Tetrahedron Letters 58 (2017) 2385–2388

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Divaccinosides A–D, four rare iridoid glucosidic truxillate esters from the leaves of *Vaccinium bracteatum*

Yong-Mei Ren [a,b,c], Chang-Qiang Ke [a], Chunping Tang [a], Sheng Yao [a], Yang Ye [a,b,c,*]

[a] State Key Laboratory of Drug Research, and Natural Products Chemistry Department, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China
[b] School of Life Science and Technology, ShanghaiTech University, Shanghai 201203, China
[c] University of Chinese Academy of Sciences, No.19A Yuquan Road, Beijing 100049, China

ARTICLE INFO

Article history:
Received 1 April 2017
Revised 4 May 2017
Accepted 5 May 2017
Available online 10 May 2017

Keywords:
Vaccinium bracteatum
Ericaceae
Iridoid glucosides
Divaccinosides
[2+2] cycloaddition

ABSTRACT

Divaccinosides A–D (**1**–**4**), four rare iridoid glucoside cyclodimers in the truxillate forms, were characterized from the leaves of *Vaccinium bracteatum*. They were presumably biosynthesized from two known iridoid glucoside monomers, vaccinoside (**5**) and 10-*O*-*trans*-*p*-coumaroyl-6α-hydroxyl-dihydromonotropein (**6**), via a [2+2] cycloaddition reaction. The structures of the new compounds were established by comprehensive spectroscopic measurements, combined with chemical conversions and single crystal X-ray crystallographic analyses.

Vaccinium bracteatum Thunb. (Ericaceae), known as "Nan Zhu" in Chinese, is an evergreen shrubby tree distributed in mountainous regions of southern China and recognized as both an edible and a medicinal product in daily life. As a medicinal herb, *V. bracteatum* was reported to possess significant health benefits, such as antifatigue, antianemia, antioxidant, and immunomodulate effects.[1] An array of phytochemical investigations have revealed the existence of fatty acids, flavonoids and triterpenes as the main chemical components in this plant,[2,3] as well as iridoid glucosides as one type of minor constituents.[4–6]

Iridoids are a large group of secondary metabolites found both in a variety of plants and selected animal species. In the past decades, considerable attention has been paid to naturally occurring iridoids, since they display an interesting spectrum of biological activity, such as cardiovascular,[7] antihepatotoxic,[8] anti-inflammatory,[9] and antiviral activities.[10] Structurally, iridoids are cyclopentanopyran monoterpenoids and usually exist in form of glycosides. The most common structural feature of these compounds is the H-5/H-9 *β*, *β*-*cis*-fused cyclopentanopyran ring system,[11] and only several enantiomer[12] and H-5/H-9 *trans*-fused iridoids[13–17] are reported.

As part of systematical searching for iridoids with novel structure or bioactivity, the leaves of *V. bracteatum* were investigated, which led to the isolation of four novel iridiod glucoside dimers named divaccinoside A–D (**1**–**4**). Their structures could come presumably from the dimerization of the known compounds, vaccinoside (**5**) and 10-*O*-*trans*-*p*-coumaroyl-6α-hydroxyl-dihydromonotropein (**6**), through a [2+2] cycloaddition reaction.[18] The absolute configurations of the cyclobutane nucleus of the new compounds was finally determined by spectroscopic analysis, chemical conversion, and single crystal X-ray crystallographic diffraction as well. Herein, we describe the isolation and structural elucidation of compounds **1**–**4** (Fig. 1).

Compound **1** was obtained as yellow amorphous powder. Its molecular formula $C_{50}H_{56}O_{26}$ with 23 double-bond equivalents (DBEs) was determined by HRESIMS at *m*/*z* 1071.2986 $[M-H]^-$ (calcd 1071.2987). The IR spectrum displayed absorption bands for hydroxyl (3420 cm^{-1}), conjugated carbonyl (1718 cm^{-1}) and aromatic functionalities (1642, 1518 cm^{-1}). In the ^1H NMR spectrum of **1** (Table 1), signals at δ_H 7.42 (d, 1.2)/7.41 (d, 1.2), 6.17 (dd, 5.7, 2.6)/6.14 (dd, 5.7, 2.5), 5.66 (d, 2.3)/5.47 (d, 3.3), 5.37 (dd, 5.7, 1.9)/5.36 (dd, 5.7, 1.8), 3.47 (dd, 8.7, 0.8)/3.45 (dd, 8.6, 1.5), 2.40 (dd, 8.7, 2.3)/2.22 (dd, 8.6, 3.3), 3.97 (m)/3.96 (m), 3.40 (m)/3.58 (d, 10.9), 4.69 (d, 8.0)/4.67 (d, 7.9), 3.95 (dd, 12.0, 1.4)/3.88 (dd, 12.0, 2.0), 3.79 (m)/3.71 (dd, 12.0, 5.1), and 3.28–3.40 (8H, m) were typical of two sets of monotropein moieties.[9] In addition, proton signals at δ_H 7.18/7.20 (each 2H, d, J = 8.4), 6.79/6.76 (each 2H, d, J = 8.4), corresponding to carbons at δ_C

* Corresponding author at: State Key Laboratory of Drug Research, and Natural Products Chemistry Department, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China.
E-mail address: yye@mail.shcnc.ac.cn (Y. Ye).

http://dx.doi.org/10.1016/j.tetlet.2017.05.013
0040-4039/

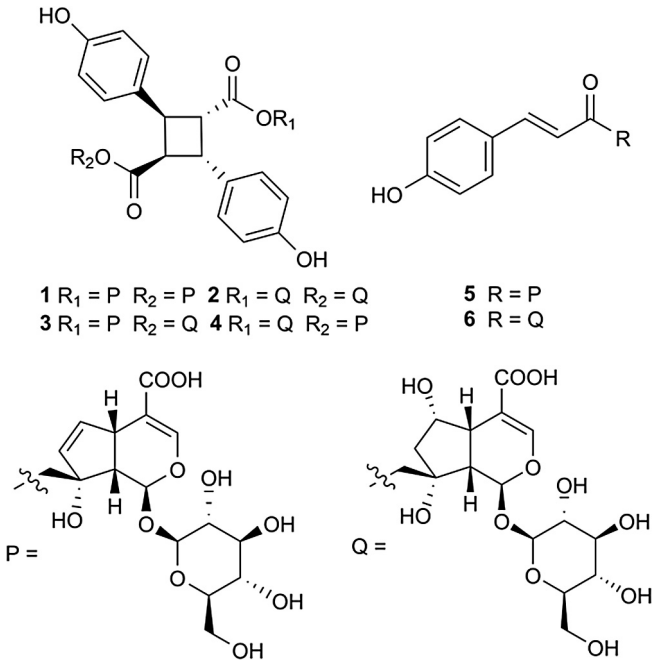

Fig. 1. Structures of compounds **1**–**6**.

129.9/130.0 and 116.5/116.2, revealed the existence of two aromatic rings with a typical AA′BB′ spin system, which were further assigned as two *p*- hydroxyphenyl groups. Detailed analysis of all the ^{1}H and ^{13}C NMR data revealed that these signals were similar to those of the known compound vaccinoside (**5**), except for the absence of the characteristic olefinic proton signals with a *ca.* ~16 Hz coupling constant for the *trans*-double bond. Instead, four methine signals resonating at δ_H 3.91 (m)/3.93 (m), 4.32 (dd, 10.9, 7.4)/4.39 (dd, 10.9, 7.6) were observed. The ^{1}H-^{1}H COSY experiment showed that these four methine protons were mutually coupled to one another in the sequence of δ_H 3.91, 4.32, 3.93, and 4.39; the last one was found in turn to correlate with the first one, indicative of a rare cyclobutane ring moiety. Such a segment was further confirmed by the 1D TOCSY experiment (Fig. S12). HMBC correlations (Fig. 2) from the methine protons at δ_H 4.32 (H-3″, unit Ⅰ) to the carbons at δ_C 173.8 (C-1″, unit Ⅰ), 173.7 (C-1″, unit Ⅱ), and 129.9 (C-5″, unit Ⅰ), and those from the proton at δ_H 4.39 (H-3″, unit Ⅱ) to the carbons at δ_C 173.8 (C-1″, unit Ⅰ), 173.7 (C-1″, unit Ⅱ), and 130.0 (C-5″, unit Ⅱ), along with the ^{1}H-^{1}H COSY data aforementioned, suggested that the carbonyl carbons and the *p*-hydroxyphenyl groups were connected to the cyclobutane ring, which were arranged in the truxillic type (head-to-tail). HMBC correlations from the proton signals at δ_H 3.40/3.97 (H-10, unit Ⅰ) to the carbonyl carbon at δ_C 173.8 (C-1″, unit Ⅰ) and those from δ_H 3.58/3.96 (H-10, unit Ⅱ) to δ_C 173.7 (C-2″, unit Ⅱ) implied that the cyclobutane ring was connected to C-10 of the iridoid skeletons by forming two ester bonds. Accordingly, compound **1** was established to be a truxillic type dimer of vaccinoside containing a cyclobutane nucleus.

The ROESY correlations (Fig. 3) of H-2″ (unit Ⅰ)/H-5″ (unit Ⅰ) and H-2″ (unit Ⅱ)/H-5″ (unit Ⅱ) established *trans*-configurations for H-2″ and H-3″ of units Ⅰ and Ⅱ, respectively. However, the relative configuration of the cyclobutyl ring remained unclear. So compound **1** was hydrolyzed with 1% potassium hydroxide to produce a di-carboxyl derivative **1a** (Scheme S1), which was then crystalized from a mixture solvent of water and methanol. The whole structure of **1a** including the absolute configuration was confirmed by the X-ray diffraction analysis (Fig. 4, CCDC 1534627). Thus, the stereochemistry of the cyclobutane ring in **1** was determined. To further confirm the absolute configuration of the iridoid glucoside moieties, vaccinoside (**5**) was treated with acetic anhydride and the reaction mixture was purified by preparative HPLC to yield the acetylated derivative **5a** (Scheme S2). The single crystal of **5a** was obtained from a mixture of water and acetonitrile. The absolute configuration of **5a** was confirmed firstly by the X-ray diffraction experiment (Fig. 5, CCDC 1534622). Based on the unambiguous structures of these two crystals, the whole structure of compound **1** was finally established, and named divaccinoside A.

Compound **2** was isolated as yellow amorphous powder and its molecular formula was assigned to be $C_{50}H_{60}O_{28}$ from the quasi-molecular negative ion at *m*/*z* 1107.3212 $[M-H]^-$ (calcd 1107.3198) in the HRESIMS, requiring 21 degrees of unsaturation. The IR spectrum displayed absorption bands for hydroxyl (3430 cm^{-1}), conjugated carbonyl (1716 cm^{-1}) and aromatic groups (1640, 1520 cm^{-1}). The ^{1}H and ^{13}C NMR data of **2** (Tables 1 and 2) showed high similarity to those of **1**, except that a methylene (δ_C 44.9/45.0) and an oxygenated methine (δ_C 76.5/77.1)

Table 1
^{1}H NMR Data for Compounds **1**–**4** (600 MHz in CD_3OD, δ in ppm, *J* in Hz).

No.	**1**		**2**		**3**		**4**	
	Unit Ⅰ	Unit Ⅱ	Unit Ⅰ	Unit Ⅱ	Unit Ⅰ	Unit Ⅱ	Unit Ⅰ	Unit Ⅱ
1	5.66 (d, 2.3)	5.47 (d, 3.3)	5.60 (d, 3.2)	5.41 (d, 4.9)	5.48 (d, 3.3)	5.60 (d, 3.3)	5.40 (d, 3.3)	5.66 (d, 1.8)
3	7.42 (d, 1.2)	7.41 (d, 1.2)	7.52 (d, 1.0)	7.50 (d, 1.1)	7.42 (d, 1.1)	7.50 (br s)	7.51 (br s)	7.41 (d, 1.1)
5	3.47 (dd, 8.7, 0.8)	3.45 (dd, 8.6, 1.5)	2.76 (dd, 9.2, 3.1)	2.65 (dd, 9.5, 6.3)	3.45 (dd, 8.5, 1.4)	2.76 (dd, 9.4, 3.8)	2.64 (br s)	3.47 (dd, 8.5, 1.4)
6	6.17 (dd, 5.7, 2.6)	6.14 (dd, 5.7, 2.5)	4.26 (q, 4.9)	4.17 (q, 6.3)	6.15 (dd, 5.7, 2.5)	4.26 (q, 4.9)	4.16 (m)	6.17 (br d, 5.6)
7	5.37 (dd, 5.7, 1.9)	5.36 (dd, 5.7, 1.8)	1.81 (dd, 13.9, 5.5) 1.65 (dd, 10.5, 5.0)	1.85 (dd, 13.5, 6.2) 1.67 (dd, 13.1, 7.5)	5.38 (dd, 5.7, 2.0)	1.65 (dd, 13.9, 5.0) 1.81 (dd, 13.9, 5.6)	1.67 (dd, 13.4, 7.1) 1.85 (dd, 13.4, 5.7)	5.36 (d, 5.4)
9	2.40 (dd, 8.7, 2.3)	2.22 (dd, 8.6, 3.3)	2.38 (dd, 9.2, 3.2)	2.01 (dd, 9.5, 4.9)	2.22 (dd, 8.5, 3.3)	2.38 (dd, 9.4, 3.3)	2.00 (dd, 9.4, 3.3)	2.40 (br d, 8.5)
10	3.40 (m) 3.97 (m)	3.58 (d, 10.9) 3.96 (m)	3.50 (d, 10.9) 3.98 (m)	3.61 (d, 10.8) 3.94 (m)	3.58 (d, 10.9) 3.97 (m)	3.50 (d, 10.9) 3.94 (m)	3.60 (d, 10.9) 3.94 (m)	3.37 (m) 3.98 (m)
1′	4.69 (d, 8.0)	4.67 (d, 7.9)	4.68 (d, 7.9)	4.67 (d, 7.9)	4.67 (d, 8.1)	4.68 (d, 8.0)	4.67 (d, 8.6)	4.69 (d, 8.3)
2′	3.30 (m)	3.28 (m)	3.28 (dd, 9.0, 7.9)	3.26 (dd, 8.9, 7.9)	3.28 (dd, 8.9, 8.1)	3.28 (dd, 9.0, 8.0)	3.26 (t, 8.3)	3.30 (m)
3′	3.40 (m)	3.36 (m)	3.38 (m)	3.37 (m)	3.39 (m)	3.39 (m)	3.38 (m)	3.38 (m)
4′	3.31 (m)	3.31 (m)	3.34 (m)	3.34 (m)	3.34 (m)	3.34 (m)	3.35 (m)	3.35 (m)
5′	3.32 (m)	3.32 (m)	3.32 (m)	3.32 (m)	3.38 (m)	3.38 (m)	3.33 (m)	3.33 (m)
6′	3.79 (m) 3.95 (dd,12.0, 1.4)	3.71 (dd, 12.0, 5.1) 3.88 (dd, 12.0, 2.0)	3.76 (dd, 11.9, 5.5) 3.94 (dd, 11.9, 2.1)	3.68 (dd, 11.9, 4.7) 3.86 (br d, 11.7)	3.71 (dd, 12.1, 5.1) 3.88 (dd, 12.1, 2.0)	3.76 (dd, 11.9, 5.8) 3.94 (dd, 12.0, 2.2)	3.67 (dd, 12.0, 4.9) 3.86 (br d, 11.8)	3.80 (dd, 11.9, 4.5) 3.93 (m)
2″	3.91 (m)	3.93 (m)	3.96 (m)	4.00 (m)	3.96 (m)	3.93 (m)	3.99 (m)	3.93 (m)
3″	4.32 (dd, 10.9, 7.4)	4.39 (dd, 10.9, 7.6)	4.35 (dd, 10.8, 6.9)	4.44 (dd, 10.8, 7.1)	4.41 (dd, 10.8, 7.2)	4.33 (dd, 10.8, 6.8)	4.34 (dd, 10.8, 6.7)	4.43 (dd, 10.8, 7.3)
5″/8″	7.18 (d, 8.4)	7.20 (d, 8.4)	7.21 (d, 8.6)	7.24 (d, 8.6)	7.22 (d, 8.4)	7.18 (d, 8.4)	7.20 (d, 8.3)	7.22 (d, 8.3)
6″/9″	6.79 (d, 8.4)	6.76 (d, 8.4)	6.77 (m)	6.75 (m)	6.75 (d, 8.4)	6.79 (d, 8.4)	6.77 (m)	6.76 (m)

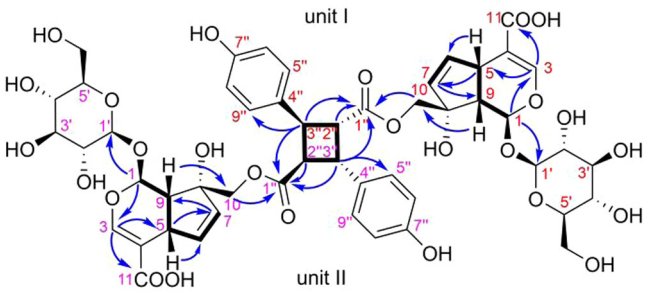

Fig. 2. ^{1}H-^{1}H COSY (H—H) and key HMBC (H→C) correlations of compound **1**.

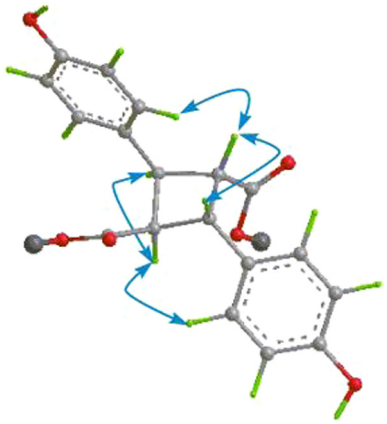

Fig. 3. Key ROESY correlations of the cyclobutane nucleus of compounds **1**–**4**.

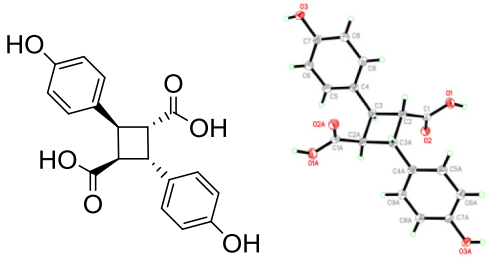

Fig. 4. Structure and ORTEP drawing of compound **1a**.

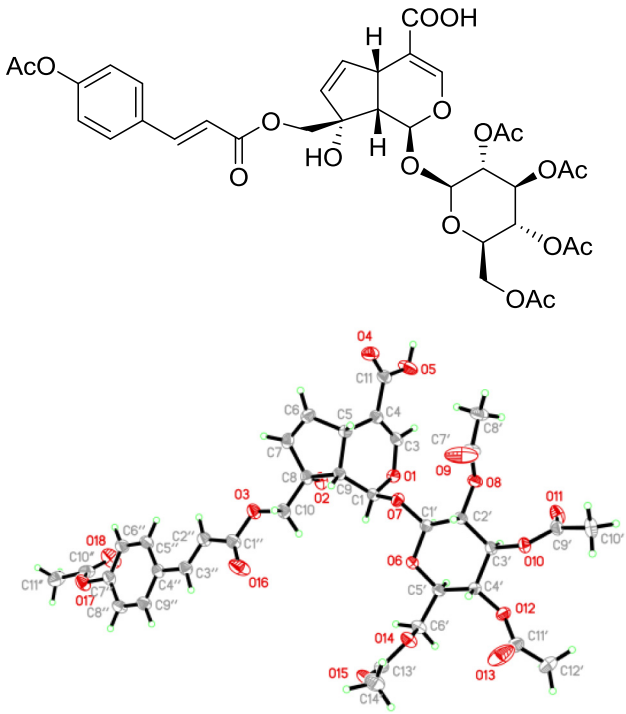

Fig. 5. Structure and ORTEP drawing of compound **5a**.

rather than a double bond were present at C-6 and C-7 of the iridoid skeleton. These signals of the iridoid glycoside moieties were similar to those reported for the known 10-*O*-*trans*-*p*-coumaroyl-6α-hydroxyl-dihydromonotropein (**6**).[5] The signals at δ_H 3.96 (m)/4.00 (m) and 4.35 (dd, 10.8, 6.9)/4.44 (dd, 10.8, 7.1) in **2** indicated the same splitting patterns and coupling constants of the cyclobutane ring with those in **1**. In addition, the cyclobutyl moiety of **2** and **1** showed the same ROESY correlation patterns (Fig. 3). These evidences supported that they had the same relative configuration. Thus, compound **2** was established and named divaccinoside B.

Compounds **3** and **4**, both isolated as yellow amorphous powder, had the same molecular formula of $C_{50}H_{58}O_{27}$ based on the HRESIMS data. Detailed comparison of the NMR data of **3** and **4** (Tables 1 and 2) with those of **1** and **2** revealed that they were also analogs with structural differences happening only in the iridoid glycoside moieties. As mentioned above, the structure of **1** contained two monotropein moieties while **2** contained two 6α-hydroxyl-dihydromonotropein moieties. The ^{1}H NMR spectrum of **3** (Table 1) showed signals at δ_H 6.15 (1H, dd, J = 5.7, 2.5 Hz, H-6) and 5.38 (1H, dd, J = 5.7, 2.0 Hz, H-7), and signals at δ_H 4.26 (1H, q, J = 4.9 Hz, H-6), 1.65 (1H, dd, J = 13.9, 5.0 Hz, H-7) and 1.81 (1H, dd, J = 13.9, 5.6 Hz, H-7), suggesting the presence of both a monotropein and a 6α-hydroxyl-dihydromonotropein moiety. These signals were also found in the ^{1}H NMR spectrum of **4**. Further analysis of 1D and 2D NMR spectra of **3** and **4** revealed that they shared the same planar structure. The splitting patterns and coupling constants, as well as the ROESY correlation patterns (Fig. 3) of the cyclobutyl protons of **3** and **4** were exactly the same with those of **1** and **2**, suggesting that these two compounds possessed the same α-truxillic acid diester skeleton. Therefore, these two compounds could only differ at the stereochemistry of the cyclobutyl moiety. Given the cyclobutyl nucleus, compounds **3** and **4** were identified accordingly as a pair of enantiomers, and named divaccinosides C and D, respectively.

V. bracteatum has been proven as a rich resource of fatty acids, flavonoids and triterpenes, however, it is the first time to report the existence of iridoid glucoside cyclodimers regardless of four iridoid glucoside monomers in previous literatures. To the best of our knowledge, naturally occurring iridoid glucoside cyclodimers in the truxillate form (head-to-tail arrangement, 2,4-diphenyl-1, 3-cyclobutanedicarboxylic acid derivative) or truxinate form (head-to-head arrangement, 3,4-diphenyl-1,2-cyclobutane-dicarboxylic acid derivative) are quite rare. So far only 4,4′-dimethoxy-β-truxinic acid catalpol diester[19] from the leaves of *Premna subscandens* and coelobillardin[20] (α-truxillate skeleton) from *Coelospermum billardieri* have been reported. The findings of four cyclobutyl dimers in the form of α-truxillate skeleton in our study significantly enriched the diversity of this kind of iridoid glucosidic dimers. Considering that these compounds may be dimerized from iridoid glucoside monomers via a [2+2] cycloaddition reaction, other types of isomers, especially differing at the relative

Table 2
^{13}C NMR Data for Compounds **1**–**4** ([a]150 MHz, [b]125 MHz, in CD_3OD).

No.	**1** [a]		**2**[b]		**3** [a]		**4** [a]	
	Unit Ⅰ	Unit Ⅱ	Unit Ⅰ	Unit Ⅱ	Unit Ⅰ	Unit Ⅱ	Unit Ⅰ	Unit Ⅱ
1	94.3	94.6	95.1	95.9	94.6	95.1	95.8	94.3
3	152.5	152.6	154.4	154.4	152.6	154.4	154.5	152.5
4	111.9	111.9	109.9	110.2	110.9	109.9	110.1	110.9
5	39.0	39.6	42.0	42.6	39.6	42.0	42.6	39.0
6	138.5	138.0	76.5	77.1	138.0	76.5	77.1	138.5
7	133.0	132.6	44.9	45.0	133.0	44.9	44.9	132.6
8	83.7	84.1	79.5	79.2	84.1	79.5	79.2	83.7
9	46.0	46.0	45.2	45.0	46.0	46.0	45.0	46.0
10	71.7	71.4	72.4	71.1	70.0	72.4	71.1	70.9
11	170.4	170.4	171.9	171.4	170.4	171.4	170.4	170.4
1′	99.3	99.6	99.9	100.6	99.6	99.9	100.6	99.3
2′	74.5	74.5	74.7	74.6	74.6	74.7	74.7	74.6
3′	78.0	78.0	78.0	77.9	77.9	78.0	78.0	77.8
4′	71.4	71.4	71.7	71.3	71.4	71.7	71.6	71.3
5′	78.3	78.3	78.4	78.35	78.3	78.4	78.4	78.3
6′	62.7	62.6	63.0	62.6	62.5	63.0	62.5	62.9
1″	173.8	173.7	174.0	173.9	173.8	173.9	173.9	173.8
2″	48.7	48.2	48.7	48.2	48.6	48.2	48.2	48.7
3″	42.2	42.0	42.2	42.0	42.0	42.2	42.2	42.0
4″	131.1	130.9	131.2	131.0	131.1	130.9	131.0	131.2
5″/9″	129.9	130.0	129.9	130.0	129.9	129.9	129.8	130.1
6″/8″	116.5	116.2	116.6	116.4	116.4	116.5	116.6	116.2
7″	157.6	157.5	157.5	157.4	157.4	157.6	157.4	157.5

configuration of the cyclobutylic nucleus, are most likely to be found in the further investigations.

Acknowledgments

We are thankful for the financial supports from the National Natural Science Funds (81673327), the Youth Innovation Promotion Association CAS, and the Bureau of Science & Technology for Development, Chinese Academy of Sciences (ZSTH-009, ZSTH-014).

A. Supplementary data

These data include experimental procedures, physicochemiscal data of compounds **1**–**4**, and X-ray data for compounds **1a** and **5a** (CIF). Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2017.05.013.

References

1. Chen SK, Chen BY, Li H, editors. *Flora of China (Zhongguo Zhiwu Zhi)*, vol. 57. Beijing: Science Press; 1999:107.
2. Xu Y, Wang L, Chen Z, Chen Y. *Sci Technol Food Industry*. 2013;34:372–376.
3. Yao HY, Wang L, Chen ZX. *Chem Industry Forest Prod.*. 2007;27:121–123.
4. Sakakibara J, Kaiya T, Yasue M. *Chem Pharm Bull*. 1971;19:1979.
5. Qu J, Chen X, Niu CS, Yu SS. *China J Chin Materia Medica*. 2014;39:684–688.
6. Li ZL, Zhou WM. *China J Chin Materia Medica*. 2008;33:2087–2089.
7. Hansen K, Adsersen A, Christensen SB, Jensen SR, Nyman U, Smitt UW. *Phytomedicine*. 1996;2:319–325.
8. Dhawan BN. *Med Chem Res*. 1995;5:595–605.
9. Choi J, Lee KT, Choi MY, et al. *Biol Pharm Bull*. 2005;28:1915–1918.
10. Rathore A, Rivastava V, Srivastava K. *Phytochemistry*. 1990;29:1917–1920.
11. Dinda B, Debnath S, Banik R. *Chem Pharm Bull*. 2011;59:803–833.
12. Murai F, Tagawa M, Damtoft S, Jensen SR, Nielsen BJ. *Chem Pharm Bull*. 1984;32:2809–2814.
13. Foderaro TA, Stermitz FR. *Tetrahedron Lett*. 1992;33:2953–2954.
14. Tagawa M, Murai F. *Planta Med*. 1980;39:144–147.
15. Foderaro TA, Stermitz F. *Phytochemistry*. 1992;31:4191–4195.
16. Krull RE, Stermitz FR, Franzyk H, Jensen SR. *Phytochemistry*. 1998;49:1605–1608.
17. Krull RE, Stermitz FR. *Phytochemistry*. 1998;49:2413–2415.
18. Ma GL, Xiong J, Yang GX, et al. *J Nat Prod*. 2016;79:1354–1364.
19. Sudo H, Ide T, Otsuka H, et al. *Chem Pharm Bull*. 2000;48:542–546.
20. Magiatis P, Skaltsounis AL, Tillequin F, Seguin E, Cosson JP. *Phytochemistry*. 2002;60:415–418.