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Natural Sciences: Report

One triterpenoid and five phenolic compounds from the liverwort *Bazzania sp.*

Nguyen Xuan Khanh[1], Tran Quoc Tan[2], Nguyen Nhat Nam[1], Nguyen Thi Bich Tram[1], Nguyen Hoang Cam[1], Phan Thanh Tung[1], Le Hoang Khang[1], Nguyen Kim Phi Phung[1], Duong Thuc Huy[3], Huynh Ngoc Vinh[1,*]

ABSTRACT

Introduction: The *Bazzania genus*, a type of liverwort known as a low-level herbaceous organism, comprises approximately 250 species. Only 8 species have been chemically and biologically studied worldwide, and none have been studied in Vietnam. This paper described the isolation and structure elucidation of six compounds isolated from the liverwort *Bazzania sp.* collected in Lac Duong district, Lam Dong Province, Vietnam, in July 2020. **Methods:** Phytochemical investigations of the n-hexane and chloroform extracts of *Bazzania sp.* led to the isolation of six pure compounds. Their chemical structures were elucidated by HR-ESI-MS, 1D and 2D-NMR spectroscopic analysis and comparison with previously published data. **Results:** From the studied liverwort *Bazzania sp.*, six compounds, namely, cycloartenol (**1**) and five phenolic derivatives, p-coumaric acid (**2**), p-hydroxybenzoic acid (**3**), methyl 2,4-dihydroxy-3,6-dimethylbenzoate (**4**), barbatic acid (**5**), and methyl 2-hydroxy-4-methoxy-6-tetradecylbenzoate (**6**), were isolated and structurally elucidated. **Conclusions:** Although these compounds are known in other species, this is the first time they have been reported in *Bazzania sp.*

Key words: Bazzania sp., phenolic derivatives, triterpenoid

[1]*Department of Organic Chemistry, University of Science, National University-Ho Chi Minh City, Vietnam*

[2]*Department of Plant Biotechnology and Biotransformation, University of Science, National University-Ho Chi Minh City, Vietnam*

[3]*Department of Chemistry, Ho Chi Minh City University of Education, Ho Chi Minh City, Vietnam*

Correspondence

Huynh Ngoc Vinh, Department of Organic Chemistry, University of Science, National University-Ho Chi Minh City, Vietnam

Email: hnvinh@hcmus.edu.vn

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INTRODUCTION

Liverworts, the species of the division Marchantiophita, belong to Bryophytes, which are known as nonvascular plants. They are mostly spread in high moisture areas, humid tropical forests, and the banks of rivers and streams.[1] The *Bazzania* genus, liverwort, comprises approximately 250 species.[2,3] In the world, only a few of them, 8 species, have been chemically and biologically studied, and none have been studied in Vietnam.[4–18] Previous chemical studies on the eight mentioned species showed that they contained sesquiterpenes, diterpenes, and lignans.[4–18] Some possess cytotoxic[14] and nitric oxide inhibitory activity.[17] Herein, we report the investigation of the n-hexane and chloroform extracts of *Bazzania sp.* collected in Lac Duong district, Lam Dong province, Vietnam. We isolated six compounds, including a triterpenoid compound, cycloartenol (1), and five phenolic derivatives, p-coumaric acid (2), p-hydroxybenzoic acid (3), methyl 2,4-dihydroxy-3,6-dimethylbenzoate (4), barbatic acid (5), and methyl 2-hydroxy-4-methoxy-6-tetradecylbenzoate (6). Six compounds were isolated via silica gel chromatographic processes, and their chemical structures were elucidated by HR-ESI-MS, 1D, and 2D NMR spectroscopic methods.

MATERIALS AND METHODS

General experimental procedures

The HR–ESI–MS was recorded on an MicrOTOF–Q mass spectrometer. ^{1}H-NMR (600 MHz) and ^{13}C-NMR (150 MHz) spectra were measured on a Bruker Avance 600 spectrometer using $CDCl_3$ (*d* 7.26 and 77.16); $(CD_3)_2CO$ (*d* 2.05 and 206.26, 29.84) and CD_3OD (*d* 4.87 and 49.0) were used both as solvents and internal references.

Thin layer chromatography (TLC) was carried out on precoated silica gel 60 F_{254} (Merck), and the isolated compounds were visualized by spraying with vanillin + H_2SO_4 in ethanol or methanol followed by heating. Gravity column chromatography was performed on silica gel 60 (0.040–0.063 mm, Himedia). Organic solvents were distilled prior to use.

Plant material

The liverwort *Bazzania sp.* was collected on Lang Biang Mountain, Lac Duong District, Da Lat city, Lam Dong Province, at an altitude of 150 meters in July 2020. The sample of *Bazzania sp.* was authenticated by MSc. Luong Thien Tam, Department of Ecology and Evolution Biology. University of Science, National University–Ho Chi Minh City. A voucher specimen, coded BazlB, was deposited in the herbarium of

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the Laboratory of Plant Biotechnology, Department of Plant Biotechnology and Biotransformation, Faculty of Biology and Biotechnology.

Extraction and isolation

The ground powder sample (0.37 kg) of *Bazzania sp.* was extracted with methanol by maceration at room temperature to obtain a crude methanolic residue (29.59 g), which was subjected to silica gel solid-phase extraction and eluted consecutively with *n*-hexane, chloroform, ethyl acetate, and methanol at ambient temperature. Then, each type of eluent was evaporated at low pressure to afford *n*-hexane (3.97 g), chloroform (6.43 g), ethyl acetate (6.82 g), and methanol (10.24 g) residues.

The *n*-hexane residue was chromatographed over silica gel with *n*-hexane: $CHCl_3$ (10:0) to (0:10) to give 10 fractions, coded HE1 (283 mg), HE2 (560 mg), HE3 (495 mg), HE4 (233 mg), HE5 (170 mg), HE6 (400 mg), HE7 (590 mg), HE8 (201 mg), HE9 (487 mg), and HE10 (228 mg). The residue of fraction HE7 was further chromatographed (CC) over silica gel with *n*-hexane:chloroform (100:0, 90:10, 0:100) to give five subfractions (HE7.1-HE7.5). The subfraction HE7.2 (192 mg) was silica gel chromatographed with a gradient system of *n*-hexane:toluene (90:10, 80:20, 70:30, 10:90) to afford compound **6** (6 mg). Subfraction HE7.5 (152 mg) was subjected to CC using the mobile phase *n*-hexane:chloroform (90:10, 85:15, 50:50, 10:90) to afford compound **1** (5 mg).

The chloroform residue was silica gel chromatographed and eluted by *n*-hexane:chloroform (80:20, 50:50, 0:100) and chloroform:ethyl acetate (90:10, 80:20, 0:100) to give seven fractions (CL1−CL7). The fraction CL4 (840 mg) was further fractionated by silica gel chromatography using *n*-hexane:ethyl acetate (stepwise, 98:2, 95:5, 90:10, 0:100) to obtain six subfractions (CL4.1−CL4.6). Further fractionation of CL4.2 (187 mg) and elution with *n*-hexane:ethyl acetate (90:10 to 0:100) afforded compound **4** (7 mg). The same procedure was applied to subfraction CL4.6 (200 mg), eluted by *n*-hexane:ethyl acetate (90:10 to 0:100) to obtain compound **5** (4 mg). Fraction CL6 (540 mg) was divided into four subfractions (CL6.1−CL6.4) by CC using *n*-hexane:acetone (99:1, 95:5, 90:10, 80:20, 0:100). Subfraction CL6.4 (213 mg) was repeatedly chromatographed followed by preparative TLC to afford compound **2** (3 mg) and compound **3** (4 mg).

RESULTS

Using silica gel column chromatography, the chemical investigation of the -hexane and chloroform extracts of the liverwort *Bazzania* sp. led to the isolation of six compounds. Their structures are shown in Figure 1 by using 1D and 2D NMR and HR-ESI-MS.

DISCUSSION

The chemical structures of six isolated compounds were elucidated based on modern spectroscopic methods such as HR-ESI-MS and 1D and 2D NMR, and their data were compared with those in the literature. Compounds possessing a similar framework were structurally discussed.

Compound **1** (5 mg) was obtained as a white solid that was highly soluble in chloroform. The HR-ESI-MS spectrum (positive mode) showed a pseudomolecular ion peak at *m/z* 427.4030 $[M+H]^+$ (calcd. for $C_{30}H_{50}O$+H, 427.3934). The ^{1}H-NMR spectrum showed signals of seven methyl groups [δ_H 0.81 (3H, *s*, H-29), 0.96 (6H, *s*, H-18, H-28), 0.91 (3H, *d*, 6.5 Hz, H-21), 0.93 (3H, *s*, H-30), 0.96 (3H, *s*, H-18), 1.60 (3H, *s*, H-27), 1.66 (3H, *s*, H-26)], two nonequivalent methylene protons [δ_H 0.37 (1H, *d*, 3.5 Hz, H-19a) and 0.58 (1H, *d*, 3.5 Hz, H-19b)], one oxymethine [δ_H 3.22 (1H, *dd*, 10.0, 5.5 Hz, H-3)] and one olefinic proton [δ_H 5.12 (1H, *m*, H-24)]. The ^{13}C-NMR spectrum showed thirty signals with two olefinic carbons [δ_C 125.98 (C-24) and 131.25 (C-25)], one oxygenated methine carbon [δ_C 78.45 (C-3)], and many signals in the high-field zone (14−53 ppm). The good compatibility of its NMR and MS data with those in the literature [19,20] proposed that **1** was cycloartenol.

Compound **2** (3 mg) was obtained as colorless needles and was highly soluble in methanol. The NMR data (Tables 2 and 3) showed two aromatic signals [δ_H 6.84 (2H, *d*, 8.6 Hz; δ_C 130.3, C-3, C-5); 7.43 (2H, *d*, 8.6 Hz; δ_C 116.1, C-2, C-6)] of a 1,4-disubstituted benzene ring and two olefinic protons [δ_H 6.25 (1H, *d*, 15.6 Hz, H-8) and 7.63 (1H, *d*, 15.6 Hz; H-7)]. The large coupling constants suggested the *E*-configuration for this double bond. The other chemical shifts for the remaining carbons are presented in Table 3. The molecular formula of **2** was confirmed by the pseudomolecular ion peak (negative mode) at *m/z* 163.0241 $[M-H]^-$ (calcd. for $C_9H_8O_3$−H, 163.0401), as well as the good compatibility in the comparison of its NMR data with those in the literature [21], suggested that **2** was -coumaric acid.

Compound **3** (4 mg) was isolated as a white solid that was highly soluble in MeOH. Its HR-MS spectrum showed a deprotonated molecular ion peak at *m/z* 137.0103 $[M-H]^-$ (calcd. for $C_7H_6O_3$−H, 137.0244), as well as good compatibility in the comparison of its NMR data with those in the literature [22], suggested that **3** was *p*-hydroxybenzoic acid.

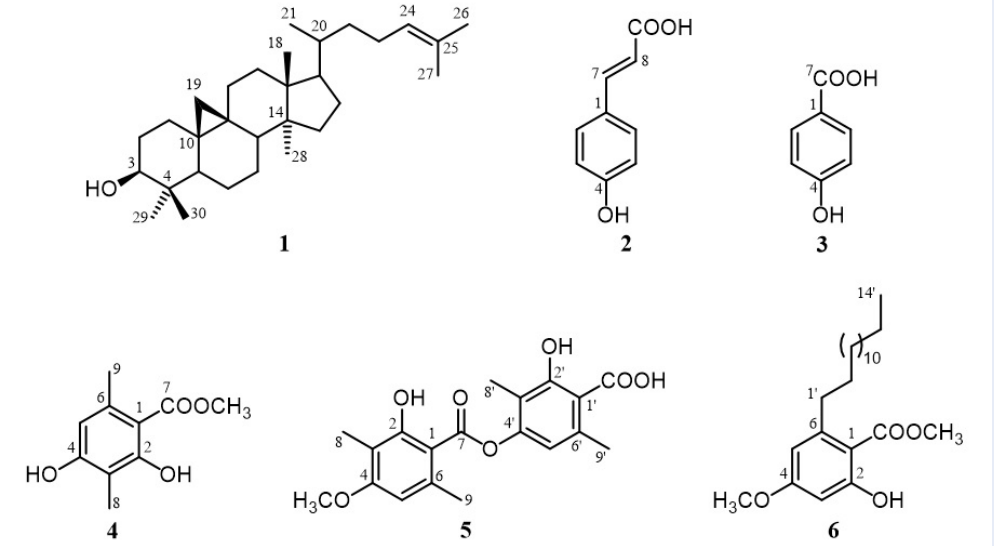

Figure 1: Chemical structures of six compounds isolated from the liverwort Bazzania sp.

Compound **4** (7 mg) was isolated as a white amorphous powder. Its molecular formula was determined to be $C_{10}H_{12}O_4$ through its pseudomolecular ion peak at *m/z* 195.0673 $[M–H]^-$ (calcd. for $C_{10}H_{12}O_4$–H, 195.0662) in the HR-ESI-MS spectrum. The ^{1}H-NMR spectrum showed one aromatic methine proton at δ_H 6.21 (3H, *s*), two methyl groups at δ_H 2.10 (3H, *s*) and 2.46 (3H, *s*), and one methoxy group at δ_H 3.92 (3H, *s*). The ^{13}C-NMR spectrum showed 10 resonances of one benzene ring, two methyl groups, and one methoxycarbonyl group (Tables 2 and 3) . HMBC cross-peaks of the methyl protons at δ_H 2.10 (3H, *s*, H-8) with ^{13}C carbon signals at δ_C 108.7 (C-3), 158.2 (C-4), 163.3 (C-2), and δ_H 2.46 (3H, *s*, H-9) with δ_C 110.7 (C-5), 140.3 (C-6) and 172.7 (C-7) (Figure 2) evidenced their positions. The presence of the methoxycarbonyl group in **3** was proven by cross-peaks of δ_H 3.92 (H-10) with δ_C 172.7 (C-7). The spectrum also confirmed the position of the chelated hydroxyl group through cross-peaks of δ_H 12.00 (1H, *s*, 2-OH) with δ_C105.5 (C-1), 108.7 (C-3), and 163.3 (C-2). Therefore, **4** was identified as methyl 2,4-dihydroxy-3,6-dimethylbenzoate, as shown in the literature.[23]

Compound **5** (4 mg) was isolated as a white solid that was highly soluble in acetone. The ^{1}H-NMR spectrum showed four methyl signals [δ_H 1.99 (3H, *s*, H-8′), 2.03 (3H, *s*, H-8), 2.61 (3H, *s*, H-9′), 2.69 (3H, *s*, H-9)], one methoxy group [δ_H 3.93 (1H, *s*, 4-OCH_3)] and two aromatic protons of two benzene rings at δ_H 6.43 (1H, *s*, H-5′) and 6.59 (1H, *s*, H-5). The spectrum also showed one chelated hydroxyl group at δ_H 11.59 (1H, *s*, 2-OH). The ^{13}C-NMR spectrum (Table 3) showed 18 signals of 19 carbons of four methyl groups (δ_C 7.8, 9.4, 23.5, 24.8), one methoxy group (δ_C 55.1), nine signals of 12 aromatic carbons, two carboxyl carbons including one ester (δ_C 171.1), and one carboxylic acid (δ_C 177.2). The HMBC experiment (Figure 2) revealed that **5** was composed of two benzene rings. This was well supported by the HR-ESI-MS spectrum (negative mode) with a pseudomolecular ion peak at *m/z* 359.1129 $[M–H]^-$ (calcd. for $C_{19}H_{20}O_7$–H, 359.1125). The good compatibility of its NMR data with those in the literature[24] suggested that **5** was a barbatic acid.

Compound **6** (6 mg) was isolated as a white amorphous solid that was highly soluble in chloroform. Its NMR data (Tables 1 and 3) showed a benzene ring with four substitutions via two *meta*-coupled aromatic proton signals (δ_H 6.33, 1H, *d*, 2.7 Hz;H-3) and 6.28 (1H, *d*, 2.7 Hz; H-5), a chelated hydroxyl at δ_H 11.69 (1H, *s*, 2-OH), and two methoxy groups (δ_H 3.92, 3H, *s*, 7-OCH_3) and (δ_H 3.80, 3H, *s*; 4-OCH_3). The data also showed that 1 possessed an alkyl side chain with some methylene groups (δ_H 1.25–2.84, δ_C 22.8–37.1) and one terminal methyl group (δ_H 0.89, 3H, *t*, 6.6 Hz; δ_C 14.2). These results revealed that **6** contained one benzene ring, one methoxy group, one methoxycarbonyl group, and one aliphatic side chain. The HMBC spectrum (Figure 2)

Table 1: The ^{1}H-NMR data of compounds 1 and 6

No.	Compound 1 (Acetone-d_6)		Compound 6 ($CDCl_3$)	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	1.25 (1H; m) 1.56 (1H; m)	32.8		104.3
2	1.68 (1H; m) 1.60 (1H; m)	31.3		164.1
3	3.22 (1H; dd; 10.5; 5.0 Hz)	78.5	6.33 (1H; d, 2.7)	98.9
4	–	41.3		165.7
5	1.31 (1H; m)	48.2	6.28 (1H; d, 2.7)	110.8
6	1.60 (1H; m) 0.83 (1H; m)	21.9		148.3
7	1.09 (1H; m) 1.34 (1H; m)	26.1		170.3
8	1.56 (1H; m)	49.0		
9	–	20.6		
10	–	26.9	2.84 (2H; t, 6.5)	37.1
11	2.03 (1H; m) 1.18 (1H; m)	27.1	1.52 (2H)	32.1
12	1.66 (2H; m)	33.8	1.29 (2H)	30.0
13	–	46.1	1.29 (2H)	30.0
14	–	49.6	1.29 (2H)	30.0
15	1.33 (2H; m)	36.3	1.29 (2H)	30.0
16	1.33 (1H; m) 1.93 (1H; m)	28.8	1.29 (2H)	30.0
17	1.64 (1H; m)	53.1	1.29 (2H)	30.0
18	0,94 (3H; s)	18.6	1.29 (2H)	30.0
19	0.37 (1H; d; 3.5 Hz; H-19a) 0.58 (1H; d; 3.5 Hz; H-19b)	30.5	1.29 (2H)	30.0
20	1.43 (1H; m)	36.7	1.29 (2H)	30.0
21	0.91 (3H; d; 6.5 Hz)	18.7	1.29 (2H)	30.0
22	1.07 (1H; m) 1.48 (1H; m)	37.1	1.25 (2H)	22.8
23	1.90 (1H; m) 2.06 (1H; m)	25.5	0.89 (3H; t; 6.6)	14.2
24	5.12 (1H; m)	125.9	11.69	55.4
25	–	131.2	3.80 (3H; s)	52.0
26	1.60 (3H; s)	17.7	3.92 (3H; s)	
27	1.66 (3H; s)	25.9		
28	0.93 (3H; s)	19.8		
29	0.96 (3H; s)	25.6		
30	0.81 (3H; s)	14.7		

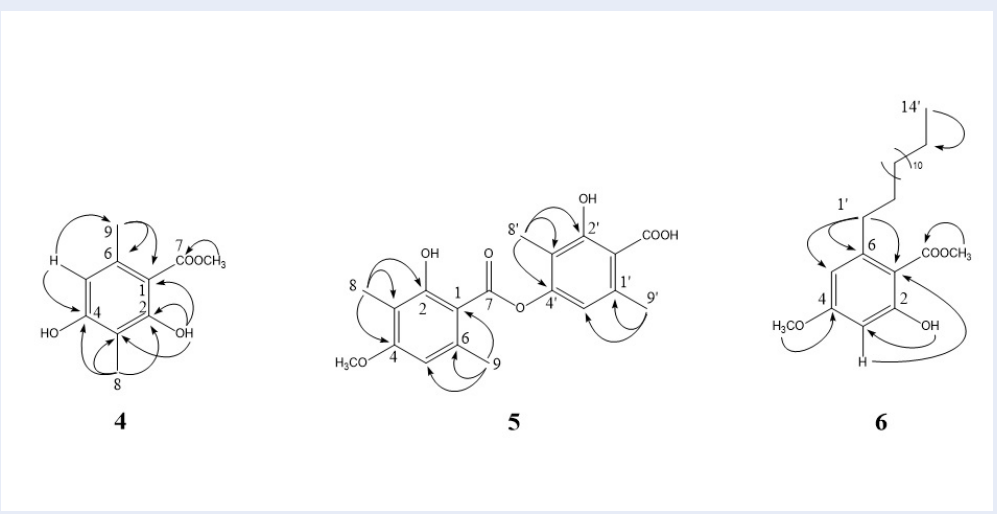

Figure 2: Selected HMBC correlations of compounds **4**, **5**, and **6**

suggested that the chemical structure of **6** was similar to that of **4,** except that it further possessed a side chain. The HR-ESI-MS spectrum of **6** showed a pseudomolecular ion peak at *m/z* 377.2642 $[M–H]^-$ (calcd. for $C_{23}H_{38}O_4$–H, 377.2682), which suggests that the alkyl chain moiety is a tetradecyl group. Therefore, **6** was elucidated as methyl 2-hydroxy-4-methoxy-6-tetradecylbenzoate. It is a new compound from the plant.

CONCLUSION

Six compounds were isolated from the *n*-hexane and chloroform extracts of the liverwort *Bazzania sp.*, collected at Lac Duong district, Lam Dong province, Vietnam, using chromatographic methods. Those compounds are cycloartenol (**1**) and five phenolic derivatives, -coumaric acid (**2**), -hydroxybenzoic acid (**3**), methyl 2,4-dihydroxy-3,6-dimethyl benzoate (**4**), barbatic acid (**5**), and methyl 2-hydroxy-4-methoxy-6-tetradecyl benzoate (**6**). Although **1**-**5** were known in other species, this is the first time they have been reported in *Bazzania sp*. After being checked in Scifinder (on September 30^{th}, 2022), compound **6** was determined to be new. Further research on the remaining extracts is ongoing.

ABBREVIATIONS

HR-ESI-MS: High resolution- Electrospray ionization-Mass spectrometry

^{1} **H-NMR:** Proton nuclear magnetic resonance

^{13} **C-NMR:** Carbon-13 Nuclear Magnetic Resonance

HMBC: Heteronuclear Multiple Bond Correlation

s: singlet

d: doublet

dd: doublet of doublets

t: triplet

m: multiplet

COMPETING INTEREST

The authors declare no competing financial interest.

AUTHORS' CONTRIBUTION

Tran Q. T. collected samples and prepared extracts. Nguyen N. N., Nguyen T. B. T., and Nguyen H. C. performed column chromatography on the *n*-hexane extract and isolated compounds. Nguyen X. K., Phan T. T., and Le H. K. performed column chromatography on the chloroform extract and isolated compounds. Duong T. H and Huynh N. V. interpreted the NMR and MS data. Nguyen X. K. prepared the manuscript and searched the bibliography. Nguyen K. P. P. gave the final correction for the manuscript.

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Table 2: ^{1}**H-NMR data of compounds 2-5**

No.	2 (Methanol-d_4)	3 (Methanol-d_4)	4 ($CDCl_3$)	5 (Acetone-d_6)
1				
2	7.43 (1H; d 8.6)	7.87 (1H; d 8.6)		
3	6.84 (1H; d 8.6)	6.79 (1H; d 8.7)		
4				
5	6.84 (1H; d 8.6)	6.79 (1H; d 8.7)	6.21 (1H; s)	6.59 (1H; s)
6	7.43 (1H; d 8.6)	7.87 (1H; d 8.6)		
7	7.63 (1H; d 15.6)			
8	6.25 (1H; d 15.6)		2.10 (3H; s)	2.03 (3H; s)
9			2.46 (3H; s)	2.69 (3H; s)
5′				6.43 (1H; s)
8′				1.99 (3H; s)
9′				2.61 (3H; s)
2-OH			12.00 (1H; s)	11.59 (1H; s)
4-OH			5.07 (1H; s)	
4-OCH_3				3.93 (3H; s)
7-OCH_3			3.92 (3H; s)	

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Table 3: ^{13}**C-NMR data of compounds 1-6**

No.	2 (CD_3OD)	3 (CD_3OD)	4 ($CDCl_3$)	5 (Acetone-d_6)
1	115.1	125.3	105.5	105.4
2	116.1	132.8	163.3	163.2
3	130.3	115.8	108.7	111.1
4	159.7	162.4	158.2	162.9
5	130.3	115.8	110.7	107.3
6	116.1	132.8	140.3	141.6
7	145.8	172.5	172.7	171.1
8	126.6		7.8	7.8
9	170.4		24.2	24.8
1′				111.1
2′				162.9
3′				115.5
4′				151.3
5′				114.8
6′				141.6
7′				177.2
8′				9.4
9′				23.5
10′				
11′				
12′				
13′				
14′				
4-OCH_3				55.1
7-OCH_3			51.8	

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