Progress on synthesis of benzylisoquinoline alkaloids in sacred lotus (Nelumbo nucifera)

Zhuoyin Chen, Hedi Zhao and Sha Chen

Key Laboratory of Beijing for Identification and Safety Evaluation of Chinese Medicine, Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, No.16, Namxiaojie, Dongzhimennei, Beijing 100700, China

* Corresponding author; E-mail: schen@icmm.ac.cn

Abstract

Sacred lotus (Nelumbo nucifera) is a 2,000-year-old perennial rhizome aquatic crop that is primarily employed as a food and drug dual-use crop in East Asia. One of the key bioactive components of sacred lotus is benzylisoquinoline alkaloids (BIAs). Existing research has demonstrated that they have therapeutic and preventive benefits on obesity, diabetes, cancer, and cardiovascular disease. Despite their broad pharmacological relevance, the metabolism of BIA in sacred lotus has received little attention. We reviewed the biosynthetic process of the BIA in sacred lotus in this research. We concluded that a thorough functional characterization of BIAs biosynthesis enzymes provides a wide range of significant therapeutic applications for sacred lotus.

Citation: Chen Z, Zhao H, Chen S. 2023. Progress on synthesis of benzylisoquinoline alkaloids in sacred lotus (Nelumbo nucifera). Medicinal Plant Biology 2:20 https://doi.org/10.48130/MPB-2023-0020

Introduction

China has cultivated the sacred lotus (Nelumbo nucifera), a perennial rhizome aquatic plant in the Nelumbonaceae family, for over 2,000 years on 330,000 hectares[1]. Two species of Nelumbo exist: nucifera and lutea. N. nucifera inhabits Asia and Oceania[2], and N. lutea inhabits North and Northern South America[3,4]. N. nucifera and N. lutea are only geographically separated, not reproductively[5]. Hybrid breeding of N. nucifera and N. lutea may enhance sacred lotus diversity. As a food-drug dual, sacred lotus is popular in East Asia, especially China[2]. According to the 2020 edition of the 'Pharmacopoeia of the People's Republic of China'[6], sacred lotus leaves, flowers, seeds, stamens, receptacles, and internodes are commonly used medicinal plants and have important medicinal value. For example, the lotus leaf may clear heat, relieve summer heat, and send clarity (pure) upward. Lotus has been shown to promote blood circulation and hemostasis, as well as to remove dampness and wind and nourish the heart and kidney. The lotus seed can tonify the spleen and kidney, alleviate diarrhoea, and stop bleeding[7–9].

BIAs with medical potential and healthcare benefits are being studied. BIAs are various plant-specific tyrosine-derived metabolites[10]. Most sacred lotus alkaloids are 1-benzylisoquinoline, aporphine, and bisbenzylisoquinoline. Norcoclaurine, a typical 1-benzylisoquinoline alkaloid, treats heart failure, arrhythmia, bradycardia, myocardial ischemia-reperfusion injury, and cardiac fibrosis in traditional Chinese medicine[11,12]. Norcoclaurine has anti-inflammatory, anti-arrhythmic, and antithrombotic properties and is a β2-adrenergic receptor agonist[13]. Neferine and isoisouloise, the main bisbenzylisoquinoline alkaloids in sacred lotus plumule extract, are pharmacologically significant[14]. Neferine possesses anti-inflammatory, anti-oxidative, anti-hypertensive, anti-arrhythmic, anti-platelet, anti-thrombotic, anti-amnesic, anti-anxiety, and anti-cancer characteristics. Isoliensinine is anti-tumor, cardoprotective, antioxidant, antidepressant, anti-HIV, and anti-Alzheimer's[14,15]. According to recent research, bisbenzylisoquinoline alkaloids may cure new coronavirus pneumonia[16]. Lotus leaves contain high-purity aporphine alkaloid nuciferine (NF). NF is anti-obesity, anti-hyperlipidemia, hypoglycemia, hypouricemic, anti-inflammatory[17], and otherwise therapeutic[18–20].

The metabolic pathways, biosynthesis, and corresponding enzymes involved in the formation of benzylisoquinoline alkaloids derived from the sacred lotus plant have yet to be elucidated, despite their significant pharmacological properties. Currently, the primary focus of research on the biosynthesis of benzylisoquinoline alkaloids (BIAs) lies in opium poppy (Papaver somniferum) and other related species within the Ranunculales order. Extensive investigations have successfully revealed the complete biosynthetic pathways of various alkaloids possessing significant pharmacological properties, including morphine (morphinan), noscapine (phthalideisoquinoline), and sanguinarine (benzophenanthridine)[21]. Although the structure of BIAs in members of the Ranunculales order is characterised by complexity and diversity, it is important to note that all BIAs share a common biosynthetic origin. Specifically, metabolites derived from L-tyrosine, dopamine, and 4-hydroxyphenylacetaldehyde (4-HPAA) undergo a Pictet-Spengler condensation catalysed by norcoclaurine synthase (NCS), resulting in the formation of (S)-norcoclaurine. Subsequently, this compound is transformed into the key intermediate (S)-reticuline through the action of three methyltransferases (6OMT, CNMT, 4'OMT) and one cytochrome P450 monoxygenase (CYP), known as N-methylcoclaurine 3'-hydroxylase (NMCH)[22–26]. The

© The Author(s)
processes outlined above are often known as the upstream universal synthesis pathway. Subsequently, a series of oxidative enzymes facilitate the specific coupling of C-C and C-O bonds, leading to the transformation of (S)-reticuline into protoberberine, which serves as a precursor for the synthesis of benzophenanthridines and phthalideisoquinolines. Additionally, the conversion of (S)-reticuline gives rise to the formation of aporphine and morphinan alkaloids.

In the BIAs biosynthetic pathway of sacred lotus, from L-tyrosine to dopamine and 4-hydroxyphenylacetaldehyde (4-HPAA) to the formation of N-methylcoclaurine and reticuline is common to the synthesis pathway of Ranunculales species such as opium poppy, and the synthesis pathway is clear. However, the synthesis of bisbenzylisoquinolines (liensinine, neferine), the different methylation modifications between bisbenzylisoquinoline alkaloids, and the synthesis of aporphine compounds (nuciferine, etc.) are not clear. Therefore, it is crucial to investigate the pharmacological importance of these particular chemicals by studying the sacred lotus' functional enzymes. Hence, it is essential to conduct a comprehensive investigation on the functional enzymes present in the sacred lotus in order to elucidate the pharmacological potential of these distinct substances. Furthermore, it is worth noting that several benzylisoquinoline alkaloids (BIAs) derived from the sacred lotus have a conformation mostly composed of the R-enantiomer. This is in stark contrast to the prevalent S-enantiomer conformation seen in BIAs derived from opium poppy and plants connected to the Ranunculales order. Hence, the investigation of the atypical stereochemistry of BIAs in the sacred lotus has significance in terms of its molecular and biochemical aspects. Furthermore, the study of the metabolism and biosynthesis of angiosperms, which constitute the fundamental group of flowering plants, has significant implications for the understanding of plant evolution.

**Occurrence of BIAs in sacred lotus**

The sacred lotus contains three forms of BIAs: 1-benzylisoquinoline, aporphine, and bisbenzylisoquinoline alkaloids (Table 1). Their structure, concentration, and physiological functions in sacred lotus have been extensively studied. Their chemical formula, stereo configuration and distribution in sacred lotus organs are shown in Table 1.

**1-Benzylisoquinoline alkaloids**

1-Benzylisoquinoline alkaloids are traced in lotus leaves, flowers, embryos, and seeds (Table 1). The 1-benzylisoquinoline alkaloids in sacred lotus mainly include norcoclaurine, coicoclaurine, norjorjirine, isococlaurine, N-methylcoclaurine, 6-demethyl-4′-O-methyl-N-methylcoclaurine, norarmepavine, N-methylisococlaurine, norroeferactine, juziphrine, arnepavine, 4′-O-methyl-N-methylcoclaurine, lotusine, isolotusine, 4′-O-methylarmepavine.

The pharmacological effects of these 1-benzylisoquinoline alkaloids are diverse. Norcoclaurine’s pharmacological action is one of the most extensively researched. It possesses anti-oxidant, anti-HIV, and anti-Alzheimer’s disease pharmacological actions[46], as well as cardiovascular pharmacological activities such as treating heart failure, lowering myocardial ischemia injury, and reducing pathological cardiac fibrosis and dysfunction[47,48]. Other 1-benzylisoquinoline alkaloids’ pharmacological properties are also noteworthy. Arnepavine, for example, inhibits melanin formation and regulates the immunological system[48]. Furthermore, it has been shown that this therapeutic approach may be used for the treatment of autoimmune disorders, including systemic lupus erythematosus and crescentic glomerulonephritis[47]. Lotusine contains anti-wrinkle, neuroprotective, and liver-protective properties[48,49].

**Aporphines**

The aporphine and pre-aporphine compounds found in sacred lotus are caaverine, asimilobine, glaziovine, O-nornuciferine, N-norncocinerve, nirinidine, N-methylsimilobine, roemeine, dehydroconcurine, dehydroanonaune, dehydroeroemerine, pronuciferine, nuciferine, 7-hydroxidehydroconcurine, lysi-camine, cepharadione B, anonaine, liridoneine. Among them, the pharmacological effect of NF is the most concerning which has anti-obesity, anti-hyperlipidemia, anti-diabetes, anti-arteriosclerosis, anti-tumor and other effects[50–52].

Among them, aporphine and pre-aporphine chemicals found in sacred lotus, such as nirinidine, asimilobine, N-methylsimilobine, and pronuciferine, O-nornuciferine, have anti-Alzheimer’s disease properties[53,54]; Nirinidine in lotus petals has an anti-cervical cancer effect[53].

**Bisbenzylisoquinolines**

Bisbenzylisoquinoline alkaloids are mainly accumulated in the seed embryo of sacred lotus. The main bisbenzylisoquino-line compounds are included nelumboferine, liensinine, isoliensinine, daucilicine, 6-hydroxyoriosiensinine, N-noriso-liensinine, nelumborine, dauricinoline, neferine, dauricine. There are several investigations being conducted on bisbenzylisoquinoline alkaloids at the moment. The most noteworthy is that bisbenzylisoquinoline alkaloids have the potential to be exploited as therapeutic agents for new coronavirus pneumonia. Neferine, in particular, can prevent SARS-CoV-2 infection by inhibiting Ca²⁺-dependent membrane fusion[10]. Furthermore, neferine possesses anti-tumor, anti-inflammatory[23], anti-hypertension, anti-diabetes, anti-arrhythmia, anti-platelet, anti-thrombosis, neuroprotective, anti-amesia, anti-anxiety, and other properties[55–58]. Neferine anti-tumor research has been on the rise in recent years. Isoliensinine and liensinine have notable pharmacological actions. Isoliensinine provides several health benefits, including anti-tumor, heart protection, anti-oxidation, anti-depression, anti-HIV, and anti-Alzheimer’s disease[14,15].

**BIAs biosynthesis**

**The latest research progress and difficulties of BIAs biosynthesis**

Because of the monophyletic evolution of BIAs biosynthesis in angiosperms, the selection of genes related to BIAs biosynthesis in sacred lotus can be guided by the opium poppy BIAs metabolic pathway. Hence, it is anticipated that the biosynthetic route of benzylisoquinoline alkaloids (BIAs) in the sacred lotus involves the condensation of dopamine and 4-hydroxyphenylacetaldehyde (4-HPAA) catalysed by NCS, followed by the enzymatic conversion of (R,S)-norcoclaurine into various substituted 1-benzylisoquinoline, protoaporphine, aporphine, and bisbenzylisoquinoline alkaloids. This conversion is facilitated by specific enzymes such as O-methyltransferase (OMT), N-methyltransferase (NMT), cytochrome P450 oxidoreductases (CYPs), and others, which belong to a restricted enzyme
Synthesis of benzylisoquinoline alkaloids in lotus

Table 1. Benzylisoquinoline alkaloids (BIAs) were identified in several organs of *Nelumbo nucifera*, together with their respective chemical formulas and stereochemical properties. L, lotus leaf; E, lotus embryo; F, lotus flower; S, lotus seed; R, lotus rhizome.

<table>
<thead>
<tr>
<th>No.</th>
<th>Alkaloid</th>
<th>Formula</th>
<th>Enantiomer</th>
<th>Organ</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Norcoclaurine</td>
<td>C_{17}H_{19}NO_{3}</td>
<td>(+)-R and (-)-S</td>
<td>L, E</td>
<td>[27–30]</td>
</tr>
<tr>
<td>2</td>
<td>Coclaurine</td>
<td>C_{17}H_{19}NO_{3}</td>
<td>(+)-R</td>
<td>L, E, F</td>
<td>[27,29,31]</td>
</tr>
<tr>
<td>3</td>
<td>Norjuzuinine</td>
<td>C_{17}H_{19}NO_{3}</td>
<td>NS</td>
<td>F</td>
<td>[32]</td>
</tr>
<tr>
<td>4</td>
<td>Isococlaurine</td>
<td>C_{17}H_{19}NO_{3}</td>
<td>NS</td>
<td>F</td>
<td>[33]</td>
</tr>
<tr>
<td>5</td>
<td>N-Methylcoclaurine</td>
<td>C_{17}H_{19}NO_{3}</td>
<td>(-)-R</td>
<td>L, E, F</td>
<td>[27,29,31]</td>
</tr>
<tr>
<td>6</td>
<td>6-Demethyl-4’-O-methyl-N-methylcoclaurine</td>
<td>C_{17}H_{19}NO_{3}</td>
<td>NS</td>
<td>E</td>
<td>[29]</td>
</tr>
<tr>
<td>7</td>
<td>Norarmepavine</td>
<td>C_{17}H_{19}NO_{3}</td>
<td>(+)-R</td>
<td>L, F</td>
<td>[31]</td>
</tr>
<tr>
<td>8</td>
<td>N-Methylisococlaurine</td>
<td>C_{17}H_{19}NO_{3}</td>
<td>NS</td>
<td>L, E</td>
<td>[29,34]</td>
</tr>
<tr>
<td>9</td>
<td>Norroefractine</td>
<td>C_{17}H_{19}NO_{3}</td>
<td>NS</td>
<td>F</td>
<td>[33]</td>
</tr>
<tr>
<td>10</td>
<td>Juziphine</td>
<td>C_{17}H_{19}NO_{3}</td>
<td>NS</td>
<td>F</td>
<td>[33]</td>
</tr>
<tr>
<td>11</td>
<td>Armeapavine</td>
<td>C_{17}H_{19}NO_{3}</td>
<td>(-)-R and (+)-S</td>
<td>L, E, S</td>
<td>[29,31,35,36]</td>
</tr>
<tr>
<td>12</td>
<td>4’-O-Methyl-N-methylcoclaurine</td>
<td>C_{17}H_{19}NO_{3}</td>
<td>NS</td>
<td>E</td>
<td>[29]</td>
</tr>
<tr>
<td>13</td>
<td>Lotusine</td>
<td>C_{17}H_{19}NO_{3}</td>
<td>NS</td>
<td>E</td>
<td>[29]</td>
</tr>
<tr>
<td>14</td>
<td>Isolotusine</td>
<td>C_{17}H_{19}NO_{3}</td>
<td>NS</td>
<td>E</td>
<td>[29]</td>
</tr>
<tr>
<td>15</td>
<td>4’-O-Methylarmepavine</td>
<td>C_{17}H_{19}NO_{3}</td>
<td>NS</td>
<td>L</td>
<td>[37]</td>
</tr>
<tr>
<td>16</td>
<td>Caaverine</td>
<td>C_{17}H_{19}NO_{3}</td>
<td>(-)-R</td>
<td>L</td>
<td>[35,38]</td>
</tr>
<tr>
<td>17</td>
<td>Asimilobine</td>
<td>C_{17}H_{19}NO_{3}</td>
<td>(-)-R</td>
<td>L, F</td>
<td>[31,38,39]</td>
</tr>
<tr>
<td>18</td>
<td>Glaziovine</td>
<td>C_{17}H_{19}NO_{3}</td>
<td>N/A</td>
<td>F</td>
<td>[33]</td>
</tr>
<tr>
<td>19</td>
<td>O-Nonclicferine</td>
<td>C_{17}H_{19}NO_{3}</td>
<td>(-)-R</td>
<td>L, F</td>
<td>[13,38,40]</td>
</tr>
<tr>
<td>20</td>
<td>N-Nonclicferine</td>
<td>C_{17}H_{19}NO_{3}</td>
<td>(-)-R</td>
<td>L, E, F</td>
<td>[13,29,38]</td>
</tr>
<tr>
<td>21</td>
<td>Lirinidine</td>
<td>C_{17}H_{19}NO_{3}</td>
<td>(+)-R</td>
<td>L, F</td>
<td>[13]</td>
</tr>
<tr>
<td>22</td>
<td>N-Methyleneisobiline</td>
<td>C_{17}H_{19}NO_{3}</td>
<td>N/A</td>
<td>F</td>
<td>[32]</td>
</tr>
<tr>
<td>23</td>
<td>Roemerine</td>
<td>C_{17}H_{19}NO_{3}</td>
<td>(-)-R</td>
<td>L, F</td>
<td>[38–40–42]</td>
</tr>
<tr>
<td>24</td>
<td>Dehydroclicferine</td>
<td>C_{17}H_{19}NO_{3}</td>
<td>N/A</td>
<td>L, R</td>
<td>[13,34,41]</td>
</tr>
<tr>
<td>25</td>
<td>Dehydroanamine</td>
<td>C_{17}H_{19}NO_{3}</td>
<td>N/A</td>
<td>L</td>
<td>[34]</td>
</tr>
<tr>
<td>26</td>
<td>Dehydroroemerine</td>
<td>C_{17}H_{19}NO_{3}</td>
<td>N/A</td>
<td>L</td>
<td>[34]</td>
</tr>
<tr>
<td>27</td>
<td>Pronclicferine</td>
<td>C_{17}H_{19}NO_{3}</td>
<td>(+)-R and (-)-S</td>
<td>L, E, F</td>
<td>[13,29,35,37]</td>
</tr>
<tr>
<td>28</td>
<td>Nuclicferine</td>
<td>C_{17}H_{19}NO_{3}</td>
<td>(-)-R</td>
<td>L, E, F</td>
<td>[29,31,38,40]</td>
</tr>
<tr>
<td>29</td>
<td>7-Hydroxydehydroclicferine</td>
<td>C_{17}H_{19}NO_{3}</td>
<td>N/A</td>
<td>L</td>
<td>[38]</td>
</tr>
<tr>
<td>30</td>
<td>Lycicamine</td>
<td>C_{17}H_{19}NO_{3}</td>
<td>N/A</td>
<td>L, F</td>
<td>[13]</td>
</tr>
<tr>
<td>31</td>
<td>Cephardione B</td>
<td>C_{17}H_{19}NO_{3}</td>
<td>N/A</td>
<td>L</td>
<td>[32]</td>
</tr>
<tr>
<td>32</td>
<td>Anonaine</td>
<td>C_{17}H_{19}NO_{3}</td>
<td>(-)-R</td>
<td>L</td>
<td>[38,41]</td>
</tr>
<tr>
<td>33</td>
<td>Liridenine</td>
<td>C_{17}H_{19}NO_{3}</td>
<td>N/A</td>
<td>L</td>
<td>[38]</td>
</tr>
<tr>
<td>34</td>
<td>Nelumboferine</td>
<td>C_{17}H_{19}NO_{3}</td>
<td>NS</td>
<td>E, S</td>
<td>[41,43]</td>
</tr>
<tr>
<td>35</td>
<td>Liensine</td>
<td>C_{17}H_{19}NO_{3}</td>
<td>1R,1′R</td>
<td>L, E, F, S</td>
<td>[39–41,44]</td>
</tr>
<tr>
<td>36</td>
<td>Isoiensine</td>
<td>C_{17}H_{19}NO_{3}</td>
<td>1R,1′S</td>
<td>E</td>
<td>[40,44]</td>
</tr>
<tr>
<td>37</td>
<td>Dauculin</td>
<td>C_{17}H_{19}NO_{3}</td>
<td>NS</td>
<td>S</td>
<td>[5]</td>
</tr>
<tr>
<td>38</td>
<td>6-Hydroxynorisosilensine</td>
<td>C_{17}H_{19}NO_{3}</td>
<td>NS</td>
<td>E</td>
<td>[29]</td>
</tr>
<tr>
<td>39</td>
<td>N-Norsoilensine</td>
<td>C_{17}H_{19}NO_{3}</td>
<td>NS</td>
<td>E</td>
<td>[29]</td>
</tr>
<tr>
<td>40</td>
<td>Nelumbonine</td>
<td>C_{17}H_{19}NO_{3}</td>
<td>NS</td>
<td>E</td>
<td>[43]</td>
</tr>
<tr>
<td>41</td>
<td>Daucinoline</td>
<td>C_{17}H_{19}NO_{3}</td>
<td>NS</td>
<td>S</td>
<td>[5]</td>
</tr>
<tr>
<td>42</td>
<td>Neferine</td>
<td>C_{17}H_{19}NO_{3}</td>
<td>1R,1′S</td>
<td>E, S</td>
<td>[40,41,44]</td>
</tr>
<tr>
<td>43</td>
<td>Daurine</td>
<td>C_{17}H_{19}NO_{3}</td>
<td>NS</td>
<td>S, R</td>
<td>[45]</td>
</tr>
</tbody>
</table>

Bisbenzylisoquinoline

<table>
<thead>
<tr>
<th>No.</th>
<th>Alkaloid</th>
<th>Formula</th>
<th>Enantiomer</th>
<th>Organ</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>34</td>
<td>Nelumboferine</td>
<td>C_{17}H_{19}NO_{3}</td>
<td>NS</td>
<td>E, S</td>
<td>[41,43]</td>
</tr>
<tr>
<td>35</td>
<td>Lieninsine</td>
<td>C_{17}H_{19}NO_{3}</td>
<td>1R,1′R</td>
<td>L, E, F, S</td>
<td>[39–41,44]</td>
</tr>
<tr>
<td>36</td>
<td>Isoieninsine</td>
<td>C_{17}H_{19}NO_{3}</td>
<td>1R,1′S</td>
<td>E</td>
<td>[40,44]</td>
</tr>
<tr>
<td>37</td>
<td>Dauculin</td>
<td>C_{17}H_{19}NO_{3}</td>
<td>NS</td>
<td>S</td>
<td>[5]</td>
</tr>
<tr>
<td>38</td>
<td>6-Hydroxynorisolensine</td>
<td>C_{17}H_{19}NO_{3}</td>
<td>NS</td>
<td>E</td>
<td>[29]</td>
</tr>
<tr>
<td>39</td>
<td>N-Norsoilensine</td>
<td>C_{17}H_{19}NO_{3}</td>
<td>NS</td>
<td>E</td>
<td>[29]</td>
</tr>
<tr>
<td>40</td>
<td>Nelumbonine</td>
<td>C_{17}H_{19}NO_{3}</td>
<td>NS</td>
<td>E</td>
<td>[43]</td>
</tr>
<tr>
<td>41</td>
<td>Daucinoline</td>
<td>C_{17}H_{19}NO_{3}</td>
<td>NS</td>
<td>S</td>
<td>[5]</td>
</tr>
<tr>
<td>42</td>
<td>Neferine</td>
<td>C_{17}H_{19}NO_{3}</td>
<td>1R,1′S</td>
<td>E, S</td>
<td>[40,41,44]</td>
</tr>
<tr>
<td>43</td>
<td>Daurine</td>
<td>C_{17}H_{19}NO_{3}</td>
<td>NS</td>
<td>S, R</td>
<td>[45]</td>
</tr>
</tbody>
</table>

Tribenzylisoquinoline

<table>
<thead>
<tr>
<th>No.</th>
<th>Alkaloid</th>
<th>Formula</th>
<th>Enantiomer</th>
<th>Organ</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Neoilensine</td>
<td>C_{17}H_{19}NO_{3}</td>
<td>1R,1′S,1′R</td>
<td>E</td>
<td>[44]</td>
</tr>
</tbody>
</table>

In contrast to the preponderance of S-conformational BIAs in Ranunculaceae, the majority of BIAs observed in sacred lotus are R-conformational. As a result of the presence of this anomalous stereochemistry in sacred lotus, it is possible that the biosynthesis of sacred lotus will contain unique pathways or homologous enzymes. The most recent study conducted by Menéndez-Perdomo and J. Facchini has provided further validation that dopamine and 4-HPAA, both derived from L-tyrosine, serve as the precursors for the synthesis of (R,S)-norcoclaurine in the sacred lotus plant. Conversely, it was observed that in other plant species, the production of (R)-norcoclaurine by-products was predominantly favoured due to the presence of R-enantiomeric methyltransferase and CYPs. The presence of these enzymes has been shown to have a role in the synthesis of diverse 1-benzylisoquinolines inside the sacred lotus plant. The study also showed that the enzymes accountable for the production of R-enantiomers of pre-aporphine (NnCYP80Q1) and bisbenzylisoquinoline (NnCYP80Q2), as well as the incorporation of methylenedioxy bridges on the aporphine substrate (NnCYP719A22), exhibit identical characteristics[59].
Nevertheless, there are still unresolved matters pertaining to the examination of the biosynthetic pathway of the sacred lotus’ benzylisoquinoline alkaloids (BIAs). Firstly, it is observed that BIAs mostly occur in R-enantiomers, whereas S-enantiomers are more prevalent in the order Ranunculales. The enantioselective synthesis of (S)-norcoclaurine has been facilitated by NCS catalysis. However, it has been shown that both (R)-norcoclaurine and (S)-norcoclaurine are present in sacred lotus, suggesting the presence of diastereoselective enzymes or two distinct NCS orthologs that selectively favour either the R or S enantiomer. Secondly, it was observed that no benzylisoquinoline alkaloids (BIAs) bearing a 3’-hydroxyl group were detected in the benzyl moiety. This absence may be attributed to the absence of the NMCH enzyme. This suggests that N-methylcoclaurine serves as a pivotal intermediary in the production of proaporphine, aporphine, and bisbenzylisoquinoline alkaloids in the sacred lotus. Thirdly, in contrast to the direct conversion of (S)-reticuline bases to aporphine alkaloids through C-C and C-O coupling in plants of the order Ranunculales, the appearance of proaporphine in the sacred lotus plant indicated that 1-benzylisoquinoline substrates were indirectly transformed into apophrine in the absence of ortho or para-substituents in the phenyl moiety. Fourthly, it was shown that bisbenzylisoquinolines exist as head-to-tail dimers in the sacred lotus, but only tail-to-tail couplings were detected in plants belonging to the Ranunculales order. The occurrence of aporphine and bisbenzylisoquinoline alkaloids in Ranunculales plants is attributed to the intramolecular C-C and intermolecular C-O coupling of BIAs. These coupling reactions are facilitated by enzymes belonging to the CYP80 family. The major aporphine alkaloid found in sacred lotus has an isoquinoline component that is characterised by the presence of a methylenedioxy bridge. The production of protoberberine in Ranunculales involves the participation of many enzymes belonging to the CYP719A subfamily, which catalyse the formation of methylenedioxy bridges. Notably, this biosynthetic pathway is absent in the sacred lotus plant.

Possible biosynthetic pathways
According to the study of BIAs biosynthesis, the possible BIAs biosynthesis pathway of sacred lotus can be obtained, which is mainly divided into three parts (Fig. 1). Firstly, the common biosynthetic pathway of sacred lotus and Ranunculales species is the Pictet-Spengler condensation of two L-tyrosine derivatives dopamine and 4-hydroxyphenylacetaldehyde (4-HPAA) to produce (R,S)-norcoclaurine. It was catalyzed by NnOMT1 to generate coclaurine, which was then catalyzed by NnNMT to generate N-methylcoclaurine. It also served as a central branch point for the biosynthesis of various BIAs. N-methylcoclaurine was catalyzed by cytochrome P450 monooxygenase 80B to generate 3’-hydroxy-N-methylcoclaurine, and it was catalyzed by 4’-O-methyltransferase to generate coacurine, which was then catalyzed by NnOMT1 to generate norcoclaurine. This was followed by the synthesis pathway of bisbenzylisoquinoline alkaloids in sacred lotus heart and aporphine alkaloids in sacred lotus leaves: N-methylcoclaurine was catalyzed by NnCYP80Q1 in sacred lotus to nelumboferine (bisbenzylisoquinoline alkaloids). N-methylcoclaurine is catalyzed by NnCYP80Q2 to N-methylcrotsparine (proaporphine), which may generate lirinidine through reduction, dehydration and aromatic ring rearrangement, and generate

![Fig. 1 Possible biosynthetic pathways of BIAs in sacred lotus.](image-url)
anomaine under the action of NnCYP719A22. In addition, it is speculated that the aporphine alkaloids in sacred lotus may also come from reticuline and generate various aporphine alkaloids under the catalysis of CYP80G, 7OMT, ODM, NDM and other enzymes. Thirdly, the synthesis of 1-benzylisoquinoline alkaloids, N-methylcoclaurine was catalyzed by NnOMT5 / 7 (7OMT) to armepavine.

**BIA biosynthetic genes and enzymes in the sacred lotus**

**Norcoclaurnine synthase**

The enzymatic pathway leading to the surprising diversity of benzylisoquinoline derivatives has been shown to originate from a common route, in which the first step is the NCS-catalyzed Pictet-Spengler condensation of dopamine with (4-HPAA) to produce (S)-norcoclaurnine[60,61]. However, (R)- and (S)-norcoclaurnine were both detected in sacred lotus. NCS selectively catalyzed the formation of (S)-norcoclaurnine, which indicated that there may be diastereoselective enzymes or two different R- and S-enantiomerically selective NCS orthologs[59]. Recently, the study of Menéndez-Perdomo & Facchini proposed a new possibility that the formation of (R)- and (S)-desmethylhengzhouaconitine in lotus is a spontaneous, non-enzymatic Pictet-Spengler condensation reaction of dopamine and 4-HPAA[58]. It can be seen that the study of NCS in lotus is of great significance to the interpretation of lotus-specific R configuration, and the study of NCS needs to be further promoted.

**Methylation**

Methylation, a frequent biological change in plants, plays an important role in the structural and functional diversity of BIAs. By adding methyl groups, BIAs’ chemical characteristics, including as steric effects, overall hydrophobicity, and electronic properties, can be altered, resulting in a shift in biological activity. Methylation processes known as methyltransferases employed S-adenosyl-L-methionine as a methyl donor[62]. The widespread terminal alteration on BIAs of sacred lotus by methyltransferases, including O-methylation and N-methylation, is also a source of its variety.

**OMT**

So far, OMTs in sacred lotus have largely been studied in terms of gene expression, with little functional characterisation of the encoded proteins[63–65]. Despite the fact that BIAs were largely active metabolites in N. nucifera, only three OMTs engaged in the 1-BIA upstream biosynthetic pathway in N. nucifera were discovered in vitro[66]. Two OMTs implicated in BIA metabolism in sacred lotus, which catalysed the 6-O and 7-O-methylation of the 1-benzylisoquinoline backbone, have been functionally characterised. In sacred lotus, the 1-benzylisoquinoline backbone was mostly O-methylated at the C6, C7, and/or C4’ locations, yielding a range of 1-benzylisoquinoline alkaloid compounds[66]. Our lab discovered a new and regiospecific O-methyltransferase (NnOMT6) that methylated monobenzylisoquinoline 6-O/7-O, aporphine skeleton 6-O, phenylpropanoid 3-O, and protoberberine 2-O[67]. Mono-benzylisoquinoline was converted into aporphine and bisbenzylisoquinoline alkaloids in sacred lotus. However, no reports of OMTs catalysing the aporphine and bisbenzylisoquinoline backbones in sacred lotus have been found.

**NMT**

It is unknown how BIAs are N-methylated in sacred lotus. According to chemical structural suggestions, the N position occurred in once or twice methylation to form tertiary amine or quaternary amine (e.g., N-methylcoclaurine and lotusine). Based on transcriptome analysis, two N-methyltransferases, NnCNMT1 and NnCNMT2, were identified from sacred lotus[68]. However, the role of the N-methyltransferase involved in the production of BIAs in sacred lotus has not yet been determined. It is critical to identify the NMT in the biosynthesis of BIAs.

**Cytochrome P450 monoxygenases**

Cytochrome P450 monoxygenases (CYPs) include a heterogeneous collection of heme proteins that facilitate a multitude of reactions within plant-specific metabolic pathways. NADPH-cytochrome P450 reductase is an enzyme responsible for transferring a pair of electrons from NADPH, facilitates the activation of these enzymes[69]. The formation of sacred lotus benzylisoquinoline alkaloids (BIA) is believed to be influenced by two primary cytochrome P450 (CYP) families, namely CYP80 (subfamilies A and G) and CYP719A[69,70].

Menéndez-Perdomo & Facchini’s most recent study characterised the functions of NnCYP80Q1, NnCYP80Q2, and NnCYP719A22, which were responsible for the formation of pre-aporphine R-enantiomers, dibenzylisoquinoline R-enantiomers, and the formation of methylenedioxy bridges on the aporphine substrate[59]. Based on predictions, the catalytic mechanism of cytochrome P450 enzymes (CYPs) involves several key reactions. Firstly, an intramolecular C-C phenol coupling occurs between the C8 and C1’ positions of 1-benzylisoquinoline substrates, resulting in the formation of the corresponding pro-aporphine compound. Additionally, an intermolecular head-to-tail C-O phenol coupling reaction takes place between the C7-hydroxyl and C3’ positions of two 1-benzylisoquinoline substrates, leading to the production of the corresponding bisbenzylisoquinoline compound. Furthermore, the oxidative cyclisation of the ortho-hydroxyl group of the isoquinoline moiety in the aporphine substrate, along with the methoxy-substituted aromatic ring, results in the formation of a methylenedioxy bridge[69].

**Conclusions**

The extraordinary therapeutic potential of BIAs is one of the reasons why they have garnered so much interest. In contrast to the S-conformation seen in Ranunculaceae, the sacred lotus, which belongs to an ancient group of aquatic basal plants, has an exceptionally high number of BIAs that have an R-conformation. The investigation of the in vitro synthesis and the pharmacological efficacy of BIAs will be helped along by the discovery of important genes and functional enzymes connected to the BIAs biosynthesis.

**Author contributions**

The authors confirm contribution to the paper as follows: conceptualization and supervision: Chen S; draft manuscript and figure preparation: Chen Z; manuscript review and editing: Zhao H. All authors reviewed and approved the final version of the manuscript.
Data availability

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

Conflict of interest

The authors declare that they have no conflict of interest.

Dates

Received 16 August 2023; Accepted 3 November 2023; Published online 18 December 2023

References

loids of Nelumbo nucifera. Phytochemistry. 12:699−701
35. Do TCMV, Nguyen TD, Tran H, Stupnner H, Ganzera M. 2013. Analy- 
sis of alkaloids in Lotus (Nelumbo nucifera Gaertn.) leaves by non-
aqueous capillary electrophoresis using ultraviolet and mass spec- 
trometric detection. Journal of Chromatography A 1302:174−80
36. Ka SM, Kuo YC, Ho PJ, Tsai PY, Hsu YJ, et al. 2010. (S)−armpavine 
from Chinese medicine improves experimental autoimmune cres-
cent glomerulonephritis. Rheumatology 49:1840−51
37. Guo Y, Chen X, Qi J, Yu B. 2016. Simultaneous qualitative and 
quantitative analysis of flavonoids and alkaloids from the leaves of 
Nelumbo nucifera Gaertn. using high-performance liquid chro- 
matography with quadrupole time-of-flight mass spectrometry. 
Journal of Separation Science 39:2409−507
and anticancer aporphine alkaloids from the leaves of Nelumbo 
nucifera Gaertn. cv. Rasa-plena. Molecules 19:17829−38
ether-à-go-go related gene (hERG) channel blocking aporphine 
alkaloids from lotus leaves and their quantitative analysis in dietary 
weight loss supplements. Journal of Agricultural and Food 
Chemistry 63:5634−29
and comparison of anti-inflammatory ingredients from different 
organs of Lotus nelumbo by UPLC/Q-TOF and PCA coupled with a 
NF-κB reporter gene assay. PLoS ONE 8:81971
of isoquinoline alkaloid composition and wound-induced variation in Nelumbo using HPLC-MS/MS. Journal of Agricultural 
and Food Chemistry 64:1130−36
Constituents of Nelumbo nucifera leaves and their antimarial and 
antifungal activity. Phytochemistry Letters 1:89−93
Bisbenzylisoquinoline alkaloids from Nelumbo nucifera. 
Chemical Pharmaceutical Bulletin 59:947−51
identification of a tricyclicbenzylisoquinoline alkaloid from 
Nelumbo nucifera Gaertn., a novel potential smooth muscle relaxant. 
Fitoterapia 124:58−65
45. Zhao X, Shen J, Chang KJ, Kim SH. 2014. Comparative analysis of 
antioxidant activity and functional components of the ethanol 
extract of lotus (Nelumbo nucifera) from various growing regions. 
Journal of Agricultural and Food Chemistry 62:6227−30
Alzheimer and antioxidant effects of Nelumbo nucifera L. alkaloids, 
nuciferine and norcoclaurine in alloxan-Induced diabetic albino 
rats. Pharmaceuticals 15:1205
47. Liu CP, Tsai WJ, Shen CC, Lin YL, Liao JF, et al. 2006. Inhibition of 
(S)−armpavine from Nelumbo nucifera on autoimmune disease of 
MRL/Mp−lpr/lpr mice. European Journal of Pharmacology 531:270−79
sine and Puerarin in Rehabilitating Alcohol-Induced Metabolic 
Disorder Based on UPLC-MS/MS. International Journal of Molecular 
Sciences 23:10385
on solar UV-induced matrix metalloproteinase-1 expression. Plants 
11:773
muciniphila: A potential novel mechanism of nuciferine to improve 
hyperlipidemia. BioMedicine & Pharmacotherapy 133:111014
enhances insulin sensitivity in insulin resistant rats. Journal of 
Ethnopharmacology 124:98−102
uates atherosclerosis by regulating the proliferation and migra-
tion of VSMCs through the Calm4/MMPI2/AKT pathway in ApoE−/− mice fed with High-Fat-Diet. Phytochemistry 108:154536
alkaloid from Nelumbo nucifera as an acetylcholinesterase inhibitor 
and the primary investigation for structure–activity correlations. 
Natural Product Research 26:387−92
permeable aporphine-type alkaloids in Nelumbo nucifera flowers 
with accelerative effects on neurite outgrowth in PC-12 cells. 
Journal of Natural Medicines 74:212−18
Protective effect of nuciferine in permanent cerebral ischemic 
rats via anti-oxidative and anti-apoptotic mechanisms. 
Neurotoxicity Research 40:1348−59
an alkaloid from lotus seed embryos, exerts antiseizure and neuro-
protective effects in a kainic acid-induced seizure model in rats. 
International Journal of Molecular Sciences 23:4130
cular endothelial inflammation by inhibiting the NF-κB signaling 
pathway. Archives of Biochemistry and Biophysics 696:108595
lotus seed embryo, inhibits human lung cancer cell growth by 
MAPK activation and cell cycle arrest. Biofactors 40:121−31
59. Menéndez-Perdomo IM, Facchini PJ. 2023. Elucidation of the (R)- 
enantiospecific benzylisoquinoline alkaloid biosynthetic path-
ways in sacred lotus (Nelumbo nucifera). Scientific Reports 13:2955
60. Facchini PJ, St-Pierre B. 2005. Synthesis and trafficking of alkaloid 
synthetic enzymes. Current Opinion In Plant Biology 8:657−66
61. Stadler R, Zenk MH. 1990. A revision of the generally accepted 
pathway for the biosynthesis of the benzyltetrahydroisouquinoline 
alkaloid reticuline. Liebig’s Annalen der Chemie 6:555−62
62. Liscombe DK, Louie GV, Noel JP. 2012. Architectures, mechanisms 
and molecular evolution of natural product methyltransferases. 
Natural Product Reports 29:1238−50
analysis provides insight into the transcript profile of the genes 
involved in aporphine alkaloid biosynthesis in lotus (Nelumbo 
nucifera). Frontiers in Plant Science 8:80
Coregulation of biosynthetic genes and transcription factors for 
aporphine-type alkaloid production in wounded lotus provides 
insight into the biosynthetic pathway of nuciferine. ACS Omega 3:8794−802
tion of benzylisoquinoline alkaloid biosynthetic pathway and its 
transcriptional regulation in lotus. Horticulture Research 5:29
66. Menéndez-Perdomo IM, Facchini PJ. 2020. Isolation and character-
ization of two O-methyltransferases involved in benzylisouquin-
one alkaloid biosynthesis in sacred lotus (Nelumbo nucifera). Journal Of Biological Chemistry 295:1598−612
terization and key residues engineering of a regiopromiscuity 
O-methyltransferase involved in benzylisoquinoline alkaloid bio-
synthesis in Nelumbo nucifera. Horticulture Research 10:100276
Distribution and structure. Annals of Botany 39:713−19
Genomics 4:59
70. Nelson DR, Schuler MA. 2013. Cytochrome P450 genes from the 
sacred lotus genome. Tropical Plant Biology 6:138−51

Chen et al. Medicinal Plant Biology 2023, 2:20