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A new acorane sesquiterpenes of *Lysionotus pauciflorus* maxim. Form Guizhou province, China

Jiayu Zhang [a,b,c], Ping Yi [a,b], Yan Xiong [a,b], Caixia Du [a,b], Yu Zhang [d], Chunmao Yuan [a,b], Liejun Huang [a,b], Wei Gu [a,b,*], Xiaojiang Hao [a,b,d,**]

[a] State Key Laboratory of Functions and Applications of Medicinal Plants, Guizhou Medical University, Guiyang, 550014, China
[b] Key Laboratory of Chemistry for Natural Products of Guizhou Province, Chinese Academy of Sciences, Guiyang, 550014, China
[c] School of Pharmaceutical Sciences, Guizhou Medical University, Guiyang, 550025, China
[d] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, 650201, China

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ABSTRACT

A new acorane sesquiterpene (**1**), together with fourteen known compounds were isolated from the whole plant of *Lysionotus pauciflorus* Maxim. The chemical structures of the compounds were identified by physio-chemical properties and 1D-NMR, 2D-NMR, ESI-MS, IR and ECD spectra. Among them, six compounds (**1**, **4**, **6**, **7**, **13**, **15**) were first found in the genus *Lysionotus*. The chemotaxonomic significance of the isolated compounds in the genus *Lysionotus* and family Gesneriaceae were discussed.

1. Subject and source

The genus *Lysionotus* (Gesneriaceae) embraces about 25 species, which are naturally distributed from northern India, Nepal, China, Thailand to southern Japan. There are about 23 species found in China (Flora of China, 1998) . *Lysionotus pauciflorus* Maxim. is widely distributed in southern China, such as Yunnan, Guizhou, Zhejiang and other places (Editorial Committee of Chinese Flora, 1998). As a traditional Chinese medicine, the whole plant of *L. pauciflorus* is often used to treat cough and phlegm (Committee of National Pharmacopoeia, 2015).

In the present study, the whole plants of *L. pauciflorus* were collected from Leishan County, Guizhou Province of China in June 2019, which were identified by Dr. Wei Gu. A voucher specimen (GH-2019-QDN-009) was deposited at the Key Laboratory of Chemistry for Natural Products of Guizhou Province and Chinese Academy of Sciences.

2. Previous work

The majority of previous phytochemical investigations on *L. pauciflorus* were focused on the separation of flavonoids (Feng et al., 2007; Zhang et al., 2009; Wang et al., 2016). Among them, lysionotin is the characteristic component of *L. pauciflorus*. In addition, diverse types of compounds were also reported from this plant, including phenylethanol glycosides (Liu et al., 1995; Zhang et al., 2017), volatile oils (Li et al., 2011; Zhang et al., 2015), sterols and triterpenoids (Terreaux et al., 1996; Li et al., 2007). Recently, there were two studies reported on the sesquiterpenoids from *L. pauciflorus* (Wen et al., 2013, 2014).

3. Present study

The air-dried whole plant of *L. pauciflorus* (66.0 kg) was extracted with 75% ethanol (3 × 80 L) under reflux. The crude extract was evaporated at reduced pressure to remove ethanol (4.8 kg) and suspended with water to successively partitioned into petroleum ether (PE, 3 × 5 L), ethyl acetate (EtOAc, 3 × 5 L) and n-butyl alcohol (n-BuOH, 3 × 5 L). The EtOAc extract (1.07 kg) was eluted by silica gel column chromatography (40–80 mesh) using a gradient elution of PE-EtOAc (50:1–0:50, v/v) and CH_2Cl_2–CH_3OH (50:1–0:50, v/v) to submit 10 fractions (Fr. 1-Fr. 10).

Fr. 6 (25.06 g) was applied to an MCI gel column, eluted with a gradient CH_3OH/H_2O (50:50 to 100:0) to submit fractions 6A-6E. Fr. 6B (5.62 g) was then subjected to column chromatography over silica gel eluted with PE-EtOAc (10:1 to 2:1) to have five subfractions (6Ba–6Be). Subsequently, Fr. 6Bb (661.3 mg) was purified using Sephadex LH-20

* Corresponding author.State Key Laboratory of Functions and Applications of Medicinal Plants, Guizhou Medical University, Guiyang, 550014, China.
** Corresponding author. State Key Laboratory of Functions and Applications of Medicinal Plants, Guizhou Medical University, Guiyang, 550014, China.
E-mail addresses: 631968054@qq.com (J. Zhang), yiping2100@ailiyun.com (P. Yi), 1136360806@qq.com (Y. Xiong), 574278239@qq.com (C. Du), zhangyu@mail.kib.ac.cn (Y. Zhang), yuanchunmao01@126.com (C. Yuan), 2267740517@qq.com (L. Huang), guwei2009@126.com (W. Gu), haoxj@mail.kib.ac.cn (X. Hao).

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eluted with CH_3OH, and then subjected to column chromatography over silica gel eluted with CH_2Cl_2-EtOAc (8:1–1:1, v/v) to have Fr. 6Bb-3 (26.8 mg), and further purified by semi-preparative HPLC on Zorbax SB-C_{18} preparative column with CH_3OH/H_2O (2.0 mL/min, CH_3OH:H_2O = 45:55, v/v) to afford compound **1** (9.8 mg, t_R = 38.45 min). Fr. 6Bd (1.15 g) was fractionated by column chromatography over silica gel with PE-EtOAc (8:1 to 1:1, v/v) gradient elution to give four subfractions (Fr. 6Bd-1 ~ Fr. 6Bb-4). Fr. 6Bb-4 (788.3 mg) was purified using Sephadex LH-20 eluted with CH_3OH to afford compound **2** (138.2 mg). Fr. 6Be (841.9 mg) was subjected to Sephadex LH-20 eluted with CH_3OH, and then purified by semi-preparative HPLC on Zorbax SB-C_{18} preparative column with CH_3OH/H_2O (2.0 mL/min, CH_3OH:H_2O = 43:57, v/v) to afford compound **3** (10.1 mg, t_R = 43.41 min). Fr. 6C (247.7 mg) was fractionated by column chromatography over silica gel with PE-Acetone (15:1 to 2:1, v/v) gradient elution to afford compound **13** (17.8 mg). Fr. 6D (6.54 g) was purified to Sephadex LH-20 eluted with CH_3OH to yield six subfractions (Fr. 6Da-6Df). Fr. 6Da purified by subjected to column chromatography over silica gel eluted with PE-EtOAc (8:1–2:1, v/v) to afford compound **6** (8.6 mg). Fr. 6Dc purified by subjected to column chromatography over silica gel eluted with PE-Acetone (15:1–2:1, v/v) to afford compound **7** (13.4 mg). Fr. 7 (155.24 g) was applied to an MCI gel column, eluted with a gradient CH_3OH/H_2O (50:50 to 100:0, v/v) to submit fractions 7A-6J. Fr. 7E (3.16 g) was purified to Sephadex LH-20 eluted with CH_3OH to yield ten subfractions (Fr. 7Ea-7Ej). Fr. 7 Eb was then subjected to column chromatography over silica gel eluted with CH_2Cl_2–CH_3OH (200:1–80:1, v/v) to afford compound **14** (12.1 mg), compound **8** (20.3 mg) and compound **9** (18.3 mg). Fr. 7Ee was subjected to column chromatography over silica gel eluted with CH_2Cl_2–CH_3OH (15:1–1:1, v/v) to afford compound **15** (20.2 mg) and compound **10** (312.6 mg). Fr. 7F (1.0304 g) was fractionated by column chromatography over silica gel with CH_2Cl_2–CH_3OH (8:1 to 1:1, with 0.5% formic acid, v/v) gradient elution to afford compound **11** (12.4 mg) and compound **12** (19.7 mg). Fr. 7I (3.0552 g) was subjected to column chromatography over silica gel eluted with CH_2Cl_2–CH_3OH (10:1–2:1, with 0.5% formic acid, v/v) to yield seven subfractions (Fr. 7Ia-7Eg). Fr. 7Ie (1.03 g) purified by semi-preparative HPLC on Zorbax SB-C_{18} preparative column with CH_3OH:H_2O (with 0.023% formic acid) = 35:65 (v/v) to afford compound **4** (78.6 mg, t_R = 18.23 min). Fr. 7If (631.2 mg) was then subjected to column chromatography over silica gel eluted with CH_2Cl_2–CH_3OH (5:1–2:1, with 0.5% formic acid, v/v) to afford compound **5** (20.5 mg).

Three acorane sesquiterpenes (**1**–**3**), four phenylethanol compounds (**4**–**7**), five flavonoids (**8**–**12**), and three triterpenoids (**13**–**15**) were isolated from the EtOH extract of *L. pauciflorus*, and compound **1** was a new one. The structures were identified by 1D-NMR, 2D-NMR, ESI-MS, IR and ECD spectra as well as physio-chemical properties.

The fourteen known compounds were identified as 3, 10-dihydroxyacoronene (**2**) (Wen et al., 2013), 1R,3S,4R,5R, 10R-3,10-dihydroxyacoronene-3-*O*-β-*D*-glucoside (**3**) (Wen et al., 2014), forsythoside B (**4**) (Saracoglu et al., 2002), paucifloside (**5**) (Liu et al., 1995), tyrosol (**6**) (Bianco et al., 2004), 4-hydroxyphenethyl-2′ -(R)-hydroxypropanoate (**7**) (Lu et al., 2012), acerosin (**8**) (Wollenweber et al., 2003), 5,7, 4′-trihydroxy-6,8-dimethoxyflavone (**9**) (Wang et al., 2016), lysionotin (**10**) (Niu et al., 2013), nevadensin-7-*O*-β-glucopyranoside (**11**) (Bui et al., 2004), 6-hydroxyluteolin-7-*O*-glucoside (**12**) (Kraut et al., 1993), 3β,17β-dihydroxyandrost-5-ene (**13**) (Yildirim et al., 2013), barbinervic

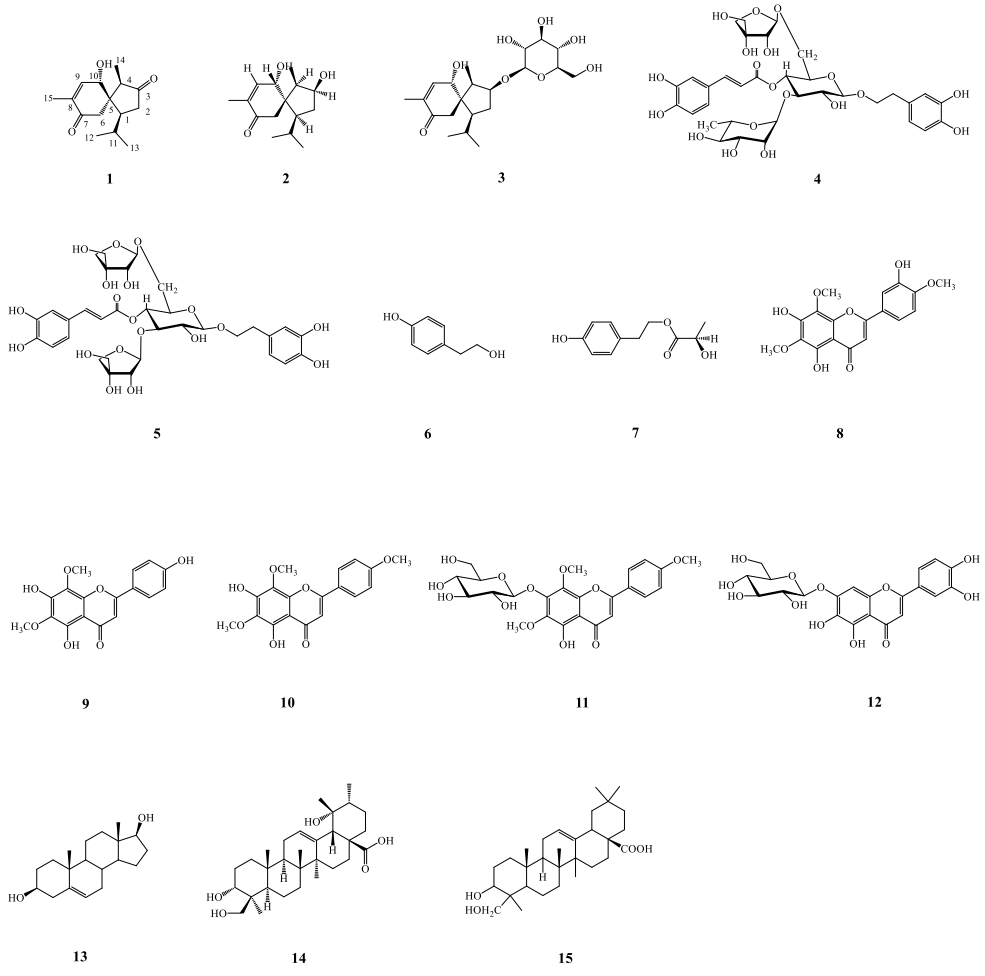

Fig. 1. Chemical structures of compounds **1**–**15**.

acid (**14**) (Zhang et al., 2011) and scutellaria acid (**15**) (Li et al., 2009) (Fig. 1), respectively. Compounds **4**, **6**, **7**, **13**, and **15** were found from the genus *Lysionotus* for the first time.

Compound **1** had a molecular formula of $C_{15}H_{22}O_3$ by HR-ESI-MS *m/z*: 273.1457 $[M+Na]^+$ (calcd for 273.1461, $C_{15}H_{22}O_3Na$). The IR absorption bands at 3437, 1727, 1679, and 1043 cm^{-1} indicated the presence of hydroxy, carbonyl, aromatic, and olefinic groups, respectively. Both ^1H and ^{13}C NMR data analyses (Table 1) of **1** revealed the presence of four methyls (δ_H 1.78, 1.13, 1.04, 0.98; δ_C 15.1, 24.5, 22.0, 13.0), a trisubstituted olefin conjugated to a carbonyl group [δ_H 6.61 (1H, s), 1.78 (3H, s); δ_C 136.0, 150.7, 200.7], a oxygenated methine [δ_H 4.90 (1H, s), δ_C 71.5], and the spiro carbon at δ_C 54.5 (s) of acorane-type sesquterpene. Detailed analysis of its NMR data indicated that **1** was similar to the known compound 3, 10-dihydroxyacoronene (Wen et al., 2013). The HMBC correlations from H-14, H-2 to C-3 and the ^1H–^1H COSY spectrum of H-1 and H-11/H-2, H-14 and H-4 indicated that a carbonyl group attached to C-3 (see Fig. 2). Therefore, the planar configuration of compound **1** was determined by the ^1H–^1H COSY and HMBC spectra.

The ROESY spectrum (Fig. 3) showed that H-1 is related to H-4, Me-12, H-10 is related to Me-12, combined with the literature (Hao et al., 2012) can be inferred that Me-14, H-10 of compound **1** is β configuration, while H-6, H-4 and H-1 are α configuration. The absolute configuration of **1** was further confirmed by comparison of the calculated electronic circular dichroism (ECD) with experimental ECD (Fig. 4). The ECD spectrum of **1** showed the n→π* electron transition effect of cyclohexanone, the cotton effect appeared at 245 nm (△ - 7.0) and 310 nm (△ + 3.0), indicated that the configuration of compound **1** is 1S,4R, 5R,10S.

The structure of compound **1** was identified as (1S,4R,5R, 10S)-10-hydroxy-1-isopropyl-4,8-dimethylspiro [4.5]dec-8-ene-3,7-dione, and given the name as Lysionoene A.

Lysionoene A (**1**): colorless powder; $[\alpha]_D^{21}$ −4.8° (c = 0.8, CH_3OH); UV (CH_3OH) λ_{max} (log ε): 210 (2.88), 201(3.06), 237 (3.36) nm; IR (KBr) ν_{max} 3436, 2962, 2923, 2873, 1727, 1679, 1650, 1462, 1367, 1232, 1090, 1042, 985 and 925 cm^{-1}; ^1H-NMR (CD_3OD, 600 MHz) and ^{13}C-NMR (CD_3OD, 150 MHz) data is shown in Table 1; ESI-MS *m/z*: 273.2 $[M+Na]^+$, HR-ESI-MS *m/z*: 273.1457 $[M+Na]^+$(calcd for 273.1461, $C_{15}H_{22}O_3Na$).

4. Chemotaxonomic significance

Compound **1**, a new acorane sesquiterpene isolated for the first time from the family Gesneriaceae. Up to now, compound **2** and **3** have been reported only from the *L. pauciflorus* (Wen et al., 2013, 2014). Therefore, compounds **1**–**3** can be considered as the characteristic components of *L. pauciflorus*, and these findings provide new insights on occurrence of sesquiterpenes in the genus *Lysionotus* and the family Gesneriaceae.

Phenylethanoid glycosides were widely distributed in Gesneriaceae plants (Li et al., 2008). There were obvious differences in the distribution of phenylethanoid glycosides in different plant groups (Bai et al., 2013). Hence, the distribution of phenylethanoid glycosides in different plants of Gesneriaceae can provide clear chemical basis for further plant classification. Forsythoside B (**4**) was first isolated from *Forsythia koreana* (Rehder) Nakai (Katsuya et al., 1982). Furthermore, it was also isolated from *Ruellia tuberosa* L. (Phakeovilay et al., 2012), *Teucrium chamaedrys* L. (Lamiaceae) (Frezz et al., 2017) and *Callicarpa nudiflora*

Table 1
^1H-NMR (600 MHz) and ^{13}C-NMR (150 MHz) of compound **1** in CD_3OD (δ in ppm).

NO	δ_C	δ_H
1	50.5	1.94 (1H, m)
2	41.5	2.38 (2H, m)
3	223.2	
4	49.9	2.50 (1H, m)
5	54.5	
6	45.8	2.80 (1H, d, *J* = 16.2 Hz), 2.52 (1H, d, *J* = 4.2 Hz)
7	200.7	
8	136.0	
9	150.7	6.61 (1H, s)
10	71.5	4.90 (1H, s)
11	29.8	2.09 (1H, m)
12	24.5	1.13 (3H, d, *J* = 6.7 Hz)
13	22.0	1.04 (3H, d, *J* = 6.6 Hz)
14	13.0	0.98 (3H, d, *J* = 7.9 Hz)
15	15.1	1.78 (3H, s)

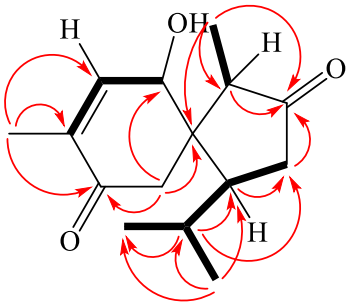

Fig. 2. Key HMBC (→) and ^1H–^1H COSY (—) correlations of compound **1**.

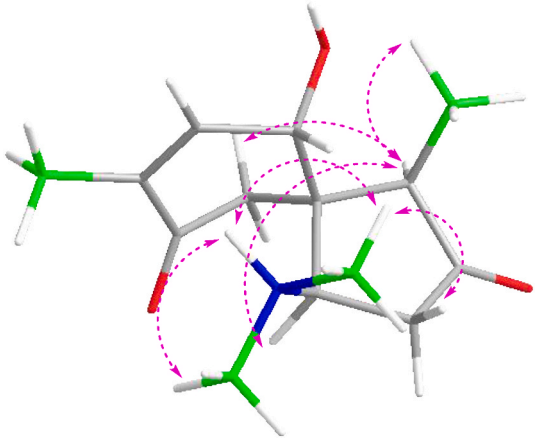

Fig. 3. Key ROESY correlations of compound **1**.

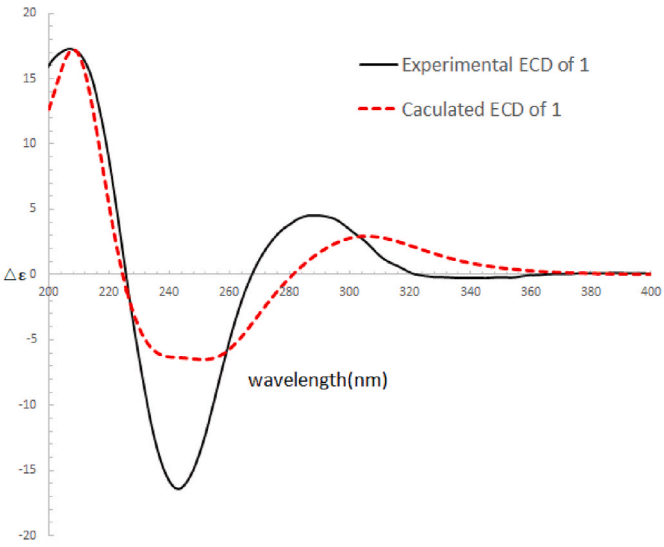

Fig. 4. ECD spectra of compound **1**.

Hook. et Arn. (Lamiaceae) (Wu et al., 2020). Herein, compound **4** was identified from the plant in the genus *Lysionotus* for the first time, which indicated the close relationships of those four species. Paucifloside (**5**) was isolated for the first time from *L. pauciflorus* (Liu et al., 1995), and also found from *Haberlea rhodopensis* Friv. (Gesneriaceae) (Ebrahimi et al., 2011) and *R. tuberosa* (Phakeovilay et al., 2012). Thus, compound **5** can be considered a characteristic component of family Gesneriaceae. Tyrosol (**6**) was widely found in many plants and it was also common in Gesneriaceae species, such as *Aeschynanthus bracteatus* Wall. ex A. DC (Li et al., 2008). and *Boea hygrometrica* (Bunge) R. Br. (Feng et al., 2011). Therefore, compound **6** might have a certain chemotaxonomic value of Gesneriaceae. 4-hydroxyphenethyl-2′-(R)-hydroxypropanoate (**7**) was first reported from *Cephalotaxus hainanensis* Li (Lu et al., 2009, 2012). In the present study, **7** was isolated from *L. pauciflorus*. These findings demonstrate the correlations between the two genera.

Acerosin (**8**) and 5,7,4′-trihydroxy-6,8-dimethoxyflavone (**9**) are also typical compounds in *L. pauciflorus* (Luo et al., 2016; Liang et al., 2018), and which were also common in *Helianthus annuus* L. (Asteraceae) (Denise et al., 2017; Spring et al., 2015). These findings suggest that there is a correlation between the two families. Lysionotin (**10**) was isolated from *Iva nevadensis* M.E. Jones (Farkas et al., 1966) for the first time. However, to our knowlege, compound **10** is a common flavonoid in the genus *Lysionotus* and appeared as a major component in *L. pauciflorus*, the content of this compound can reach as high as 0.9% in the plant. (Zhang et al., 2009). Therefore, compound **10** can be used as the main chemical identification composition of *L. pauciflorus*. Several studies on *Ocimum basilicum* L. (Lamiaceae) (Bernhardt et al., 2015; Zengin et al., 2019) also found the presence of lysionotin. Therefore, compound **10** can be used as a strong evidence to confirm the relationship between the two families. Nevadensin-7-*O*-β-glucopyranoside (**11**) was isolated for the first time from *L. pauciflorus* (Liu et al., 1998), and it was also reported from *Limnophila aromatica* (Lam.) Merr. (Bui et al., 2004), and Scrophulariaceae species (Harborne et al., 1992), it proves that these three families have close relationship. Compound **12** was first reported in *Stereospermum suaveolens* (Roxb.) DC. (Subramanian et al., 1972), and it was also isolated from *Rhaponticum uniflorum* (L.) DC. (Asteraceae) (Olennikov et al., 2019), *Salvia officinalis* L. (Lamiaceae) (Dent et al., 2015; Lee et al., 2018). So, it can be inferred that there may have certain relationship of these families.3β,17β-dihydroxyandrost-5-ene (**13**) was first isolated from the urine of a woman suffering from an adrenocortical carcinoma (Schiller et al., 1945). This compound was associated with endocrine hormones and mould transformation (Loux et al., 2020; Yildirim et al., 2013, 2015), which is rarely isolated from plants. Thus, compound **13** can be used as a characteristic component for identification of *L. pauciflorus*. Barbinervic acid (**14**) was first isolated from *Clethra barbinervis* Sieb. et Zucc. (Takani et al., 1977). This compound was also isolated from the *Verbena officinalis* L. (Zhang et al., 2011), *Pyrola incarnata* Fisch. ex DC (Liu et al., 2020). and *L. pauciflorus*. (Wei et al., 2011). Accordingly, compound **14** has slight taxonomic significance for *L. pauciflorus*. Scutellaria acid (**15**) was first isolated from *Scutellaria rivularis* Wall. ex Benth. (Lamiaceae) (Kuo et al., 1988; Li et al., 2009), Herein, compound **15** was identified from the plant in the family Gesneriaceae for the first time. All of these compounds can help extend the chemotaxonomic knowledge of *L. pauciflorus* and Gesneriaceae.

In conclusion, **1**, **2**, **3**, **4**, **5**, **10**, **13** and **15** can be used as important chemical taxonomic markers of *L. pauciflorus*. This study not only enriched the chemical constituents of *L. pauciflorus*, but also found the evidence of relationships for the families of Gesneriaceae, Lamiaceae and Asteraceae. It is noteworthy that compounds **1**, **4**, **6**, **7**, **13**, and **15** have not been reported from the genus *Lysionotus* previously, therefore it provides new evidences on chemotaxonomic information of this genus.

Authors statement

The corresponding author is responsible for ensuring that the descriptions are accurate and agreed by all authors.

CRediT authorship contribution statement

Jiayu Zhang: Methodology, Data curation, Writing - original draft. **Ping Yi:** Software, Validation. **Yan Xiong:** Formal analysis. **Caixia Du:** Formal analysis. **Yu Zhang:** Formal analysis. **Chunmao Yuan:** Validation. **Liejun Huang:** Validation. **Wei Gu:** Supervision, Funding acquisition, Conceptualization, Resources, Writing - review & editing. **Xiaojiang Hao:** Supervision, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bse.2020.104165.

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