**2**) (10.5 mg), and 5,5″-*bis*[2,3-dicarboxy-6,7-dihydroxy-l-(3′,4′-dihydroxyphenyl)-1,2-dihydronaphthalene] (**3**) (59 mg). (Spherisorb ODS 1, 5 μm, MeOH–H_2O, 3:17+1% HCO_2H.)

Methylation of compound **7**

A mix. of **7** (20 mg), CH_3I (0.1 ml) and K_2CO_3 (80 mg) in DMF was stirred for 15 h at 50°. The insol. material was then filtered off and filtrate evapd to dryness to afford **7a** (23 mg).

Compound **3**. $[\alpha]_D^{20}$ −106.1 (MeOH; *c* 0.59). IR *v* cm^{-1}: 3300, 1700, 1610, 1590, 1525, 1450, 1360, 1285, 1200. UV λ_{max} nm 252, 329; λ_{min} 240, 298. ^1H NMR: Table 1. ^{13}C NMR: δ (each signal correspond to 2C): 47.5 (*d*), 49.8 (*d*), 115.6 (*d*), 116.6 (*d*), 117.0 (*d*), 120.3 (*d*), 123.4 (*s*), 123.6 (*s*), 124.2 (*s*), 131.7 (*s*), 136.5 (*s*), 137.7 (*d*), 144.1 (*s*), 144.9 (*s*), 146.0 (*s*), 149.3 (*s*), 170.9 (*s*), 176.8 (*s*). FAB-MS: $[M-H]^-$ *m/z* 713.

Compound **4**. $[\alpha]_D^{20}$ −85.3 (MeOH; *c* 0.79). IR *v* cm^{-1}: 3300, 1700, 1610, 1590, 1520, 1450, 1360, 1285, 1230, 1200. UV λ_{max} nm 252, 338; λ_{min} 236, 278. ^1H NMR: Table 2. ^{13}C NMR: Table 3. FAB-MS: $[M+H]^+$ *m/z* 563.

Compound **5**. $[\alpha]_D^{20}$ −75.3 (MeOH; *c* 0.27). IR *v* cm^{-1}: 3300, 1700, 1610, 1590, 1520, 1450, 1360, 1285, 1230, 1200. UV λ_{max} nm 253, 338; λ_{min} 235, 277. ^1H NMR: Table 2. ^{13}C NMR: Table 3. FAB-MS: $[M+H]^+$ *m/z* 577.

Compound **6**. $[\alpha]_D^{20}$ −35.9 (MeOH; *c* 0.12). IR *v* cm^{-1}: 3300, 1700, 1610, 1590, 1520, 1450, 1360, 1285,

1230, 1200. UV λ_{max} nm 253, 334; λ_{min} 228, 280. ^{1}H NMR: Table 2. ^{13}C NMR: Table 3. FAB-MS: $[M+H]^+$ m/z 549.

Compound **7**. $[\alpha]_D^{20}$ −80.1 (MeOH; *c* 0.67). IR *v* cm^{-1}: 3300, 1700, 1610, 1590, 1520, 1450, 1360, 1285, 1230, 1200. UV λ_{max} nm 247, 318; λ_{min} 236, 274. ^{1}H NMR: Table 4. ^{13}C NMR: Table 3. FAB-MS: $[M-H]^-$ m/z 535.

Compound **7a**. ^{1}H NMR: Table 4. ^{13}C NMR: Table 3. FAB-MS: $[M+H]^+$ m/z 649.

Compound **8**. $[\alpha]_D^{20}$ −16.5 (MeOH; *c* 0.24). IR *v* cm^{-1}: 3300, 1700, 1600, 1510, 1450, 1360, 1285, 1200. UV λ_{max} nm 276; λ_{min} 244. ^{1}H NMR: Table 5. ^{13}C NMR: Table 3. DCIMS: $[M]^+$ m/z 408.

Acknowledgements—We thank Dr R. Graf, Universität des Saarlandes, for recording MS and Dr R. Speicher, Universität des Saarlandes, for carrying out methylation of trilobatin C.

REFERENCES

1. Thiel, R., Adam, K.-P. and Becker, H., *Pharm. Pharmacol. Lett*., 1997, **7**, 103.
2. Martini, U., Zapp, J. and Becker, H., *Phytochemistry*, 1998, **47**, 89.
3. Cullmann F., Adam, K. P. and Becker, H., *Phytochemistry*, 1993, **34**, 831.
4. Rischmann, M., Mues, R., Geiger, H., Laas, H. J. and Eicher, Th., *Phytochemistry*, 1989, **28**, 867.
5. Kalinowski, H. D., Berger, S. and Braun, S., *^{13}C-Spektroskopie*. Georg Thieme, Stuttgart, 1984.
6. Agata, I., Kusakabe, H., Tsutomu, H., Nishibe, S. and Okuda, T., *Chem. Pharm. Bull*., 1993, **41**, 1608.
7. Zhang, H.-J. and Li, L.-N., *Planta Med*., 1994, **60**, 70.