

•Review•

## Chemical constituents and biological activities of lycophytes and ferns

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Available online 20 Dec., 2019

**[ABSTRACT]** Lycophytes and ferns are unique and charismatic members of many terrestrial ecosystems and occupy the pivotal position in land plant origin and evolution. The Chinese lycophytes and ferns flora, with approximately 2000 species, contributes a substantial component to the global lycophytes and ferns diversity, with estimates of 12 000 species. Among them, about 433 species are medicinally recorded and researches based on their phytochemical properties are important topics in natural medicines. This paper reviewed the research history and current status of chemical constituents and biological activities of lycophytes and ferns, which had highlighted the research progress of our group.

**[KEY WORDS]** Lycophytes; Ferns; Chemical constituents; Biological activities; *Lycopodium* alkaloids

**[CLC Number]** R284    **[Document code]** A    **[Article ID]** 2095-6975(2019)12-0887-05

### Introduction

Pteridophytes are conventionally divided into four major groups, Psilotatae, Lycopodiatae (lycophytes or club mosses), Equisetatae (horse tails), and Filicatae (ferns). Molecular data shows that the lycophytes, characterized by microphylls and protostelic or polystelic vascular strands should be separated from the euphyllophytes. Lycophytes include lycopodiaceae, isoëtaceae and selaginellaceae and ferns include leptosporangiates, core leptosporangiates, polypods, eupolypods and eupolypods I, II<sup>[1]</sup>. The lycopods and ferns resources in China is very rich, accounting for 20% of the total in the world, and over 1000 of them are in Yunnan Province<sup>[2]</sup>. About 433 species of lycopods and ferns have pharmaceutical value. Among them, *Lycopodium japonicum*, *Selaginella tamariscina*, *Equisetum hyemale*, *Lygodium japonicum*, *Drynaria*

*Fortunei*, *Pyrrosia sheareri* and *Dryopteris crassirhizoma* are included in the Chinese Pharmacopoeia<sup>[3]</sup>. Huperzine A, an acetylcholinesterase inhibitor was found in *Huperzia serrata*, is a self-developed natural medicines drug in China<sup>[4]</sup>. In hunting for structurally interesting and bioactive natural products, our research group has been making great efforts to phytochemical and biological investigations of lycopods and ferns since 2006, leading to some important progresses. This paper mainly described our perseverant researches which might offer the reference for following works.

### Phytochemistry

#### *Lycopodium* alkaloids

*Lycopodium* alkaloids, which were isolated only from lycopodiaceae in lycophytes, are a group of alkaloids with structural and biological diversity. Up to now, more than 500 alkaloids have been isolated and identified. Our research group have investigated chemical constituents of 12 species of *Lycopodium* in southwestern China, and collected more than 300 purified *lycopodium* alkaloids, which covered all the known structural types of them.

In 2009, a novel C<sub>16</sub>-N-type *Lycopodium* alkaloid lycopodine A (**1**) was isolated by HE Jun *et al.* from the club moss *L. japonicum*, which possesses a unique 6/6/6/7 tetracyclic skeleton with an unprecedented carbinolamine lactone motif<sup>[5]</sup>. Its inhibitory activity on acetylcholinestrase and anti-HIV-1 activity were also evaluated. Lycojapodine A was

**[Received on]** 14-Nov.-2019

**[Research funding]** This work was supported by the National Natural Science Foundation of China (No. 21837003) and Open fund from the State Key Laboratory of Phytochemistry and Plant Resources in West China (No. P2017-KF13).

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These authors have no conflict of interest to declare.

Dedicated to Professor SUN Han-Dong on the Occasion of His 80th Birthday

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considered as a target molecule of total synthesis for organic chemists. The total chemical synthesis of lycopodine A have been accomplished by LEI Xiao-Guang *et al.* [6] and TU Yong-Qiang *et al.* [7]. Lycopalhine A (**2**), a novel sterically congested *Lycopodium* alkaloid that possesses a fused hexacyclic (5/5/5/6/6/6) ring system comprising a 5, 9-diaza-tricyclo[6.2.1.0<sup>4,9</sup>]undecane moiety and a tricyclo[5.2.1.0<sup>4,8</sup>]decane moiety which was isolated by DONG Liao-Bin *et al.* from *Palhinhaea cernua* in 2012 [8]. This molecule has been successfully synthesized by Trauner and Fukuyama groups by different strategies [9–11]. A new pentacyclic (5/6/6/6/7) *Lycopodium* alkaloid named isopalhinine A (**3**), with a sterically congested architecture built with a tricyclo[4.3.1.0<sup>3,7</sup>]decane (isotwistane) moiety and a 1-azabicyclo[4.3.1]decane moiety, was isolated by DONG Liao-Bin *et al.* from the same plant [12]. The absolute configuration of **3** was elucidated by X-ray diffraction experiment and a possible biogenetic pathway was also proposed. HSIEH Hsing-Pang successfully accomplished the first total synthesis of **3** [13] by biomimetic strategy through a bio-inspired 5/6/6/9 tetracyclic intermediate, it mimics the amino ketone form of palhinine D. Lycospidine A (**4**), a first example of *Lycopodium* alkaloid with five-membered A ring, was isolated from *L. complanatum* by CHENG Jin-Tang [14]. The unique five-membered A ring in **4** indicates that carbons 2–5 in **4** are presumably derived from proline instead of the lysine biosynthetically, which suggests that **4** represent a new class of *Lycopodium* alkaloids. In 2016, the first asymmetric total synthesis of (–)-lycospidine A was accomplished by SHE Xue-Gong in only 10 steps with 21.6% overall

yield from the known conveniently available sulfoxide [15]. In our continuing efforts to find structurally and biogenetically interesting *Lycopodium* alkaloids, a new C<sub>16</sub>-N<sub>2</sub>-type *Lycopodium* alkaloid named casuarine A (**5**), which has a cage-like structure featured with a fused 6/6/6/6/6 pentacyclic ring system was isolated by LIU Fei *et al.* from *L. casuarinoides* in 2013 [16]. Cernupalhine A (**6**) is a trace *Lycopodium* alkaloid (0.7 mg) possessing a new C<sub>17</sub>-N skeleton with an unusual hydroxydihydrofuranone motif newly isolated from *P. cernua* [17]. We elucidated its absolute stereochemistry by computational theoretical prediction and the first asymmetric total synthesis. In 2016, five new *Lycopodium* alkaloids, phlegghenines A–D (**7–10**) and neophlegghenine A (**11**) were isolated by DONG Liao-Bin *et al.* from *Phlegmariurus henryi*. The structures and absolute configurations of these new compounds were determined by extensive spectroscopic data coupled with computational calculations which revealed that **7–10** possesses a bicyclo[3.2.2]nonane core, whereas **11** possesses an unprecedented 9-azaprotoadamantane core [18]. Lycoplanine A (**12**) with a 6/9/5 tricyclic ring skeleton fused with the  $\gamma$ -lactone ring and featuring an unusual 1-oxa-6-azaspiro[4.4]nonane moiety and an unprecedented 3-azabicyclo[6.3.1]dodecane unit, was isolated by ZHANG Zhi-Jun *et al.* [19] from the club moss *L. complanatum* in 2017. Recently, phlegmadine A (**13**) with a unique cyclobutane ring and featuring a complex tetracyclo[4.2.2.0<sup>3,8</sup>.0<sup>3,10</sup>]decane-bridged system was isolated from the *P. phlegmaria*. Moreover, phlegmadine A was synthesized by our group from a known compound by biomimetic synthesis method [20].

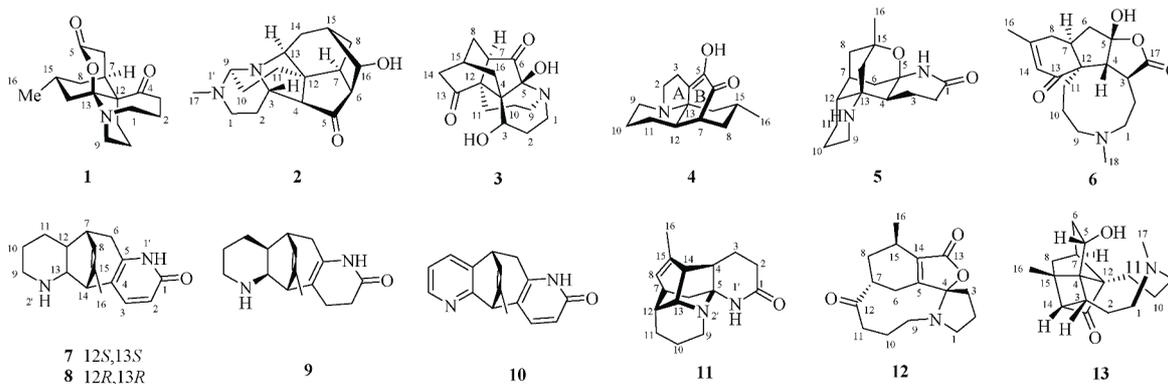


Fig. 1 Structures of the new types of alkaloids

### Terpenoids

As a part of phytochemical investigation of lycophytes and ferns, the chemical constituents of *Hicriopteris glauca*, *H. laevisissima*, *Diplopterigium rufopilosum*, *Dicranopteris dichotoma*, *D. ampla* belonging to family Gleicheniaceae were investigated, leading to the isolation of some new tetranor-clerodanes, clerodane-type diterpene glycosides [21–22], labdane-type diterpenoid glycosides [23–24] and *ent*-kauranoid diterpenoid [25]. In addition, phytochemical investigation on the fronds of *Dennstaedtia scabra* leads to the isolation of two new sesquiterpene glucosides [26]. It is worth pointing out that diterpenoid glycosides are widely distributed in some

genera of ferns while rarely in other land plants. Our study has found that abundant diterpenoid glycosides existed in the Gleicheniaceae family of ferns, which indicates that this family is an important diterpenoid glycosides resource.

### Phenols and flavones

Lycophytes and ferns are rich in phenols and flavonoids. In 2006, we chemically investigated the fronds of *D. Dichotoma*, and two new highly oxygenated phenolic derivatives dichotomains A (**30**) and B (**31**) that feature a spirodilactone moiety in their structures were isolated. Their structures were elucidated on the basis of NMR and MS spectroscopic data, and the stereochemistry of dichotomain A was finally

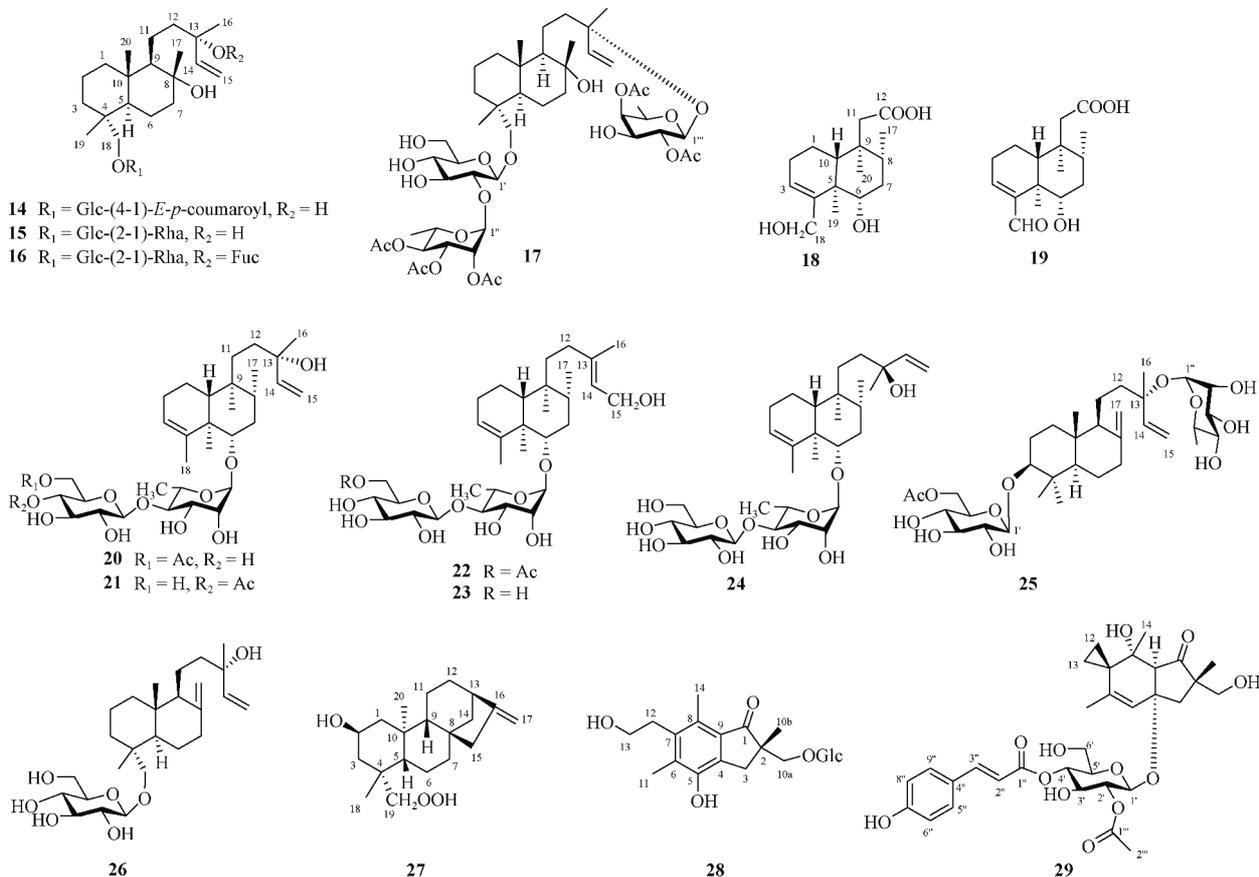


Fig. 2 Structures of new terpenoids isolated from ferns

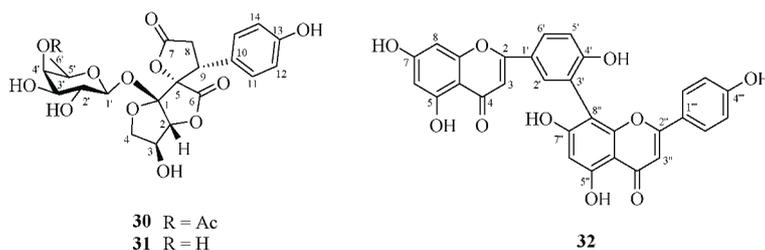


Fig. 3 Chemical structures of dichotomains A and B

determined by single-crystal X-ray diffraction [27].

Previous phytochemical studies on the constituents of *Selaginella* plants showed that this genus was rich in diflavones, represented by amentoflavone (32) [28]. The presence of a series of interesting selaginellin derivatives were also revealed in recent phytochemical investigations. Selaginellin derivatives with unique acetylene bond and *p*-quinone methide functionalities, represent a rare group of naturally occurring phenolic compounds. Their natural occurrence is hitherto confined to the genus *Selaginella*. We systematically investigated the selaginellins of *S. tamariscina*, and isolated three new compounds, namely selaginellin T (33), selaginellin U (34) and isoselagintamarlin A (35). Notably, selaginellin U (34) was a novel derivative of selaginellins, with an unprecedented 6-(4-hydroxyphenyl)-2H-pyran-2-one unit, whereas isoselagintamarlin A (35) featured with a 2-(4-hydroxy-

phenyl)-benzofuran unit. We carried out the biomimetic semi-synthesis of 34 and 35, which unambiguously confirmed their structures, clarified the possible biogenic pathways of them, and provided sufficient method of sample preparation for further bioactivity studies [29-30].

### Biological activities

Acetylcholinesterase inhibitory activity is a characteristic activity of some structural types of *lycopodium* alkaloids. Huperzine A, first isolated from *H. serrata* with potent inhibitory activity against AChE, has been marketed as a new drug in the treatment of Alzheimer's disease (AD) in China [31]. Our group has established a screening method for acetylcholinesterase and butyrylcholinesterase activities and all the isolated *lycopodium* alkaloids were tested for AChE inhibitory activities [16, 18, 32-39]. Phleghenrines A and D, isolated

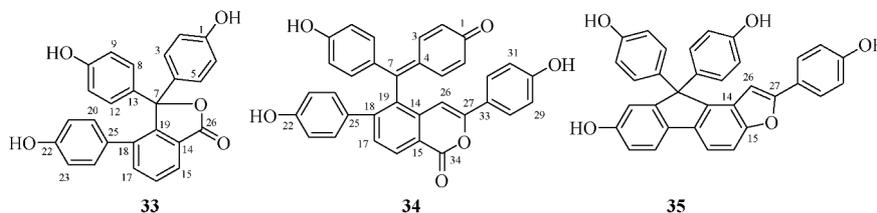


Fig. 4 Chemical structure of new selaginellin derivatives

from *P. henryi*, showed significant AChE inhibitory activities, and phleghenrine D was a potential lead natural product for the further development of novel AChE inhibitors for the treatment of Alzheimer's disease<sup>[18]</sup>. Obscurumine L from *L. obscurum*<sup>[34]</sup>, huperzine E from *H. serrata*<sup>[37]</sup> and casuarine B from *L. casuarinoides*<sup>[16]</sup> exhibited inhibitory activities of acetylcholinesterase.

Biological studies showed that lycoplanine A exhibited noticeable inhibition of Ca<sub>v</sub>3.1 TTCC peak current with an IC<sub>50</sub> value of 6.06 μmol·L<sup>-1</sup>. Meanwhile mibefradil, a classic TTCC inhibitor that was once clinically used for the treatment of hypertension, inhibited Ca<sub>v</sub>3.1 with an IC<sub>50</sub> value of 1.32 μmol·L<sup>-1</sup>. The result suggested that lycoplanine A is a potent Ca<sub>v</sub>3.1 TTCC inhibitor, a good leading compound for the treatment of diseases associated with T-type calcium channels, such as neuropathic pain, absence epilepsy, insomnia, and Parkinson's disease<sup>[19]</sup>.

14-Hydroxylobsurinol exhibited noticeable protective effects for long-term potentiation (LTP) impairment by corticosterone induced in mice<sup>[20]</sup>; Obscurumine O exhibited inhibition of the secretion of IL-2 in phytohemagglutinin (PHA) and phorbol myristate acetate (PMA) stimulated Jurkat cells, and the IC<sub>50</sub> value was 17.2 μmol·L<sup>-1</sup><sup>[34]</sup>.

## Prospective

Lycophytes and ferns, as the second diversified flora on the earth, play an important role in the origin and evolution of terrestrial plants<sup>[40]</sup>. Phylogenetically, they are sisters to the seed plants, and therefore being considered as the key to understand vascular plant diversity and evolution<sup>[41]</sup>. The resources of lycophytes and ferns, accounting for 20% of the world's species, are very rich in China. About 433 species of lycophytes and ferns have been used for treatments of various diseases as Chinese natural medicines. Lycophytes and ferns produce unique primary and secondary metabolites during the long period of adaption to unique environments, and these unique metabolites are potential sources of natural medicines. Although more than 1200 species of lycophytes and ferns are distributed in China, only few species have been investigated. To fully exploit the therapeutic value and utilize the resources of lycophytes and ferns, more attention should be paid on them. We expect that, investigations of lycophytes and ferns based on chemistry and biology will make great progress with the development of separation and identification technology, and more active natural products will be found.

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**Cite this article as:** ZHU Qin-Feng, ZHAO Qin-Shi. Chemical constituents and biological activities of lycophytes and ferns [J]. *Chin J Nat Med*, 2019, **17**(12): 887-891.



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