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Communication

Anti-NLRP3 inflammasome abietane diterpenoids from *Callicarpa bodinieri* and their structure elucidation

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ABSTRACT

Callicarpa bodinieri is a Chinese traditional medicine herb with anti-inflammatory activity in clinic. Herein, we report two new 9,10-*seco* and etherified abietane diterpenoids bodinieric acids J and K (**1** and **2**) and one known compound (**3**) isolated from the leaves and twigs of this plant. Their chemical structures were elucidated by detailed spectrometry data analysis and DP4+ NMR calculation methods. Hypothetical biosynthetic pathways of **1**–**3** were preliminarily speculated. Compound **3** inhibited inflammasome activation and exhibited blockage of NLRP3 inflammasome activation at non-cytotoxic concentrations *in vitro*.

Callicarpa bodinieri, belonging to the genus of *Callicarpa* in Verbenaceae family [1], has been widely used in traditional medicine for the treatment of inflammation [2], rheumatism, hematemesis, and gastrointestinal bleeding [3]. Previous phytochemical investigations of the leaves and twigs of *C. bodinieri* has led to the identification of flavonoids [4], lignans [5–7], glycosides [8–10], phenolic acids [11,12], triterpenoids [13,14], and diterpenoids [15–20], *etc*. In this study, two new 9,10-*seco* and etherified abietane-type diterpenoids bodinieric acids J and K (**1** and **2**) and one known compound (**3**) were isolated and identified from *Callicarpa bodinieri* (Fig. 1). In order to clarify the biological source of 9,10-*seco* and etherified abietane-type scaffold, the biosynthetic pathways of compounds **1**–**3** were preliminarily elucidated. Based on the medicinal utilization of this plant for the treatment of immunity-related diseases, inhibitory activities against NLRP3 inflammasome activation of **1**–**3** were evaluated.

Stereochemistry is a critical structural element for the presented physical and biological properties of an organic molecule. However, the incorrectly assigned structures are not rare, even with many new experimental techniques and methods emerged nowadays. DP4+ is one of the most sophisticated approaches that has been applied to the challenging task of stereo-chemical assignment using the probabilities from the differences between DFT-GIAO calculated and experimental NMR data [21,22]. Along with the extensive application of DP4+ in NMR calculations last few years [23], it has been becoming an increasingly popular method for the structure elucidation in organic and natural product chemistry.

Bodinieric acid J (**1**) was isolated as a white powder; $[\alpha]_D^{22}$ −3.2 (*c* 0.28, MeOH). Its molecular formula was deduced to be $C_{19}H_{24}O_4$ from the sodium adduct molecular ion at *m/z* 339.1571 in the positive-ion HRESIMS spectrum. The IR spectrum exhibited absorption bands at 3386, 1740, 1672, 1595, 1279, 1133, 1042 cm^{-1} (as shown in Fig. S8 in Supporting information), which reminded the presence of hydroxy, carboxyl, ether and aryl groups. These NMR data listed in Table 1 revealed that compound **1** was closely related to the abietane-type diterpenoid **3** that previously isolated from this plant by our group [24]. According to ^1H–^1H COSY spectrum, the key correlations of H-5/H_2-6/H_2-7 and H-11/H-12 were shown in Fig. 2. HMBC correlations of H_2-1 with C-2 and C-3; H-5 with C-6 and C-19; H_2-7 with C-5 and C-6; H_3-18 with C-3 and C-19; H_3-20 with C-1, C-5 and C-10; H-14 with C-7 and C-9 revealed the presence of a routine A-ring and part of B-ring structure in abietane-skeleton. The H-12 with C-11 and C-15; H-14 with C-12 and C-15; H_3-17 with C-15

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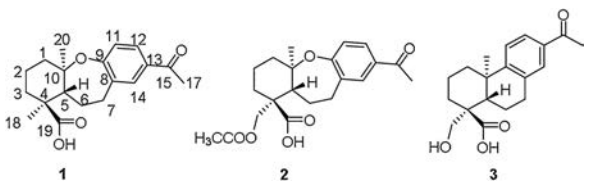

Fig. 1. Structure of compounds **1**-**3** isolated from *Callicarpa bodinieri*.

Table 1
^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data of compounds **1** and **2** (δ in ppm, *J* in Hz, acetone-d_6).

Position	**1**		**2**	
	δ_C	δ_H	δ_C	δ_H
1	37.1	1.79 (overlap) 1.75 (overlap)	37.0	1.84 (overlap) 1.77 (overlap)
2	20.5	1.78 (overlap) 1.59 (overlap)	20.1	1.84 (overlap) 1.62 (overlap)
3	37.3	1.79 (m) 1.60 (overlap)	32.4	1.91 (m) 1.65 (overlap)
4	49.0		52.4	
5	55.4	1.94 (t, 5.7)	53.4	2.12 (m)
6	27.3	1.79 (overlap) 1.70 (overlap)	26.6	1.84 (overlap) 1.75 (overlap)
7	29.5	2.71 (m)	29.6	2.73 (m)
8	129.6		129.3	
9	160.3		160.3	
10	85.0		86.5	
11	115.4	6.92 (d, 8.3)	115.5	6.93 (d, 8.4)
12	129.3	7.72 (dd, 8.4, 2.3)	129.4	7.73 (dd, 8.4, 2.3)
13	130.6		130.7	
14	131.5	7.79 (d, 2.2)	131.7	7.80 (d, 2.2)
15	196.3		196.3	
17	26.3	2.47 (s)	26.3	2.47 (s)
18	18.5	1.22 (s)	64.9	4.33 (d, 11.8) 4.21 (d, 11.8)
19	180.1		177.2	
20	22.3	1.49 (s)	22.2	1.54 (s)
AcO-			170.8, 20.5	1.98 (s)

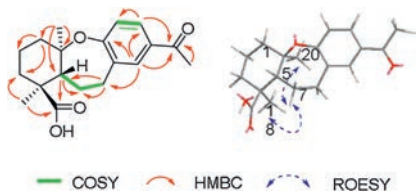

Fig. 2. Key ^{1}H–^{1}H COSY, HMBC, and ROESY correlations of **1**.

correlations undoubtedly established its C-ring and isopropyl unit. In addition, C-9 and C-10 in B-ring were speculated to be disconnected and bridged by an oxygen atom according to the downfield ^{13}C NMR chemical shift at δ_C 160.3 (C-9) and 85.0 (C-10), as well as its molecular formula. Thus, the planar structure of **1** was confirmed.

The relative configuration of **1** was preliminarily inferred from a ROESY experiment, which showed correlations between: H_3-20 and H-7α; H-7α and H_3-18 (Fig. 2). The configuration of **1** was further elucidated by the comparison of experimental and calculated NMR spectrum data. The NMR data of four different isomers that 4*S*, 5*S*, 10*R* (isomer 1), 4*S*, 5*S*, 10*S* (isomer 2), 4*S*, 5*R*, 10*R* (isomer 3) and 4*S*, 5*R*, 10*S* (isomer 4) were calculated using GIAO method [25]. The comparison of the computed results and experimental data were then processed by DP4+ probability statistical analysis [26,27]. Details of the computational procedure

Table 2
Comparison of the computed results and experimental data of compound **1** processed by DP4+ statistical analysis.

Functional mPW1Pw91	Solvent PCM		Basis set 6-311 G(d,p)	
	Isomer 1	Isomer 2	Isomer 3	Isomer 4
sDP4+ (H data)	45.45%	1.30%	52.84%	0.41%
sDP4+ (C data)	0.01%	0.00%	99.99%	0.00%
sDP4+ (all data)	0.00%	0.00%	100.00%	0.00%
uDP4+ (H data)	8.36%	0.98%	65.82%	24.85%
uDP4+ (C data)	0.01%	0.19%	99.80%	0.00%
uDP4+ (all data)	0.00%	0.00%	100.00%	0.00%
DP4+ (H data)	9.82%	0.03%	89.89%	0.26%
DP4+ (C data)	0.00%	0.00%	100.00%	0.00%
DP4+ (all data)	0.00%	0.00%	100.00%	0.00%

for NMR calculation can be found in Supporting information. As shown in Table 2, the isomer of 4*S*, 5*R*, 10*R* was 100% consistent with experimental NMR data. Thus, the structure of **1** was consequently established and named as bodinieric acid J.

Bodinieric acid K (**2**) was purified as white microcrystalline solid, with $[\alpha]_D^{22}$ −14.9 (*c* 0.60, MeOH). Its positive ion HRESIMS revealed a peak for a sodium adduct ion at *m/z* 397.1622, corresponding to a molecular formula of $C_{21}H_{26}O_6Na$. Compared the ^{1}H and ^{13}C NMR data (Table 1) with that of compound **1**, their only difference was the additional presence of a CH_3CO- group that bonded with C-18 hydroxyl to generate an ester in **2**. This assignment could be confirmed by the HMBC correlations of H_2-18 with CH_3CO-, H_2-3 and C-19; H-5 with C-18 and C-19 (Fig. 3). The ROESY correlations were also similar to those of **1**. Based on the biosynthetic considerations of natural products, the configuration of **2** was speculated to stay the same way. Consequently, the structure of **2** was then confirmed and named as bodinieric acid K.

To better understand the biosynthesis mechanism of 9,10-*seco* and etherified B-ring of abietane-type scaffold, the biosynthetic pathways of compounds **1**–**3** were preliminarily elucidated as shown in Fig. 4. Compared with the synthesis of common abietane-skeleton *in* **3**, the variant core structure of B-ring in compounds **1** and **2** were derived initially from the hydratization of 5,10-alkenyl in GGPP to form intermediate **A1**. 9,10-*seco* and etherified fragment in **B1** was further generated by the cycloaddition of 10-hydroxyl and 8,9-alkenyl. Herein, the unusual abietane-type diterpene of **C1** was finally obtained through methyl rearrangement and dehydrogenation reactions.

The leaves and twigs of *Callicarpa bodinieri* have been used as traditional Chinese medicine for thousands of years in the treatment of inflammation-related diseases, especially pyroptosis-related diseases, such as rheumatoid arthritis and gout [28]. Inflammasome plays a key role in autoinflammatory and autoimmune diseases, such as gout, rheumatoid arthritis *etc*. [29]. Its activation causes an inflammatory form of programmed cell death of macrophages called pyroptosis, which leads to maturation of Caspase-1 and release of inflammatory mediators, including IL-1β and IL-18. Excessive IL-1β production is harmful and causes immune dysfunctions [30]. Thus, the isolated compounds were

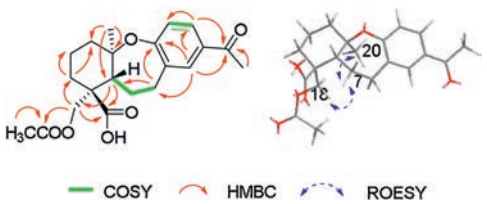

Fig. 3. Key ^{1}H–^{1}H COSY, HMBC, and ROESY correlations of **2**.

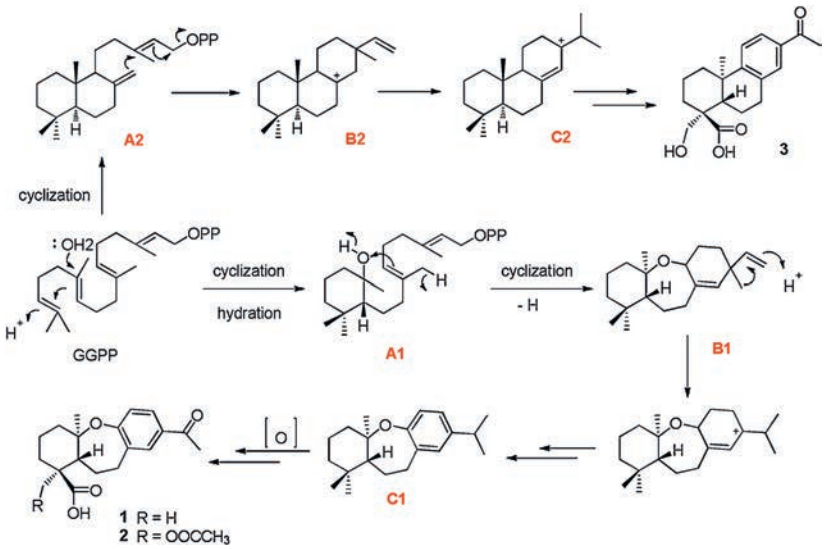

Fig. 4. Hypothetical biosynthetic pathways of compounds **1**–**3**.

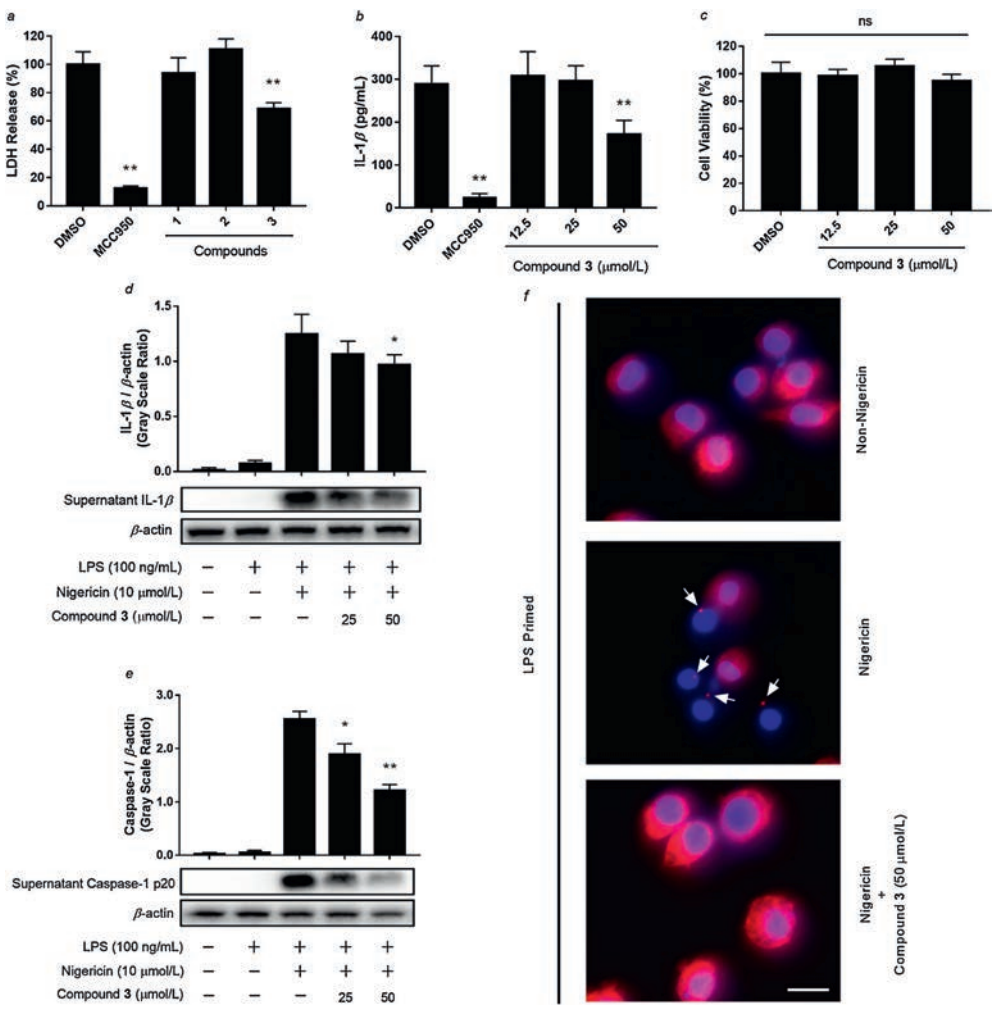

Fig. 5. Compounds from *Callicarpa bodinieri* inhibits NLRP3 inflammasome activation. (a) LDH release assay. MCC950 was used as a positive control. (b) Supernatant IL-1β inhibition. (c) Cytotoxicity of compound **3**. (d, e) Western blot assays for supernatant IL-1β and Caspase-1 treated by compound **3**. Data are shown as mean ± SD ($n = 3$). $^{*}P < 0.05$, $^{**}P < 0.01$. ns, not significant. (f) ASC formation treated by compound **3**. Blue = DAPI, red = ASC, white arrows indicated ASC specks, scale bar = 20 μm.

firstly evaluated for the inhibition of pyroptosis, which was induced by NLRP3 inflammasome activation.

The effects of compound **1**–**3** on pyroptosis inhibition were tested in mouse macrophage J774A.1 cell. Among the three compounds, compound **3** inhibited LDH (*Lactate* dehydrogenase) release in J774A.1 cell rather than compounds **1** and **2** (Fig. 5a). To furtherly detect whether compound **3** inhibits inflammasome activation, the IL-1β secretion and maturation as well as Caspase-1 maturation were evaluated. As shown in Fig. 5b, compound **3** significantly inhibited IL-1β secretion. Besides, MTT assay showed that cell viabilities were not affected by compound **3** at concentrations $12.5-50\ \mu mol/L$ (Fig. 5c), suggesting that the effects of compound **3** on J774A.1 cell were not attributable to its cytotoxicity. Furthermore, compound **3** blocked IL-1β and Caspase-1 maturation in Western blot assays (Figs. 5d and e). Compound **3** also inhibited ASC (apoptosis-associated speck-like protein containing a CARD) specks formation in macrophages (Fig. 5f). These results suggest that compound **3** inhibits LPS and Nigericin induced NLRP3 inflammasome activation in mouse macrophages.

In summary, two new 9,10-*seco* and etherified abietane diterpenoids bodinieric acids J and K (**1** and **2**) and one known compound (**3**) were isolated from the leaves and twigs of *Callicarpa bodinieri*. Their chemical structures were elucidated by detailed spectrometry data analysis as well as DP4+ NMR calculation. Hypothetical biosynthetic pathways of **1**–**3** were also preliminarily speculated. Compound **3** inhibited macrophage pyroptosis and exhibited blockage of NLRP3 inflammasome activation *in vitro*.

Acknowledgments

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.cclet.2019.09.020.

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