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Bonaspectins and neobonaspectins, first sesquilignans and sesquineolignans from a convolvulaceous species ☆

Britta Tofern [a], Kristina Jenett-Siems [a], Karsten Siems [b], Jasmin Jakupovic [b], Eckart Eich [a,*]

[a] *Institut für Pharmazie (Pharmazeutische Biologie), Freie Universität Berlin, Königin-Luise-Straße 2 + 4, D-14195 Berlin, Germany*
[b] *AnalytiCon AG, NL Potsdam, Hermannswerder Haus 17, D-14473 Potsdam, Germany*

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Abstract

Four new tetrahydrofuran-type sesquilignans, named bonaspectin A, bonaspectin B, bonaspectin C 4″-β-glucoside and bonaspectin D 4″-β-glucoside, as well as two new 8.O.4′-type sesquineolignans, named neobonaspectin A and B, were isolated from the aerial vegetative parts of *Bonamia spectabilis* (Convolvulaceae), together with the known compound *rel*-(7*S*,8*S*,7′*R*,8′*R*)-3,3′,4,4′,5,5′-hexamethoxy-7.O.7′,8.8′-lignan. Their structures were established on the basis of spectral data.

Keywords: *Bonamia spectabilis*; Convolvulaceae; Sesquilignans; Sesquineolignans; Lignan; Bonaspectin A; Bonaspectin B; Bonaspectin C 4″-β-glucoside; Bonaspectin D 4″-β-glucoside; Neobonaspectin A; Neobonaspectin B; *rel*-(7*S*,8*S*,7′*R*,8′*R*)-3,3′,4,4′,5,5′-Hexamethoxy-7.O.7′,8.8′-lignan

1. Introduction

There have been only a few reports on the occurrence of lignans in the large Convolvulaceae family, which comprises approximately 2000 species. The first lignan, pinoresinol dimethyl ether, was isolated from *Humbertia madagascariensis* LAM. in 1959 (Combes, Billet & Mentzer, 1959). Since then furofuran-type lignans were obtained from *Pharbitis nil* (L.) CHOISY (syn. *Ipomoea nil* (L.) ROTH) and *Cuscuta chinensis* LAM. (Hirai, Kojima, Koshimizu, Shinozaki & Takimoto, 1993; Hirai, Yamamuro, Koshimizu, Shinozaki & Takimoto, 1994; Yahara et al., 1994). The lignanolides (−)-arctigenin and (−)-trachelogenin, their glucosides arctiin and tracheloside as well as their corresponding 4-β-gentiobiosides were isolated from *Ipomoea cairica* SWEET (Trumm & Eich, 1989; Kayser, 1994). Lignanolides were also detected in *Ipomoea alba* L., *Jacquemontia corymbulosa* BENTH., *J. paniculata var. paniculata* (L.) HALL. f., *Merremia gemella* (BURM. f.) HALL. f. *ssp. gemella* and *Operculina codonantha* (BENTH.) HALL. f. (Henrici, 1996; Jenett-Siems, 1996; Tofern, 1999). Furthermore, tetrahydrofuran-type lignanamides were isolated from a convolvulaceous species, *J. paniculata var. paniculata* (Henrici, Kaloga & Eich, 1994).

The present study describes the isolation and structural characterization of four new sesquilignans (bonaspectin A and B, bonaspectin C 4″-β-glucoside, bonaspectin D 4″-β-glucoside), two new sesquineolignans (neobonaspectin A and B) and one known lignan from the vegetative aerial parts of *Bonamia spectabilis* (CHOISY) HALL. f., a climbing shrub occurring in Madagascar and in tropical East Africa.

2. Results and discussion

The compounds **1–7** were detected in the petrol

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* Corresponding author. Tel.: +49-30-8383724; fax: +49-30-8383729.

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Table 1
^{1}H-NMR data for compounds **2–7**[a] [400 MHz, $CDCl_3$, δ (ppm), *J* (Hz) in parentheses]

H	**2**	**3**	**4**	**5**	**6**	**7**[b]
2, 6	6.67, 2 H, s	6.56, 2 H, s	6.56, 2 H, s	6.58, 2 H, s	6.53, 2 H, s	6.46, 2 H, s
7/7a	4.52, 1 H, m	5.12, 1 H, d (8.5)	5.11, 1 H, d (8.5)	5.13, 1 H, d (8.6)	5.84, 1 H, d (3.3)	2.74, 1 H, m
7b	–	–	–	–	–	3.12, 1 H, dd (5.3, 13.5)
8	2.36, 1 H, m	2.27, 1 H, ddq (6.5, 8.5, 7.0)	2.26, 1 H, m	2.29, 1 H, ddq (6.5, 8.6, 7.0)	4.41, 1 H, m	4.37, 1 H, m
9	1.07, 3 H, d (6.4)	0.70, 3 H, d (7.0)	0.70, 3 H, d (6,9)	0.72, 3 H, d (7.0)	1.29, 3 H, d (6.5)	1.21, 3 H, d (5.6)[c]
2′, 6′	6.66, 2 H, s	6.74, 2 H, s	6.73, 2 H, s	6.79, 2 H, s	6.45, 2 H, s	6.45, 2 H, s
7′/7a′	4.52, 1 H, m	4.43, 1 H, d (9.3)	4.42, 1 H, d (8.9)	4.43, 1 H, d (9.1)	2.74, 1 H, dd (7.9, 13.5)	2.74, 1 H, m
7b′	–	–	–	–	3.11, 1 H, dd (5.3, 13.5)	3.12, 1 H, dd (5.3, 13.5)
8′	2.36, 1 H, m	1.82, 1 H, ddq (6.5, 9.3, 6.5)	1.81, 1 H, m	1.84, 1 H, ddq (6.5, 9.1, 6.4)	4.41, 1 H, m	4.37, 1 H, m
9′	1.08, 3 H, d (6.4)	1.12, 3 H, d (6.5)	1.12, 3 H, d (6.4)	1.15, 3 H, d (6.4)	1.21, 3 H, d (6.2)	1.23, 3 H, d (6.7)[c]
2″, 6″	6.47, 2 H, s	6.48, 2 H, s	6.54, 2 H, s	6.59, 2 H, s	6.56, 2 H, s	6.55, 2 H, s
7″/7a″	2.75, 1 H, m	2.75, 1 H, dd (7.8, 13.2)	2.79, 1 H, dd (7.0, 13.6)	4.79, d (2.5)	6.33, 1 H, dd (1.4, 15.7)	6.33, 1 H, dd (1.3, 15.7)
7b″	3.12, 1 H, m	3.12, 1 H, dd (5.4, 13.2)	3.10, 1 H, dd (5.3, 13.6)	–	–	–
8″	4.39, 1 H, m	4.41, 1 H, m	4.42, 1 H, m	4.35, dq (2.5, 6.4)	6.16, 1 H, m	6.16, 1 H, m
9″	1.23, 3 H, d (6.2)	1.24, 3 H, d (6.4)	1.26, 3 H, d (6.2)	1.15, 3 H, d (6.4)	1.88, 3 H, dd (1.4, 6.6)	1.87, 3 H, dd (1.3, 6.5)
1‴	–	–	4.53, 1 H, d (7.6)	4.55, 1 H, d (7.6)	–	–
2‴	–	–	3.68, 1 H, t (8.5)	3.69, 1 H, t (8.5)	–	–
3‴	–	–	3.59, 1 H, m	3.59, 1 H, t (8.5)	–	–
4‴	–	–	3.64, 1 H, m	3.67, 1 H, t (8.5)	–	–
5‴	–	–	3.38, 1 H, m	3.38, 1 H, m	–	–
6a‴, 6b‴	–	–	3.75–3.95, 2 H, m	3.77–3.95, 2 H, m	–	–
OCH_3	3.79–3.86, 24 H	3.80–3.85, 24 H	3.78–3.87, 21 H	3.80–3.90, 21 H	3.79–3.85, 21 H	3.76–3.84, 21 H
$\underline{CH_3}CO$	–	–	–	–	2.19, 3 H, s	–

[a] Compound **7** was measured at 500 MHz.
[b] 3, 5-OMe: 3.84, s, 4-OMe: 3.82, s, 3′, 5′-OMe: 3,78, s, 3″, 5″-OMe: 3,81, s. The assignment was based on HMQC, HMBC and NOE spetra.
[c] Signal assignment within a column may be interchanged.

Table 2
^{13}C-NMR data for compounds **1–7** [100.6 MHz, $CDCl_3$, δ (ppm)]

C	1	2	3	4	5	6	7
1	137.9[a]	137.8[a]	136.7	136.7	136.5[a]	n.d.[b]	134.9[a]
2, 6	103.4	103.6	104.2	104.2	104.2	104.1	106.7
3, 5	153.3	153.3	153.0	153.0	153.0	153.1[a]	152.9
4	137.5[a]	137.5[a]	137.1	137.2	137.5[a]	n.d.[b]	136.6[a]
7	87.4	87.6	83.4	83.4	83.4	76.9	43.7
8	44.3	44.3	46.1	46.1	46.1	80.1	79.8
9	13.1	13.1	14.9	14.9	15.0	14.6	19.8
1′	137.9[a]	136.4[a]	136.3	136.4	136.6[a]	n.d.[b]	134.4[a]
2′, 6′	103.4	103.5	103.7	103.7	103.6	106.7	106.6
3′, 5′	153.3	153.7	153.8	153.8	153.7	153.2[a]	153.4[a]
4′	137.5[a]	135.6[a]	135.7	135.5	137.1[a]	n.d.[b]	134.6[a]
7′	87.4	87.5	87.5	87.5	87.5	43.6	43.7
8′	44.3	44.3	47.8	47.9	48.1	79.7	79.7
9′	13.1	13.2	15.4	15.4	15.4	19.7	19.7
1″	–	134.8[a]	134.8	133.8	134.3[a]	n.d.[b]	133.5[a]
2″, 6″	–	106.6	106.6	106.8	103.2	103.1	103.1
3″, 5″	–	152.9	152.9	152.2	152.5	153.8[a]	153.8[a]
4″	–	135.5[a]	136.2	146.8	146.9	n.d.[b]	135.8[a]
7″	–	43.7	43.7	43.7	73.2	131.0	131.0
8″	–	79.8	79.8	79.4	82.2	125.2	125.2
9″	–	19.8	19.8	19.9	12.9	18.4	18.3
1‴	–	–	–	106.6	106.3	–	–
2‴	–	–	–	74.2	74.2	–	–
3‴	–	–	–	76.7	76.5	–	–
4‴	–	–	–	70.4	70.2	–	–
5‴	–	–	–	76.0	76.0	–	–
6‴	–	–	–	62.6	62.4	–	–
4,4′,4″-OCH_3	60.86	60.86	60.86	60.91	60.92	60.78	60.85
	–	–	60.90	–	–	–	–
3,3′,3″,5,5′,	56.10	56.05	56.08	56.08	56.13	56.05	56.05
5″-OCH_3	–	56.13	56.10	56.10	56.20	56.14	56.07
	–	56.18	56.13	56.34	56.42	–	56.11
$\underline{C}H_3CO$	–	–	–	–	–	21.3	–
$\overline{C}H_3CO$	–	–	–	–	–	170.2	–

[a] Signal assignment of chemical shifts with similar values within a column may be interchanged. In order to make the OCH_3-signals distinguishable, these data are listed with two decimals.
[b] n.d.: not determined.

ether and dichloromethane extracts by HPLC (characteristic UV spectra) and by TLC (orange colour with Dragendorff's reagent), both in greenhouse material and in material collected in the wild. Their structures were established on the basis of EIMS, ^{1}H-NMR, ^{13}C-NMR, DEPT, ^{1}H–^{1}H COSY, HETCOR, HMBC as well as NOE experiments. All molecular ion peaks, which could be obtained by EIMS, were confirmed by FAB mass spectrometry (positive mode), and their molecular formulae were determined by HRMS.

Compound **1** was characterized as *rel*-(7*S*,8*S*,7′*R*,8′*R*)-3,3′,4,4′,5,5′-hexamethoxy-7.O.7′,8.8′-lignan, an optically inactive tetrahydrofuran-type lignan previously isolated from *Aristolochia birostris* (Aristolochiaceae) and *Polyalthia bullata* (Annonaceae) (Conserva et al., 1990; Fun, Sivakumar, Yip, Othman & Said, 1996). Since both the Aristolochiaceae and the Annonaceae belong to the Magnoliopsida (subclass Magnoliidae), this is the first report of this compound in the Rosopsida (Sitte, Ziegler, Ehrendorfer & Bresinsky, 1998).

The EI mass spectrum of bonaspectin A (**2**) showed the $[M]^+$ at m/z 626, and the molecular formula was determined as $C_{35}H_{46}O_{10}$. The ^{1}H-NMR spectrum (Table 1) showed three aromatic signals at δ 6.47, 6.66 and 6.67 (2 H each, s) and eight methoxy groups between δ 3.79 and 3.86, belonging to three partially methoxylated aromatic systems. Five aliphatic methine, one methylene and three methyl groups pointed to three phenylpropanoid moieties. The ^{13}C-NMR signals at δ 56.05, 56.13 and 56.18 (Table 2) were attributed to three pairs of equivalent methoxy groups at positions 3/5, 3′/5′ and 3″/5″, while the signal at δ 60.86 was assigned to the remaining two methoxy groups attached to C-4 and C-4′ or C-4″, respectively. In the ^{1}H-NMR spectrum two methine groups at δ 2.36 (2 H, m, H-8, H-8′) coupled with two methine groups at δ 4.52 (2 H, m, H-7, H-7′) as well as with two nearly equivalent methyl groups at δ 1.07 (3 H, d, $J = 6.4$ Hz, H-9) and δ 1.08 (3 H, d, $J = 6.4$ Hz, H-9′) and were assigned to a substituted tetrahydrofuran moiety. The proton shifts as well as the corresponding shifts in the ^{13}C-NMR spectrum (Table 2) are characteristic for *trans*-oriented aryl/methyl and *cis*-oriented methyl/methyl substituents at the tetrahydrofuran ring (Fig. 1) (Sarkanen & Wallis, 1973; Fonseca et al., 1979).

The proton of an oxygenated methine group at δ 4.39 (H-8″) coupled with the protons of a methylene and a methyl group in the ^{1}H-NMR spectrum (Table 1). These groups belonged to the third phenylpropanoid moiety, which was thus linked via an oxygen bridge to C-4′. The structure of this sesquilignan was supported by the fragmentation pattern observed in the EI mass spectrum (Pelter et al., 1966) (Fig. 2).

The EI and HR mass spectra of bonaspectin B (**3**) were similar to those of **2**. However, the ^{1}H-NMR shifts for the tetrahydrofuran moiety as well as the corresponding ^{13}C-NMR shifts were quite different (Tables 1 and 2). These shifts are characteristic for a tetrahydrofuran ring with a *cis*-configuration of one aryl and methyl substituent as well as a *trans*-configuration of the other aryl and methyl group, whereas the two methyl groups are *trans*-oriented to each other (Fig. 1) (Sarkanen & Wallis, 1973; Fonseca et al., 1979). The ^{1}H and ^{13}C-NMR data of the third phenylpropanoid moiety were in agreement with those of **2**.

Due to the asymmetric stereochemistry of the tetrahydrofuran substituents it had to be determined which of the two aryl substituents showed a *cis*-orientation to the neighbouring methyl group. This was deduced from the HMBC spectrum and the NOE experiments. The HMBC correlations between the H-7a″/b″ signals at δ 2.75/δ 3.12 and the signals at δ 106.6 and δ 134.8 led to the assignment of C-2″/6″ and C-1″. Five ad-

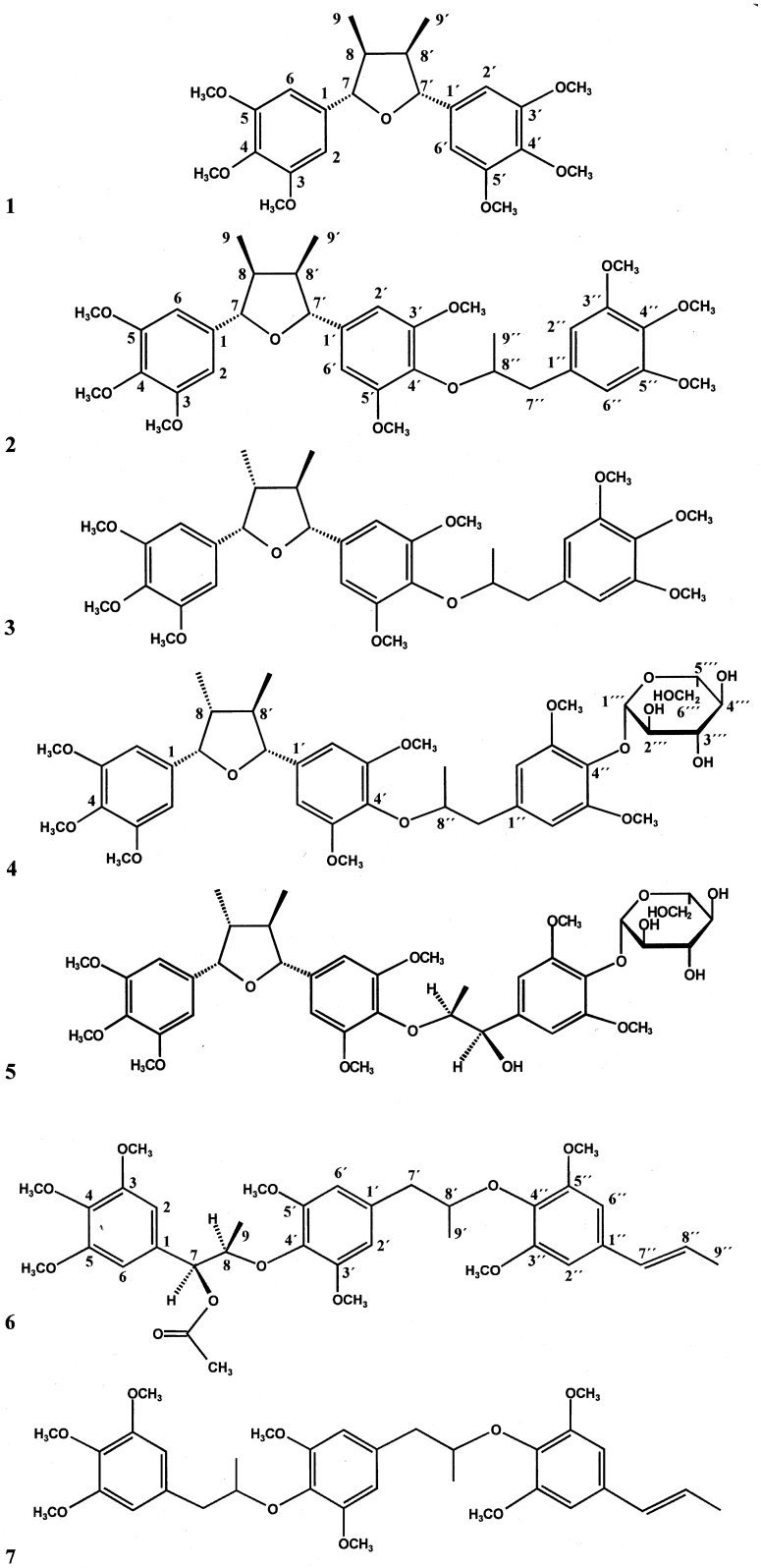

Fig. 1. Compounds isolated from *Bonamia spectabilis*.

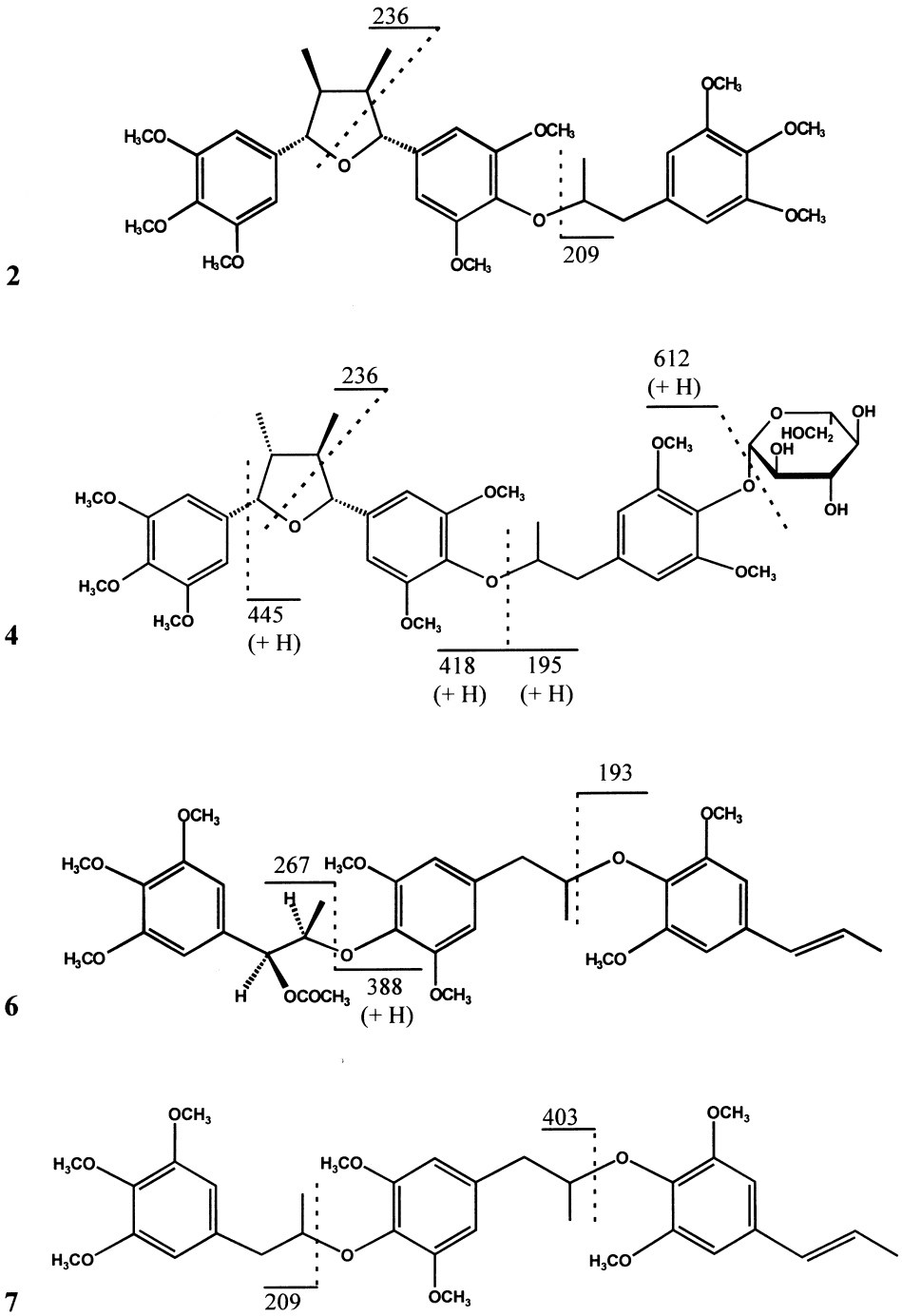

Fig. 2. EIMS fragmentation pattern of compounds **2**, **4**, **6** and **7**.

ditional singlets for C-1, C-1′, C-4, C-4′ and C-4″, respectively, appeared between 134 and 138 ppm in the ^{13}C-NMR spectrum. Two of them, viz at δ 137.1 and δ 136.2 correlated to the methoxy protons at δ 3.80–3.85 in the ^{1}H-NMR spectrum. Furthermore, a correlation between δ 136.2 and δ 6.48 (H-2″, H-6″) allowed the assignment of C-4 and C-4″. The singlets at δ 136.7 (C-1) and δ 136.3 (C-1′) could be assigned by correlations to the tetrahydrofuran proton signals. Thus, the remaining singlet at δ 135.7 could be attributed to C-4′. Starting from C-4 and C-4′ the aromatic singlets at δ 6.56 (H-2, H-6) and at δ 6.74 (H-2′, H-6′) could be assigned.

On irradiation of the signal at δ 6.56 (H-2, H-6) NOE enhancements of each of the signals for H-7 (δ 5.12), H-9 (high field methyl group at δ 0.70) and H-8′ (δ 1.82) could be observed (Table 3). Irradiation at δ 6.74 (H-2′, H-6′) also resulted in an NOE enhance-

Table 3
NOE study of compound **3** [400 MHz, $CDCl_3$, δ (ppm)]

Irradiated proton	Observed NOE
H-2, H-6	H-7, H-9, H-2′, H-6′, H-8′
H-7	H-2, H-6, H-8, H-8″
H-8	H-7, H-7′, H-9′
H-9	H-2, H-6, H-8′, H-9′
H-2′, H-6′	H-2, H-6, H-7′, H-8′, H-9′
H-7′	H-7, H-8, H-2′, H-6′
H-8′	H-2, H-6, H-8, H-9, H-2′, H-6′
H-9′	H-8, H-9, H-2′, H-6′, H-7′
H-2″, H-6″	H-7a″, H-7b″, H-8″
H-7a″	H-2″, H-6″
H-7b″	H-2″, H-6″
H-8″	H-2″, H-6″
H-9″	H-2″, H-6″, H-7a″

ment at δ 1.82 (H-8′). Therefore, the two aromatic substituents and the methyl group at δ 0.70 (H-9) are located on the same side of the tetrahydrofuran ring, while the second methyl group (H-9′) is situated on the opposite side (Fig. 1). This stereochemistry was confirmed by NOE interactions between H-9′ and H-7′ as well as between H-9′ and H-8.

The EI mass spectrum of bonaspectin C 4″-β-glucoside (**4**) exhibited an ion peak at *m/z* 612, and its formula was determined as $C_{34}H_{44}O_{10}$. A quasi-molecular ion peak was observed in the FABMS (negative mode) spectrum at *m/z* 773 $[M - H]^-$. The FABMS (positive mode) spectrum showed characteristic peaks at *m/z* 797 $[M + Na]^+$ and *m/z* 612 $[M - 162]^+$, indicating the presence of a hexose moiety. After acid hydrolysis glucose could be detected by TLC. Except for the observation of the signals for glucose and for only seven methoxy groups, the ^{1}H-NMR as well as the ^{13}C-NMR data closely resembled those of **3**. A *cis-trans-trans*-configuration of the tetrahydrofuran moiety could be assumed (Fig. 1).

The signals of the sugar carbons in the ^{13}C-NMR spectrum confirmed the presence of a glucopyranose moiety (Markham & Chari, 1982). The H-1‴/H-2‴ coupling constant (7.6 Hz) observed in the ^{1}H-NMR spectrum of **4** was indicative of an axial anomeric glucose proton (Markham & Geiger, 1993). Finally, it was obvious from the EI mass fragmentation pattern that the β-glucose moiety was attached to a hydroxy group at C-4″ and not at C-4 (Fig. 2), since otherwise the strong peak at *m/z* 236 would not be possible (Pelter et al., 1966).

The EIMS of bonaspectin D 4″-β-glucoside (**5**) exhibited a characteristic peak at *m/z* 628, and its formula was determined as $C_{34}H_{44}O_{11}$. The FABMS (positive mode) spectrum showed characteristic peaks at *m/z* 813 $[M + Na]^+$ and *m/z* 628 $[M - 162]^+$, indicating the presence of a hexose moiety, which was identified by TLC as glucose, again. The ^{1}H-NMR spectra of **4** and **5** (Table 1) were very similar. However, the spectrum of **5** lacked the signals of a methylene group (**4**: δ 2.79 and δ 3.10) and instead showed the signal of one additional methine group at δ 4.79 (1 H, d, $J = 2.5$ Hz). Since the proton of this methine group coupled with the proton of the signal at δ 4.35 (1 H, dq, $J = 2.5$, 6.4 Hz, H-8″), it was assigned to H-7″.

A comparison of the chemical shifts of H-7″, H-8″, C-7″, C-8″ and C-9″ (methyl group) with published data of related 8.O.4′-type neolignans proved to be helpful for the determination of the relative configuration of these methine groups. The *erythro*-orientation of the ether and the hydroxy group and the *threo*-configuration of these groups can be distinguished by their different chemical shift ranges: methine group bearing the hydroxy group (*erythro*: δ_H 4.75–4.85, δ_C 72.6–73.3; *threo*: δ_H 4.60–4.66, δ_C 77.5–78.7), methine group bearing the ether group (*erythro*: δ_H 4.33–4.38, δ_C 81.8–82.6; *threo*: δ_H 4.03–4.18, δ_C 82.8–86.1), methyl group (*erythro*: δ_C 12.6–13.7; *threo*: δ_C 16.2–17.3) (Braga et al., 1984; Zacchino & Badano, 1985, 1988; Zacchino, 1994). The chemical shifts of H-7″ and H-8″ as well as the shifts of C-7″, C-8″ and C-9″ of **5** were in good agreement with an *erythro*-orientation of the hydroxy and the ether group (Fig. 1). The small coupling constant ($J_{7'',8''} = 2.5$ Hz) was also in accordance with the *erythro*-configuration (Lit: $J = 2.7$–4.4 Hz, *erythro*-isomer; $J = 8.0$–8.6 Hz, *threo*-configuration) (Barata, Baker, Gottlieb & Ruveda, 1978; Braga et al., 1984; Hattori, Hada, Shu, Kakiuchi & Namba, 1987).

The missing bathochromic shift of band I (270 nm) in the UV spectrum upon addition of sodium methylate solution indicated glucosylation of the phenolic hydroxy group (Ayres & Loike, 1990). A compound with a structure related to the aglycone of bonaspectin D 4″-β-glucoside, viz saucerneol, was isolated from *Saururus cernuus* (Saururaceae) (Rao & Alvarez, 1983).

The EI mass spectrum of neobonaspectin A (**6**) showed the $[M]^+$ at *m/z* 654, corresponding to a molecular formula of $C_{36}H_{46}O_{11}$ as well as a characteristic ion at *m/z* 594 with a formula of $C_{34}H_{42}O_9$, indicating the loss of acetic acid. The ^{1}H-NMR spectrum showed the signals for three symmetrically substituted phenylpropanoid moieties, bearing seven methoxy groups, and, in accordance with the EIMS, an acetate moiety (δ 2.19, 3 H, s). The chemical shift of the methine group at δ 5.84 (1 H, d, $J = 3.3$ Hz) indicated the position of the ester group. Starting with this signal the sequence was established by the ^{1}H–^{1}H COSY spectrum (Table 1). In the second phenylpropanoid moiety one oxygenated methine coupled with a methylene group and with a methyl group. In the third phenylpropanoid moiety two olefinic protons at δ 6.33 (1 H, dd, $J = 1.4$, 15.7 Hz) and δ 6.16 (1 H, m) coupled with a methyl group at δ 1.88 (3 H, dd, $J = 1.4$, 6.6 Hz).

From the coupling constant $J = 15.7$ Hz a *trans*-orientation of the olefinic protons could be deduced. The linkage of the phenylpropanoid moieties was deduced from the EI mass fragmentation pattern (Fig. 2), which showed strong characteristic peaks at m/z 193, 267 and 388. Therefore, the phenylpropanoid containing the methylene group had to be linked to both, to the moiety with the olefinic group and to the acetylated phenylpropanoid by oxygen bridges. Finally the relative configurations of C-7 and C-8 had to be determined. As in compound **5**, the small coupling constant $J_{7,8} = 3.3$ Hz indicated an *erythro*-orientation of the acetylated hydroxy group at C-7 and the ether group at C-8 (Fig. 1).

The EI mass spectrum of neobonaspectin B (**7**) exhibited a molecular ion peak at m/z 596, corresponding to a molecular formula of $C_{34}H_{44}O_9$. In comparison to neobonaspectin A (**6**) the ^{1}H-NMR spectrum of **7** lacked the signals of an acetylated methine group, but showed the signals of two methylene groups (δ 2.74, 2 H, m and δ 3.12, 2 H, dd, $J = 5.3$, 13.5 Hz) instead. Since the ^{1}H-NMR and the ^{13}C-NMR data (Tables 1 and 2) otherwise closely resembled those of **6**, neobonaspectin B (**7**) was assigned the structure as depicted in Fig. 1.

The tetrahydrofuran-type sesquilignans **2–5** as well as the 8.O.4′-type sesquineolignans **6** and **7** have been described neither as natural nor as synthetic products so far, and this is also the first report on the occurrence of sesquilignans and sesquineolignans in the Convolvulaceae in general. To date, the presence of such compounds in *Bonamia spectabilis* can be considered as unique in this family, since they could not be detected in numerous species of the genera *Argyreia*, *Calystegia*, *Convolvulus*, *Falkia*, *Hewittia*, *Ipomoea*, *Merremia*, *Operculina* and *Turbina* (Kayser, 1994; Henrici, 1996; Mann, 1997; Tofern, 1999). While sesquilignans occur erratically in 15 plant families from the Gymnospermae to the Rosopsida, the presence of sesquineolignans seems to be even more restricted, since there have been only a few reports from the Illiciaceae (3 species), the Salicaceae (2 species), the Buddlejaceae (1 species), the Rosaceae (1 species), the Ranunculaceae (1 species) and the Pinaceae (1 species) so far (Miki, Sasaya & Sakakibara, 1979; Houghton, 1985; Kouno, Hashimoto, Kawano & Yang, 1989; Yoshinari, Shimazaki, Sashida & Mimaki, 1990; Hashimoto, Ozawa & Sasaya, 1993; Yoshikawa, Kinoshita & Arihara, 1997).

3. Experimental

3.1. Plant material

Aerial vegetative parts of *Bonamia spectabilis* were obtained from plants cultivated in the greenhouse of the Institut für Pharmazie (Pharmazeutische Biologie), Freie Universität Berlin, Germany, where a voucher specimen is deposited. The plants were grown from seeds collected about 70 km east of Morondava/Madagascar. Aerial vegetative parts were also collected in the wild. The species was identified from voucher specimens by Prof. Daniel F. Austin, Ph. D., Florida Atlantic University, Boca Raton, FL, USA.

3.2. Analytical TLC and HPLC

Analytical TLC was carried out on silica gel 60 F_{254} with cyclohexane–$CHCl_3$–MeOH (50 : 45 : 5), Et_2O–petrol (3 : 1) or $CHCl_3$–MeOH (8 : 2) as solvent systems (systems I, II, and III; system II: developed twice). The TLC sheets were checked for spots quenching UV light at 254 nm and were then sprayed with Dragendorff's reagent, causing an orange colour of those spots.

Analytical HPLC was carried out on an Eurospher RP 18 column (7 μm, 4 × 250 mm) with one of the following gradients: MeOH-aq. 0.5% H_3PO_4 (70 : 30 to 95 : 5 in 20 min) (I) or MeOH-aq. 0.5% H_3PO_4 (20 : 80 to 80 : 20 in 40 min) (II). Flow rate: 1 ml min^{-1}. Detection: diode-array-detector.

3.3. Spectroscopic methods

EIMS, HRMS and FABMS spectra were obtained using Varian MAT CH_7A, Finnigan MAT 711 and Finnigan MAT CH_5DF spectrometers, respectively. HMBC spectra were recorded in $CDCl_3$ on a Bruker DRX 500 spectrometer, NOE spectra on a Bruker AC 400 spectrometer and all other ^{1}H-NMR and ^{13}C-NMR spectra on a Bruker DPX 400 spectrometer. TMS was used as internal standard.

3.4. Extraction and isolation

Ground dried aerial vegetative parts (77 g from the greenhouse) were extracted with MeOH at room temperature (4 × 3 h each). After evaporation of the extract the residue was dissolved in 1% aq. tartaric acid and extracted with petrol and CH_2Cl_2 successively. Ground dried aerial vegetative parts (44 g collected on Madagascar) were extracted in the same way. Each extract was evaporated in vacuo and examined by TLC. The extracts of the greenhouse material and of the material collected on Madagascar showed the same results on TLC; therefore, the respective extracts obtained with petrol as well as the extracts obtained with CH_2Cl_2 were combined. The extract obtained with petrol was chromatographed on a silica gel column (45 × 3 cm), using a cyclohexane–$CHCl_3$ gradient (75 : 25 → 50 : 50), followed by cyclohexane–

$CHCl_3$–MeOH (50 : 45 : 5). The combined fractions containing a mixture of compounds **1–3**, **6** and **7** were evaporated in vacuo and dissolved in CH_3CN. After removing the unsoluble chlorophyll the compounds were separated by prep. TLC on silica gel using either solvent system I or II. **4** and **5** were isolated from the extract obtained with CH_2Cl_2 by chromatography on a silica gel column (60 × 2 cm), using a $CHCl_3$–MeOH gradient (9 : 1 → 8 : 2) as eluent. The final purification of all compounds was achieved by prep. HPLC [Eurospher RP 18, 7 μm, 16 × 250 mm, **1**: MeOH-aq. 0.5% H_3PO_4 (95 : 5), **2**, **3**, **6**, **7**: MeOH-aq. 0.5% H_3PO_4 (80 : 20), **4**, **5**: MeOH-aq. 0.5% H_3PO_4 (65 : 35), flow rate 5 ml min^{-1}, UV detection at 225 nm].

3.5. Acid hydrolysis

1 mg of compounds **4** and **5**, respectively, was dissolved in 0.5 ml MeOH and refluxed with 1 ml 3% aq. HCl for 40 min. After evaporation of MeOH and extraction with EtOAc 100 mg Ag_2CO_3 were added to the aq. layer; the mixture was filtered and the solution was evaporated in vacuo. Glucose was identified by TLC [silica gel, EtOAc–MeOH–HAc–H_2O (60 : 15 : 15 : 10)] and co-chromatography with reference compounds.

3.6. rel-(7S,8S,7′R,8′R)-3,3′,4,4′,5,5′-Hexamethoxy-7.O.7′,8.8′-lignan (*1*)

Oil, 12 mg. R_f 0.49 (II). R_t 1002 s (I). UV λ_{max}^{MeOH} nm: 217, 238 (sh), 271. EIMS 70 eV, *m/z* (rel. int.): 432 $[M]^+$ (40), 236 (100), 221 (49), 205 (53), 195 (21). (+)-FABMS *m/z*: 432 $[M]^+$. HRMS 80 eV, *m/z*: 432.21494 ($C_{24}H_{32}O_7$, calc. 432.21481), 236.14101 ($C_{14}H_{20}O_3$, calc. 236.14125), 221.11797 ($C_{13}H_{17}O_3$, calc. 221.11777), 205.12289 ($C_{13}H_{17}O_2$, calc. 205.12286), 195.06578 ($C_{10}H_{11}O_4$, calc. 195.06574). ^{1}H-NMR data were in good agreement with lit. values (Sarkanen & Wallis, 1973; Conserva et al., 1990). ^{13}C-NMR (Table 2).

3.7. Bonaspectin A (*2*)

Oil, 5 mg. R_f 0.27 (II). R_t 996 s (I). UV λ_{max}^{MeOH} nm: 210, 236 (sh), 270. EIMS 70 eV, *m/z* (rel. int.): 626 $[M]^+$ (8), 418 (7), 236 (7), 209 (100). (+)-FABMS *m/z*: 626 $[M]^+$. HRMS 80 eV, *m/z*: 626.30919 ($C_{35}H_{46}O_{10}$, calc. 626.30910), 418.19906 ($C_{23}H_{30}O_7$, calc. 418.19916), 236.14119 ($C_{14}H_{20}O_3$, calc. 236.14125), 222.12546 ($C_{13}H_{18}O_3$, calc. 222.12560), 209.11792 ($C_{12}H_{17}O_3$, calc. 209.11777). ^{1}H-NMR (Table 1). ^{13}C-NMR (Table 2).

3.8. Bonaspectin B (*3*)

Oil, 5 mg. R_f 0.25 (II). R_t 876 s (I). $[\alpha]_D^{20} + 11.5°$ ($c = 0.2$, $CHCl_3$). UV λ_{max}^{MeOH} nm: 210, 236 (sh), 270. EIMS 70 eV, *m/z* (rel. int.): 626 $[M]^+$ (8), 418 (5), 236 (5), 209 (100). (+)-FABMS *m/z*: 626 $[M]^+$. HRMS 80 eV, *m/z*: 626.30945 ($C_{35}H_{46}O_{10}$, calc. 626.30905), 418.19989 ($C_{23}H_{30}O_7$, calc. 418.19919), 209.11730 ($C_{12}H_{17}O_3$, calc. 209.11735). ^{1}H-NMR (Table 1). ^{13}C-NMR (Table 2).

3.9. Bonaspectin C 4″-β-glucoside (*4*)

Oil, 14 mg. R_f 0.50 (III). R_t 2334 s (II). $[\alpha]_D^{20} + 12.4°$ ($c = 0.23$, $CHCl_3$). UV λ_{max}^{MeOH} nm: 217, 239 (sh), 271; +NaOMe (6% in MeOH): 221, 239, 267. EIMS 70 eV, *m/z* (rel. int.): 612 $[M - 162]^+$ (48), 445 (4), 418 (43), 236 (43), 222 (74), 195 (100), 194 (50). (+)-FABMS *m/z*: 797 $[M + Na]^+$, 612 $[M - 162]^+$. (−)-FABMS *m/z*: 773 $[M - H]^-$. HRMS 80 eV, *m/z*: 612.29305 ($C_{34}H_{44}O_{10}$, calc. 612.29345), 445.22185 ($C_{25}H_{33}O_7$, calc. 445.22263), 418.19924 ($C_{23}H_{30}O_7$, calc. 418.19915), 236.14119 ($C_{14}H_{20}O_3$, calc. 236.14125), 222.12557 ($C_{13}H_{18}O_3$, calc. 222.12560), 195.10206 ($C_{11}H_{15}O_3$, calc. 195.10206), 194.09427 ($C_{11}H_{14}O_3$, calc. 194.09426). ^{1}H-NMR (Table 1). ^{13}C-NMR (Table 2).

3.10. Bonaspectin D 4″-β-glucoside (*5*)

Oil, 30 mg. R_f 0.48 (III). R_t 2190 s (II). $[\alpha]_D^{20} + 15.3°$ ($c = 0.3$, $CHCl_3$). UV λ_{max}^{MeOH} nm: 217, 238 (sh), 270; +NaOMe (6% in MeOH): 222, 238 (sh), 265. EIMS 70 eV, *m/z* (rel. int.): 628 $[M - 162]^+$ (4), 418 (80), 236 (59), 222 (93), 194 (100). (+)-FABMS *m/z*: 813 $[M + Na]^+$, 628 $[M - 162]^+$. HRMS 80 eV, *m/z*: 628.28828 ($C_{34}H_{44}O_{11}$, calc. 628.28837), 418.19915 ($C_{23}H_{30}O_7$, calc. 418.19916), 236.14130 ($C_{14}H_{20}O_3$, calc. 236.14125), 222.12546 ($C_{13}H_{18}O_3$, calc. 222.12560), 194.09427 ($C_{11}H_{14}O_3$, calc. 194.09430). ^{1}H-NMR (Table 1). ^{13}C-NMR (Table 2).

3.11. Neobonaspectin A (*6*)

Oil, 7 mg. R_f 0.31 (II). R_t 1068 s (I). $[\alpha]_D^{20} + 5.3°$ ($c = 0.23$, $CHCl_3$). UV λ_{max}^{MeOH} nm: 210, 267. EIMS 70 eV, *m/z* (rel. int.): 654 $[M]^+$ (4), 594 $[M - HAc]^+$ (1), 388 (3), 267 (55), 195 (100), 193 (50). (+)-FABMS *m/z*: 654 $[M]^+$. HRMS 80 eV, *m/z*: 654.30412 ($C_{36}H_{46}O_{11}$, calc. 654.30402), 594.28325 ($C_{34}H_{42}O_9$, calc. 594.28289), 388.18871 ($C_{22}H_{28}O_6$, calc. 388.18859), 267.12296 ($C_{14}H_{19}O_5$, calc. 267.12325), 195.10206 ($C_{11}H_{15}O_3$, calc. 195.10206), 193.08668 ($C_{11}H_{13}O_3$, calc. 193.08647). ^{1}H-NMR (Table 1). ^{13}C-NMR (Table 2).

3.12. Neobonaspectin B (7)

Oil. 4 mg. R_f 0.45 (II). R_t 528 s (I). UV λ_{max}^{MeOH} nm: 210, 235 (sh), 270. EIMS 70 eV, *m/z* (rel. int.): 596 $[M]^+$ (6), 403 (2), 221 (2), 209 (100), 195 (21), 194 (11). (+)-FABMS *m/z*: 596 $[M]^+$. HRMS 80 eV, *m/z*: 596.29814 ($C_{34}H_{44}O_9$, calc. 596.29854), 403.21211 ($C_{23}H_{31}O_6$, calc. 403.21207), 221.11786 ($C_{13}H_{17}O_3$, calc. 221.11777), 209.11772 ($C_{12}H_{17}O_3$, calc. 209.11777), 195.10244 ($C_{11}H_{15}O_3$, calc. 195.10212), 194.09466 ($C_{11}H_{14}O_3$, calc. 194.09430). ^{1}H-NMR (Table 1). ^{13}C-NMR (Table 2).

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