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Bazzanins L–R, chlorinated macrocyclic bisbibenzyls from the liverwort *Lepidozia incurvata*

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Abstract

Seven new chlorinated bisbibenzyls (bazzanins L–R), of the isoplagiochin C type, as well as isoplagiochin C have been isolated from the liverwort *Lepidozia incurvata*. The structures have been elucidated based on extensive NMR spectral evidence and by mass spectrometry.

Keywords: *Lepidozia incurvata*; Hepaticae; Liverwort; Structure elucidation; Chlorinated bisbibenzyl

1. Introduction

The liverwort genus *Lepidozia*, mainly distributed in the tropics and subtropics, is a rich source of various types of secondary metabolites. So far mostly terpenoids have been isolated from this genus (Matsuo et al., 1979, 1981, 1984a, b; Shu et al., 1994; Toyota et al., 1996; Paul et al., 2001). The only previous chemical investigation of *L. incurvata* led to the isolation of the lignans epiphyllic acid and its derivatives 3-carboxy-6,7-dihydroxy-1-(3′,4′-dihydroxyphenyl)-naphthalene and 3-carboxy-6-methoxy-1-(3′,4′-dihydroxyphenyl)-naphthalene-7-*O*-α-L-rhamnopyranoside (Cullmann et al., 1999). Continuing our studies on the secondary metabolites of liverworts (Hertewich et al., 2003; Scher et al., 2003) we now report seven new chlorinated bisbibenzyls of the isoplagiochin C type (bazzanin L–R) from the methanolic extract of *L. incurvata*.

2. Results and discussion

The air-dried plant material was first extracted with ether and then with methanol. The ethyl acetate-soluble fraction of the methanol extract was chromatographed on Sephadex LH-20. The resulting fractions were subjected to vacuum liquid chromatography (VLC) and further purified by HPLC to yield bazzanins L-R (**2**–**8**), which derive from the known bisbibenzyl isoplagiochin C (**1**).

Compound **1** was obtained as a white powder. Its EI mass spectrum (m/z 422 $[M]^+$) suggested $C_{28}H_{22}O_4$ as molecular formula. The comparison of its ^1H and ^{13}C NMR data taken in CD_3OD with those in literature (Hashimoto et al., 1996) led to isoplagiochin C as structure of **1**. For comparison with the data of compounds **2**–**8** the NMR-data in $CDCl_3$ are given in Tables 1 and 2.

The ^1H and ^{13}C NMR spectra of **2** (see Tables 1 and 2) showed signals for two benzylic methylene groups (δ_H 2.41 and 2.91, H-7a and b; δ_H 2.56 and 3.03, H-8a and b; δ_C 34.3 and 35.5, C-7 and C-8), a *cis* double bond (δ_H 6.70 and 6.53, each *d*, $J = 11.9$ Hz, H-7′ and H-8′; δ_C 131.9 and 124.7, C-7′ and C-8′) and four benzene rings. So far, these data resembled those of isoplagiochin C (**1**). But in contrast to **1**, the compound contained a phenolic methyl ether group (δ_H 3.88, *s*, 3H; δ_C 56.5, *q*) and the substitution pattern of the two benzene rings had changed from two trisubstituted benzenes to one tetra- (δ_H 7.11 and 7.01, both *d*, both $J = 7.8$ Hz, H-13′ and H-14′) and one pentasubstituted (δ_H 7.18, *s*, H-13) benzene ring, respectively. The CI mass spectrum showed a collection of peaks which are typical for the isotope pattern of three chlorine atoms in the $[M+1]$ peaks (m/z 539:540:541:542:543:544:545:546; $\%_{found}$ 95:30:100:32:39:12:5:1; $\%_{calcd}$ 99:31:100:31:36:10:5:1 (Winter, 2001). Therefore the molecular formula of **2** could be established as $C_{29}H_{21}O_4Cl_3$. The position of the methyl ether and the three chlorine atoms followed directly from the HMBC correlations (Fig. 1) indicating

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Table 1
^{1}H NMR spectral data for compounds **1**–**8** ($CDCl_3$)

H	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
H-2	6.70 *d* (8.0)	6.87 *d* (8.2)	6.78 *d* (8.1)	–	6.89 *d* (8.2)	–	–	–
H-3	6.92 *dd* (8.0, 2.1)	7.12 *dd* (8.2, 2.2)	7.05 *dd* (8.1, 2.0)	7.20 *d* (2.1)	7.18 *dd* (8.2, 2.1)	7.21 *d* (2.1)	7.17 *d* (2.0)	7.36 *d* (2.0)
H-5	6.52 *d* (2.1)	6.45 *d* (2.2)	6.42 *d* (2.0)	6.33 *d* (2.1)	6.28 *d* (2.1)	6.27 *d* (2.1)	6.16 *d* (2.0)	6.16 *d* (2.0)
H-7	2.46-2.65 (2H)	2.41 *m*	2.40 *m*	2.38 *m*	2.57 *m*	2.38 *m*	2.46 *m*	2.79 *m*
		2.91 *m*	2.91 *m*	2.90 *m*	2.76 *m*	2.89 *m*	2.70 *m*	3.20 *m*
H-8	2.46-2.65 (2H)	2.56 *m*	2.56 *m*	2.55 *m*	2.76 *m*	2.51 *m*	2.62 *m*	3.05 *m*
		3.03 *m*	3.03 *m*	3.00 *m*	2.81 *m*	3.03 *m*	2.82 *m*	3.31 *m*
H-10	6.79 *d* (2.2)	–	–	–	–	–	–	–
H-12	6.72 *dd* (8.0, 2.2)	–	–	–	–	–	–	–
H-13	7.07 *d* (8.0)	7.18 *s*	7.18 *s*	7.12 *s*	7.18 *s*	7.17 *s*	7.13 *s*	7.12 *s*
H-3′	7.20 *d* (2.1)	6.96 *d* (2.2)	7.00 *d* (2.0)	6.93 *d* (2.0)	6.78 *d* (2.0)	6.83 *d* (2.0)	6.77 *d* (2.0)	6.25 *d* (2.0)
H-5′	7.08 *dd* (8.2, 2.1)	7.20 *dd* (8.2, 2.2)	7.19 *dd* (8.2/2.0)	7.22 *dd* (8.2/2.0)	7.33 *d* (2.0)	7.33 *d* (2.0)	7.30 *d* (2.0)	7.20 *d* (2.0)
H-6′	6.83 *d* (8.2)	6.95 *d* (8.2)	6.92 *d* (8.2)	6.97 *d* (8.2)	–	–	–	–
H-7′	6.49 *d* (12.0)	6.70 *d* (11.9)	6.71 *d* (11.9)	6.70 *d* (11.9)	6.76 *d* (11.9)	6.65 *d* (11.9)	6.72 *d* (11.9)	–
H-8′	6.59 *d* (12.0)	6.53 *d* (11.9)	6.56 *d* (11.9)	6.54 *d* (11.9)	6.43 *d* (11.9)	6.61 *d* (11.9)	6.42 *d* (11.9)	–
H-10′	6.83 *m*	–	–	–	–	–	–	–
H-13′	7.09 *dd* (8.0, 2.0)	7.11 *d* (7.8)	7.11 *d* (7.8)	7.10 *d* (7.8)	7.19 *s*	7.10 *d* (7.8)	7.16 *s*	7.14 *s*
H-14′	6.83 *m*	7.01 *d* (7.8)	7.01 *d* (7.8)	6.99 *d* (7.8)	–	6.96 *d* (7.8)	–	–
OCH_3	–	3.88 *s*	–	–	3.91 *s*	–	–	–

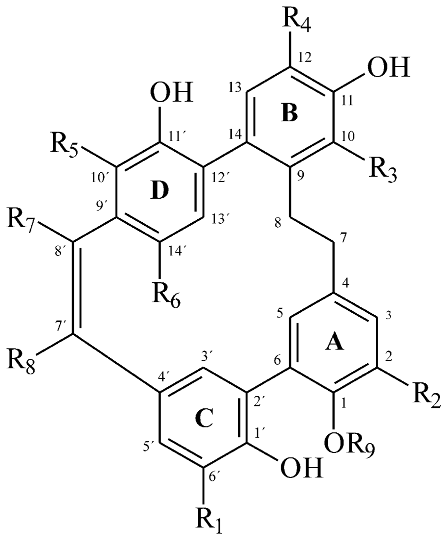
Table 2
^{13}C NMR spectral data for compounds **1**–**8** ($CDCl_3$)

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
C-1	150.4 *s*	153.0 *s*	150.7 *s*	144.7 *s*	153.0 *s*	145.7 *s*	146.4 *s*	146.4 *s*
C-2	115.6 *d*	111.4 *d*	116.8 *d*	–*	111.4 *d*	120.7 *s*	121.4 *s*	121.4 *s*
C-3	127.6 *d*	128.0 *d*	128.0 *d*	127.4 *d*	128.6 *d*	128.5 *d*	128.6 *d*	129.4 *d*
C-4	135.2 *s*	136.2 *s*	134.8 *s*	–*	136.1 *s*	136.1 *s*	135.9 *s*	–*
C-5	133.5 *d*	134.1 *d*	133.1 *d*	132.6 *d*	133.6 *d*	132.3 *d*	131.6 *d*	131.6 *d*
C-6	127.0 *s*	127.9 *s*	126.4 *s*	126.8 *s*	127.5 *s*	126.3 *s*	127.7 *s*	128.8 *s*
C-7	37.3 *t*	34.3 *t*	34.3 *t*	34.2 *t*	34.2 *t*	34.4 *t*	34.1 *t*	–*
C-8	37.8 *t*	35.5 *t*	35.6 *t*	–*	35.6 *t*	35.2 *t*	35.6 *t*	–*
C-9	143.9 *s*	139.3 *s*	139.5 *s*	139.0 *s*	139.0 *s*	139.1 *s*	138.9 *s*	138.9 *s*
C-10	115.9 *d*	121.3 *s*	121.4 *s*	–*	121.3 *s*	121.3 *s*	122.5 *s*	–*
C-11	156.5 *s*	147.5 *s*	147.8 *s*	147.6 *s*	147.8 *s*	147.7 *s*	148.3 *s*	147.9 *s*
C-12	113.2 *d*	118.2 *s*	118.3 *s*	118.3 *s*	118.3 *s*	118.4 *s*	118.8 *s*	118.4 *s*
C-13	131.8 *d*	129.2 *d*	129.2 *d*	129.4 *d*	129.1 *d*	129.5 *d*	129.2 *d*	129.3 *d*
C-14	127.5 *s*	130.0 *s*	129.9 *s*	130.0 *s*	129.5 *s*	129.8 *s*	129.3 *s*	128.8 *s*
C-1′	152.0 *s*	153.6 *s*	152.6 *s*	153.0 *s*	149.5 *s*	147.7 *s*	148.4 *s*	146.0 *s*
C-2′	125.5 *s*	125.5 *s*	125.8 *s*	124.6 *s*	128.6 *s*	125.9 *s*	126.9 *s*	126.7 *s*
C-3′	133.0 *d*	132.0 *d*	132.0 *d*	131.9 *d*	128.8 *d*	131.1 *d*	128.8 *d*	131.8 *d*
C-4′	128.3 *s*	128.6 *s*	129.2 *s*	129.3 *s*	129.9 *s*	129.9 *s*	130.5 *s*	–*
C-5′	130.2 *d*	131.0 *d*	130.5 *d*	131.3 *d*	130.2 *d*	130.7 *d*	130.3 *d*	128.3 *d*
C-6′	116.5 *d*	118.0 *d*	117.3 *d*	118.1 *d*	123.0 *s*	122.6 *s*	121.7 *s*	120.5 *s*
C-7′	129.8 *d*	131.9 *d*	131.3 *d*	131.4 *d*	132.7 *d*	130.5 *d*	132.1 *d*	125.6 *s*
C-8′	128.3 *d*	124.7 *d*	124.8 *d*	125.1 *d*	122.9 *d*	126.9 *d*	123.7 *d*	–*
C-9′	140.0 *s*	138.6 *s*	138.6 *s*	138.6 *s*	136.0 *s*	137.9 *s*	136.0 *s*	136.7 *s*
C-10′	115.0 *d*	119.8 *s*	119.8 *s*	119.9 *s*	120.4 *s*	119.8 *s*	124.7 *s*	–*
C-11′	153.6 *s*	149.2 *s*	149.5 *s*	149.3 *s*	148.2 *s*	149.4 *s*	148.7 *s*	147.9 *s*
C-12′	126.4 *s*	125.9 *s*	126.3 *s*	126.0 *s*	127.8 *s*	126.4 *s*	127.3 *s*	126.5 *s*
C-13′	131.4 *d*	129.2 *d*	129.2 *d*	129.3 *d*	130.1 *d*	129.5 *d*	130.2 *d*	129.7 *d*
C-14′	120.2 *d*	121.8 *d*	121.4 *d*	121.7 *d*	125.0 *s*	121.8 *d*	120.8 *s*	–*
OCH_3	–	56.5 *q*	–	–	56.5 *q*	–	–	–

*Not detected.

Assignments confirmed by 2D NMR HSQC, HMBC and NOESY.

that **2** is the 1-methyl ether of 10,12,10′-trichloroisoplagiochin C. Chlorinated bisbibenzyls possessing the isoplagiochin C skeleton have been found in *Bazzania trilobata* (Martini et al., 1998). Until now a total of eleven chlorinated bisbibenzyls, named bazzanins A–K, are known from this liverwort. In continuation we want to introduce the trivial name bazzanin L for the structure of compound **2**.



	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9
1	H	H	H	H	H	H	H	H	H
2	H	H	Cl	Cl	Cl	H	H	H	CH_3
3	H	H	Cl	Cl	Cl	H	H	H	H
4	H	Cl	Cl	Cl	Cl	H	H	H	H
5	Cl	H	Cl	Cl	Cl	Cl	H	H	CH_3
6	Cl	Cl	Cl	Cl	Cl	H	H	H	H
7	Cl	Cl	Cl	Cl	Cl	Cl	H	H	H
8	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	H

Compound **3**, named bazzanin M, was obtained as a pale yellow solid. The DCI mass spectrum was in agreement with the molecular formula $C_{28}H_{19}O_4Cl_3$ and showed the typical isotope pattern of three chlorine atoms. Its ^1H NMR and ^{13}C NMR spectra were very similar to those of **2** but lacked the signal for a phenolic methyl ether group at C-1. Therefore compound **3** was suggested to be 10,12,10′-trichloroisoplagiochin C which was confirmed by HSQC, HMBC and NOESY spectra.

The ^1H NMR spectrum of bazzanin N (**4**) was very close to that of **3** except for the resonance of H-2, which was missing, and that of H-3, which had changed from a *o,m*-coupled double doublet to a *meta*-coupled doublet. It was obvious that proton H-2 was replaced by a chlorine atom. This could be proven by the DCI mass spectrum (*m*/*z* 558 $[M]^+$), that showed the isotope pattern of four chlorine atoms and led to the molecular formula $C_{28}H_{18}O_4Cl_4$ and the structure of 2,10,12,10′-tetrachloroisoplagiochin C for compound **4**.

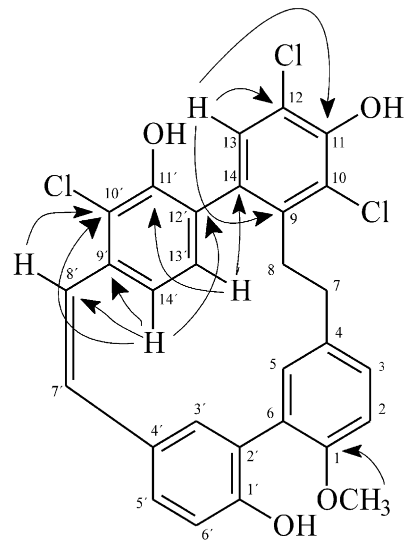


Fig. 1. Significant HMBC correlations of **2**.

Compound **5** was obtained as a yellow powder, whose molecular formula $C_{29}H_{18}O_4Cl_5$ was established by DCI mass spectrometry. The mass spectrum revealed the typical isotope pattern for five chlorine atoms in the $[M]^+$ peaks (*m*/*z* 606:607:608:609:610:611:612; $\%_{found}$ 57:23:100:40:64:21:24; $\%_{calc}$ 60:19:100:31:67:20:23). The ^1H and ^{13}C NMR data of **5** were very close to those of **2** especially for the bibenzyl subunit 1 (ring A and B) indicating that this part of the molecule bearing a methyl ether at C-1 and two chlorine atoms at C-10 and C-12 remained unchanged. Therefore the second bibenzyl subunit must contain the additional three chlorine atoms. Their positions followed by considerations of the ^1H NMR and the results of the 2D HMBC spectrum. Apart from the signals of a double bond (δ_H 6.76 and 6.43, both *d*, both $J = 11.9$ Hz, H-7′ and H-8′) the second bibenzyl subunit showed only resonances for one tetra- and one pentasubstituted benzene ring. The singlet at δ_H 7.19 belonged to H-13′ of ring D because it showed $^3J_{CH}$ couplings to C-9′, C-11′ and C-14. Therefore C-10′ (δ_c 120.4, *s*) and C-14′ (δ_c 125.0, *s*) must bear chlorine atoms. The fifth chlorine atom in the molecule must be positioned at C-6′ (δ_c 123.0, *s*) because the two remaining protons (δ_H 6.78 and 7.33, both *d*, both $J = 2.0$ Hz, H-3′ and H-5′) of ring C are *meta* to each other, which is only possible with an additional substituent at position C-6′. Therefore compound **5** was suggested to be the 1-methyl ether of 10,12,6′,10′14′-pentachloroisoplagiochin C, named bazzanin O.

Compound **6**, $C_{28}H_{17}O_4Cl_5$ (m/z 593 $[M+1]^+$), was also found to be a pentachlorinated isoplagiochin C derivative. Its ^1H NMR and ^{13}C NMR resonances for ring A, B and D resembled those of the tetrachlorinated bazzanin N (**4**), whereas the resonances for ring C fit very well with those of bazzanin O (**5**). Compound **6**, called bazzanin P, should therefore be 2,10,12,6′,10′-pentachloroisoplagiochin C, which was proven by 2D NMR H,H-COSY, HSQC and HMBC experiments.

The molecular formula of **7** was determined to $C_{28}H_{16}O_4Cl_6$ by DCI mass spectrometry (m/z 626 $[M]^+$). In the ^1H NMR spectrum the only difference to the pentachlorinated compound **6** was the lack of the signal for proton H-14′ and furthermore a reduction of the multiplicity of proton H-13′ (δ_H 7.16) from a doublet to a singlet indicating an additional chlorine atom at position C-14′. Therefore the structure of compound **7**, named bazzanin Q, is 2,10,12,6′,10′,14′-hexachloroisoplagiochin C, which was supported by careful analysis of the 2D NMR spectra.

Compound **8**, bazzanin R, represents the highest degree of chlorination within the bazzanins. The DCI mass spectrum (m/z 694 $[M]^+$) and the NMR spectral data agree with the molecular formula $C_{28}H_{14}O_4Cl_8$. The NMR spectral data of bazzanin R **8** are in good accordance with those of compound **7** but lack the resonances for the double bond protons H-7′ and H-8′. However, the double bond was still present as the UV spectrum showed a λ_{max} at 293 nm characteristic for a benzene ring conjugated with a double bond. The stereochemistry at the double bond C-7′-C-8′ is arbitrarily determined as *cis* because of the geometry of the compounds **1**–**7**. Therefore compound **8** was assigned to 2,10,12,6′,7′,8′,10′,14′-octachloroisoplagiochin C. Due to the low amount of the compound (1 mg) the signal to noise ratio was very low, so that the ^{13}C resonances given in Table 2 are incomplete.

Bisbibenzyls are not widespread in nature. Apart from one report dealing with fern constituents (Oiso et al., 1999) they have only been isolated from the Hepaticae (Asakawa et al., 2000) and were recognized as one of the most important chemical markers of this class (Asakawa, 1995). Bazzanin L-R represent the first bisbibenzyls from the genus *Lepidozia*. Martini et al. (1998) isolated the chlorinated bisbibenzyls A-K from the liverwort *Bazzania trilobata*. Even though *Bazzania* and *Lepidozia* belong to the same family, the occurence of chlorinated bisbibenzyls is no phenomenon limited to the Lepidoziaceae. Until now chlorinated derivatives of isoplagiochin C and D are also described for the liverworts *Herbertus sakuraii* (Hashimoto et al., 2000), *Plagiochila peculiaris* (Wu et al., 2001), *Plagiochila* species (Anton et al., 1997) and *Mastigophora diclados* (Hashimoto et al., 2000).

The first occurence of chlorinated bisbibenzyls in liverworts described by Martini et al. (1998) raised the question of their originality. It was considered that they might be artefacts originated from the non-chlorinated bisbibenzyls during chromatographic procedures using dichloromethane or chloroform. Hashimoto et al. (2000) could exclude this hypothesis by treatment of isoplagiochin C and D with chlorinated solvents and did not observe any chlorinated derivatives. In addition, Speicher et al. (2001) demonstrated that the chlorinated bazzanins A–K are genuine in the crude extract of *B. trilobata* using MALDI-TOF mass spectrometry. In order to clarify the biogenesis of these chlorometabolites Speicher et al. (2003) succeeded in the in vitro chlorination of isoplagiochin C with chloroperoxidase and were able to detect an enzyme of this type in *B. trilobata*.

An interesting feature of the isolated bazzanines is their optical activity. Although the structures of **1**–**8** have no chiral center, their specific optical rotation showed $[\alpha]_D$ between $+42.5°$ and $+225°$. The existence of atropisomeres is first discussed by Asakawa et al. (2000) and prompted us to perform detailed studies on the chirality of the isoplagiochin C framework which we will publish separately.

3. Experimental

3.1. General

CI-MS, DCI-MS data were recorded on a Finnigan MAT 90 spectrometer. The optical rotations were recorded on a Perkin Elmer 241 polarimeter in MeOH. IR spectra were measured on a Perkin Elmer 257 spectrometer with KBr pellets. UV spectra were recorded on a Perkin Elmer Lambda 2. ^1H (400 MHz) and ^{13}C NMR (100 MHz) data were measured on a Bruker AM 400. 2D NMR spectra were performed on a Bruker DRX 500. All NMR spectra were recorded in $CDCl_3$. Chemical shifts are given in δ values (ppm) relative to $CHCl_3$ at δ_H 7.24 or $CDCl_3$ at δ_C 77.0. Diol modified silica gel (LiChroprep Diol, 40–63 μm (Merck) and LiChroprep Diol, 15 μm (Merck) were used for vacuum liquid chromatography (VLC), respectively, while thin layer chromatography was performed on silica gel (Kieselgel 60 F_{254}, Merck), reversed phase C18 (HPTLC-Fertigplatten RP18, F_{254}, Merck), diol and cyano modified silica (HPTLC-Fertigplatten RP18, F_{254}, Merck).

3.2. Plant material

Lepidozia incurvata Lindenberg was collected in Costa Rica (near to Póas vulcano), 2550 m altitude, during September 1994. The plant was collected and identified by Mues and Gradstein. A voucher specimen is deposited at "Pharmakognosie und Analytische Phytochemie, Universität des Saarlandes", Saarbrücken, Germany.

3.3. Extraction and isolation

Air dried and powdered plant material (700 g) of *L. incurvata* was extracted with Et_2O to remove the non polar compounds, followed by MeOH. The evapd. methanolic extract was distributed between EtOAc and H_2O. The EtOAc phase was evapd. in vacuo (5.5 g) and chromatographed on Sephadex LH-20 using MeOH–CH_2Cl_2 (4:1) as eluent to yield 6 frs. Fr. 3 (200 mg) was chromatographed on diol-modified silica gel via VLC using a *n*-hexane–EtOAc gradient yielding 2 frs. Further seperation of fr. 3.2 by HPLC (Spherisorb CN, 5 µm, *n*-hexane–EtOAc, 85:15) afforded bazzanin L (**2**) (2 mg) and bazzanin O (**5**) (2 mg). Fr. 4 (220 mg) was chromatographed on diol-modified silica gel via VLC using a *n*-hexane–EtOAc gradient yielding 3 frs. (fr. 4.1-4.3). Separation of fr. 4.2 using HPLC (Spherisorb CN, 5 µm, *n*-hexane–EtOAc, 80:20), yielded bazzanin M (**3**) (2.0 mg) and bazzanin N (**4**) (1.0 mg). Further purification of fr. 4.1 and fr. 4.3 using HPLC (fr. 4.1: LiChrospher RP18 5 µm, MeOH:H_2O, 8:2; fr.4.1 and LiChrospher RP18 5 µm, MeOH–H_2O, 7:3), yielded bazzanin R (**8**) (1 mg) and isoplagiochin C (**1**) (2 mg). Fr. 5 (150 mg) was chromatographed on diol-modified silica gel via VLC using a *n*-hexane–EtOAc gradient yielding two fractions. Fr. 5.1 was separated using HPLC (Spherisorb CN, 5 µm, *n*-hexane–EtOAc, 80:20) yielding bazzanin P (**6**) (4 mg) and bazzanin Q (**7**) (25 mg).

3.3.1. Isoplagiochin C (*1*)

$[\alpha]_{D}^{20}$ +42.5 (MeOH; *c* 0.2). IR matched literature (Hashimoto et al., 1996). UV (MeOH) λ_{max} (nm): 250, 290; λ_{min}: 273. ^1H NMR: Table 1. ^{13}C NMR: Table 2. EIMS: *m*/*z* 422 $[M]^+$.

3.3.2. Bazzanin L or 1-methyl ether of 10,12,10″-trichloroisoplagiochin C (*2*)

$[\alpha]_{D}^{20}$ +126.5° (MeOH; *c* 0.2). IR ν (cm^{-1}): 3400, 2920, 1500, 1460, 1410, 1240. UV λ_{max} (nm) 250, 288; λ_{min} 268. ^1H NMR: Table 1. ^{13}C NMR: Table 2. DCIMS *m*/*z* (rel. Int.): 538 $[M]^+$, 538:539:540:541:542:543:544:545 (95:30:100:32:39:12:5:1).

3.3.3. Bazzanin M or 10,12,10′-trichloroisoplagiochin C (*3*)

$[\alpha]_{D}^{20}$ +95° (MeOH; *c* 0.2). IR ν (cm^{-1}): 3400, 2930, 1460, 1400, 1290. UV λ_{max} (nm) 247, 290; λ_{min} 272. ^1H NMR: Table 1. ^{13}C NMR: Table 2. DCIMS *m*/*z* (rel. Int.): 524 $[M]^+$, 524:525:526:527:528:529:530:531 (93:38:100:32:43:14:6:1).

3.3.4. Bazzanin N or 2,10,12,10′-tetrachloroisoplagiochin C (*4*)

$[\alpha]_{D}^{20}$ +90° (MeOH; *c* 0.1). IR ν (cm^{-1}): 3400, 2930, 1460, 1400, 1290. UV λ_{max} (nm) 250, 290; λ_{min} 273. ^1H NMR: Table 1. ^{13}C NMR: Table 2. DCIMS *m*/*z* (rel. Int.): 558 $[M]^+$, 558:559:560:561:562:563:564:565:566 (61:21:100:26:56:18:16:5:1).

3.3.5. Bazzanin O or 10,12,6′,10′14′-pentachloroisoplagiochin C (*5*)

$[\alpha]_{D}^{20}$ +54° (MeOH; *c* 0.2). IR ν (cm^{-1}): 3450, 2930, 1500, 1470, 1450, 1400, 1300. UV λ_{max} (nm) 244, 290; λ_{min} 272. ^1H NMR: Table 1. ^{13}C NMR: Table 2. DCIMS *m*/*z* (rel. Int.): 606 $[M]^+$, 606:607:608:609:610:611:612 (57:23:100:40:64:21:24).

3.3.6. Bazzanin P or 2,10,12,6′,10′-pentachloroisoplagiochin C (*6*)

$[\alpha]_{D}^{20}$ +225° (MeOH; *c* 0.7). IR ν (cm^{-1}): 3400, 2910, 1690, 1590, 1460, 1390, 1300. UV λ_{max} (nm) 252, 291; λ_{min} 277. ^1H NMR Table 1. ^{13}C NMR Table 2. CIMS *m*/*z* (rel. Int.): 593 $[M+1]^+$, 593:594:595:596:597:598:599 (58:19:100:31:61:19:23).

3.3.7. Bazzanin Q or 2,10,12,6′,10′,14′-hexachloroisoplagiochin C (*7*)

$[\alpha]_{D}^{20}$ +120° (MeOH; *c* 1.2). IR ν (cm^{-1}): 3450, 2920, 1480, 1450, 1410, 1300. UV λ_{max} nm 253, 293; λ_{min} 276. ^1H NMR: Table 1. ^{13}C NMR: Table 2. DCIMS *m*/*z*: 626 $[M]^+$, 626:628:630 (30:100:60).

3.3.8. Bazzanin R or 2,10,12,6′,7′,8′,10′,14′-octachloroisoplagiochin C (*8*)

$[\alpha]_{D}^{20}$ not measured. IR ν (cm^{-1}): 3450, 2950, 1720, 1460, 1400, 1300. UV λ_{max} (nm) 253, 293; λ_{min} 277. ^1H NMR: Table 1. ^{13}C NMR: Table 2. DCIMS *m*/*z* (rel. Int.): 694 $[M]^+$, 696:697:698:699: (90:29:100:31).

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