Organic LETTERS

Letter

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# Monoterpene Indole Alkaloid-Like Compounds Based on Diversity-Enhanced Extracts of Iridoid-Containing Plants and Their Immune Checkpoint Inhibitory Activity

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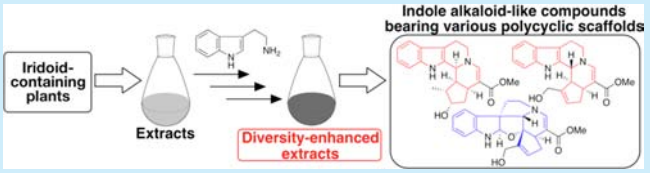
Supporting Information

**ABSTRACT:** A library of iridoid-conjugated indole alkaloid-like compounds was constructed from diversity-enhanced extracts, which constitutes an approach for increasing the chemical diversity of natural-product-like compounds by combining natural product chemistry and diversity-oriented synthesis. Pharmacological screening of the library revealed a seed compound that can be used for the development of small molecular immune checkpoint inhibitors.




Natural products and their derivatives have long played an essential role in the development of novel drugs because of their structural diversity. Nevertheless, pharmaceutical research into natural products has recently declined because of factors such as the difficulty of collecting novel compounds containing privileged structures.[1,2] Hence, new approaches to augmenting their chemical diversity are crucial for retaining the utility of natural products and their derivatives.

Recently, we proposed the use of "diversity-enhanced extracts",[3] an approach for increasing the chemical diversity of natural-product-like compounds by combining natural product chemistry and diversity-oriented synthesis.[4] Diversity-enhanced extracts are obtained from chemical reactions that remodel molecular scaffolds directly in the extracts of natural resources. The subsequent isolation of each compound produced from such reactions affords a diverse natural-product-like library bearing new molecular scaffolds. Some studies using similar methods that chemically convert natural extracts have been reported.[5−10] In these studies, functional groups are converted and are then added to the original natural products, but new molecular scaffolds are not obtained. The use of diversity-enhanced extracts is an unprecedented approach of applying reactions that form new carbon−carbon bonds and produce new molecular scaffolds.[3]

Many monoterpene indole alkaloids[11] such as ajmaline and yohimbine have been identified from natural resources (Figure 1A). Their structures contain $sp^3$-rich terpenoid scaffolds[12] and nitrogen-containing alkaloid scaffolds,[13] which confer various pharmacological activities. In their biosynthetic pathways, strictosidine is produced via the Pictet−Spengler-type reaction[14] between tryptamine and secologanin.[11] Strictosidine is important in that it is a common intermediate and that it provides many monoterpene indole alkaloids. Recently, bioengineering modification of strictosidine synthase has enabled it to accept substituted tryptamines and secologanins to produce substituted strictosidine.[15] However, monoterpenes other than secologanin cannot be accepted by strictosidine synthase. Indole alkaloids containing several monoterpene moieties other than secologanin that can be produced may be useful for constructing chemically diverse compound libraries for drug discovery.

In this letter, we report the construction of a library of iridoid-conjugated indole alkaloid-like compounds from diversity-enhanced extracts of classical medicinal plants (Figure 1B). Among these compounds, compound **1** decreases the production of IL-10 and inhibits the expression of the cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) gene, exhibiting potential as a novel small-molecule immune checkpoint inhibitor.

To produce indole alkaloid-like compounds from the diversity-enhanced extracts, we focused on iridoids, which are a type of monoterpenes typically occurring as glucosides in some plants. Many iridoids contain a hemiacetal moiety and can therefore react with tryptamine to form iminium cations. Pictet−Spengler reaction between an indole ring and iminium cations affords indole alkaloid-like compounds bearing synthetic pentacyclic skeletons (Scheme 1). Pictet−Spengler reaction of tryptamine has been extensively utilized for synthesis of indole alkaloids and their derivatives.[16] However, Pictet−Spengler reaction between tryptamine and iridoids has been reported only once.[17]

*Cornus officinalis* and *Gardenia jasminoides*, traditional medicinal plants that contain various iridoid glucosides,[18,19] were used as starting materials. First, each of the plant materials was extracted with methanol. Second, a large amount of sugars

**Received:** October 12, 2016
**Published:** November 10, 2016





DOI: 10.1021/acs.orglett.6b03057
*Org. Lett.* 2016, 18, 5948−5951

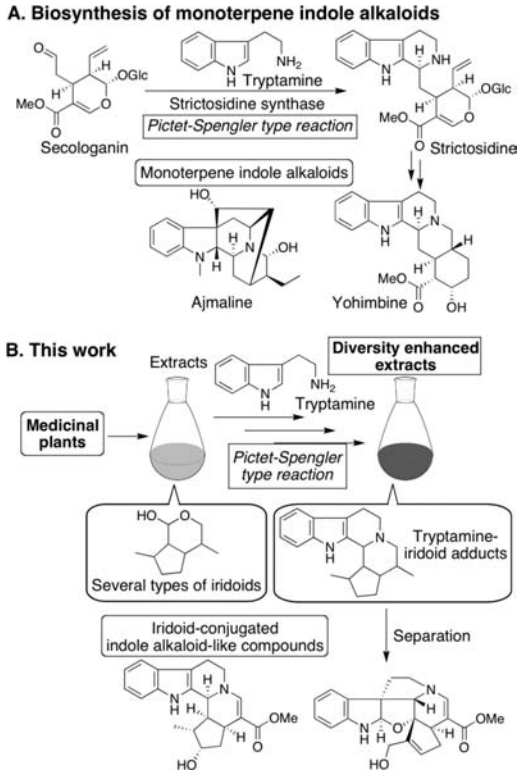
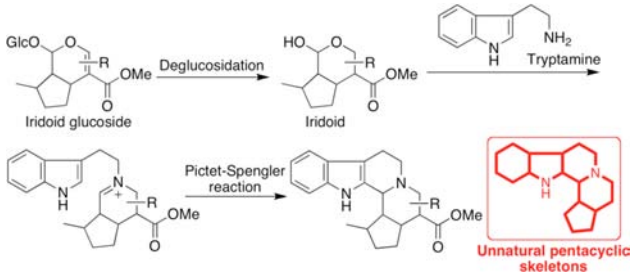



**Figure 1.** Schematic diagram for natural monoterpene indole alkaloids and iridoid-conjugated indole alkaloid-like compounds from diversity-enhanced extracts.

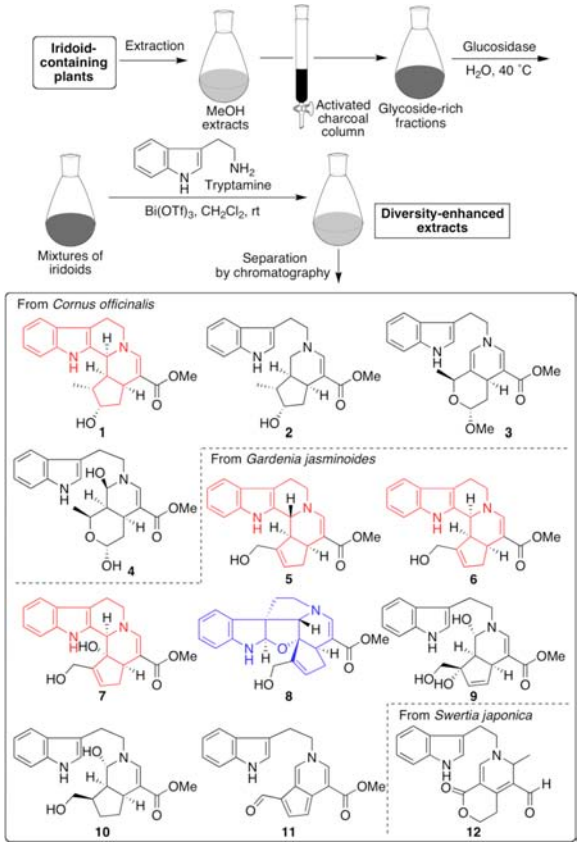
**Scheme 1. Synthetic Route for Iridoid-Conjugated Indole Alkaloid-Like Compounds**




was removed from the extracts by charcoal chromatography to produce glycoside-rich fractions. Their reactions with glucosidase resulted in the deglucosidation of iridoid glucosides, affording mixtures of iridoids. Finally, these mixtures were subjected to tryptamine treatment catalyzed by bismuth triflate followed by the Pictet–Spengler reaction to obtain diversity-enhanced extracts containing indole alkaloid-like compounds (Scheme 2). In this reaction, we used metal triflates lanthanum triflate and ytterbium triflate instead of bismuth triflate. However, these triflates are not superior to bismuth triflate.

The diversity-enhanced extracts of *C. officinalis* and *G. jasminoides* were separated by repeated column chromatography. Four indole-conjugated compounds (**1**–**4**) were isolated from the extracts of *C. officinalis* (Scheme 2), while seven compounds (**5**–**11**) were isolated from those of *G. jasminoides*. All of these compounds have not been reported so far. The structures of these compounds were established using NMR spectra (see Supporting Information). For example, $^{1}$H–$^{1}$H COSY and HMBC spectra in Figure 2A reveal the planar structure of

**Scheme 2. Iridoid-Conjugated Indole Alkaloid-Like Compounds Isolated from the Diversity-Enhanced Extracts of Iridoid-Containing Medicinal Plants**



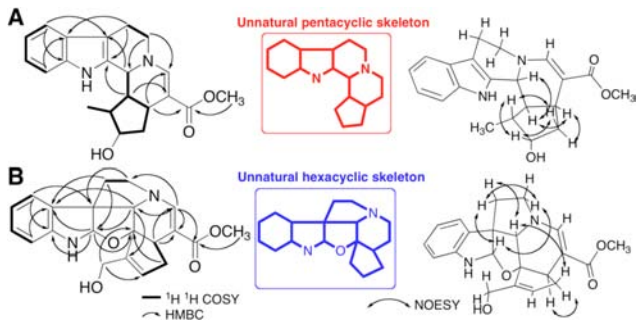



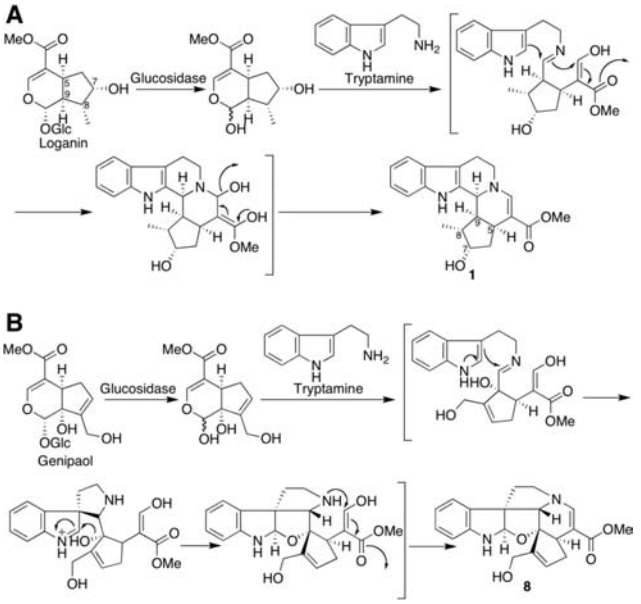

**Figure 2.** Structural elucidation of (A) the pentacyclic compound **1** and (B) the hexacyclic compound **8**.

pentacyclic compound **1**. The relative stereochemistry of **1** was determined from its NOESY spectrum. Compound **1** is assumed to be produced from loganin,[18] a constituent of *C. officinalis*, by sequential deglucosidation, imination, and Pictet–Spengler reaction (Scheme 3A). Because the stereochemistry at C-5, -7, -8, and -9 is retained through these reactions, the absolute configurations at C-5, -7, -8, and -9 in **1** are assumed to be *S*, *S*, *R*, and *S*, respectively, which are the same as those in loganin. Compound **1** has an unnatural pentacyclic skeleton, which has been reported only once.[17] Compounds **5**–**7** have also unnatural pentacyclic skeletons. Compounds **5** and **6** are assumed to originate from geniposide,[19] a constituent of *G. jasminoides*. No study about the isolation of the origin of **7** from *G. jasminoides* has been reported; nevertheless, an iridoid, genipaol isolated from



DOI: 10.1021/acs.orglett.6b03057
*Org. Lett.* 2016, 18, 5948–5951

**Scheme 3. Plausible Pathways for the Synthesis of (A) Pentacyclic Compound 1 and (B) Hexacyclic Compound 8**




*Genipa americana*,[20] corresponds to the structure of **7** (Scheme S1 in Supporting Information).

Compound **8** has an unexpected hexacyclic skeleton, which was elucidated by its NMR spectra (Figure 2B). Compound **8** possibly originates from genipaol as well, which reacts with tryptamine to produce an imine. Through its beta position (instead of the $\alpha$ position), the indole attacks the imine in a typical Pictet–Spengler reaction, producing a spiro intermediate. A tertiary alcohol attacks the $\alpha$ position in the indole, and tetrahydropyridine subsequently forms, affording **8** (Scheme 3B). This hexacyclic skeleton is unprecedented in both synthetic and natural compounds.

Using another medicinal plant that contains secoiridoid glucosides, *Swertia japonica*, we also prepared diversity-enhanced extracts, albeit on a small scale. Compound **12** thus obtained from the diversity-enhanced extracts of *S. japonica* is assumed to be produced from swertiamarin.[21]

To verify the usefulness of the library of the iridoid-conjugated indole alkaloid-like compounds for drug discovery and to discover new pharmacologically active compounds, the synthetic compounds were screened for biological activities. Cancer immunotherapy involves the use of the immune system for cancer treatment. Recently, immune checkpoint inhibitors have become an integral part to treating some types of cancers.[22] Immune checkpoints are negative regulators of the immune system, playing key roles in preventing autoimmunity and in maintaining self-tolerance. Programmed cell death-1 (PD-1) and CTLA-4 are typical immune checkpoint proteins on T cells. Sometimes, cancer cells find ways to use these checkpoints to avoid attack by the immune system. Recently, ipilimumab (Yervoy), an anti-CTLA-4 monoclonal antibody, as well as pembrolizumab (Keytruda) and nivolumab (Opdivo), anti-PD-1 monoclonal antibodies, have been approved. These antibody drugs exhibit clinically significant antitumor responses but are highly expensive and can be dosed only by parenteral routes. Thus, the development of small-molecule immune checkpoint inhibitors is urgently required.[23]

CTLA-4 is constitutively expressed by $CD4^+CD25^+FOXP3^+$ regulatory T cells (Treg); it plays important roles in immune regulation.[24] We then focused on discovery of small-molecule inhibitors against CTLA-4 to develop new immune checkpoint inhibitors. In the first screening, the effects of indole alkaloid-like compounds on the production of IL-10, an anti-inflammatory cytokine associated with $FOXP3^+$ Treg,[25] were tested. Compounds **1**, **2**, **5**, **8**, and **10** were found to exhibit inhibitory activity at 10–20 $\mu$M (Figures 3A and S1). We then examined

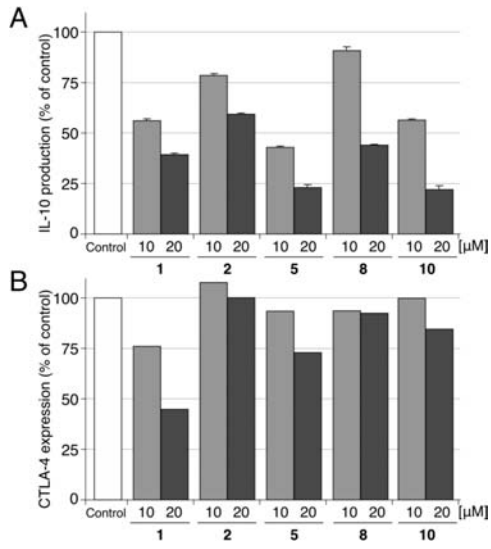



**Figure 3.** Immune checkpoint inhibitory effect of iridoid-conjugated indole-alkaloid-like compounds. (A) Effects of selected compounds on the production of IL-10 as determined by ELISA. (B) Effects of selected compounds on the gene expression of CTLA-4.

the inhibitory effects of these compounds on the gene expression of CTLA-4, which inhibits the production of IL-10. Although **2**, **8**, and **10** exhibited no effect, **1** inhibited the gene expression of CTLA-4 in a concentration-dependent manner (Figure 3B). Compound **5** also exhibited weak inhibitory activity. It has already been reported that atorvastatin and lovastatin inhibited CTLA-4 expression.[26] However, compound **1** inhibited not only CTLA-4 expression but also the production of anti-inflammatory IL-10, suggesting that compound **1** can be a seed compound for small-molecule immune checkpoint inhibitors.

In summary, we constructed a library of iridoid-conjugated indole alkaloids from diversity-enhanced extracts of medicinal plants. The library contains diverse compounds bearing various polycyclic scaffolds, of which there are few or no reports. Although the isolation of these iridoid-conjugated indole alkaloids appeared to be tedious, we utilized only two or three successive column chromatography steps for their separation, similar to the methods utilized for the isolation of natural products. In reality, the isolation of iridoid glucosides is difficult,[27] and obtaining indole alkaloid-like compounds by chemical conversion of isolated iridoid glucosides is even more difficult. Hence, the utilization of diversity-enhanced extracts can provide diverse natural-product-like compounds, which are difficult to obtain by other synthetic methods. However, if a compound is easily isolated from extracts, it is more effective to apply diversity-oriented synthesis based on a purified natural compound than to use the method of diversity-enhanced extracts. It is important to use properly both methods, diversity-oriented synthesis and diversity enhanced extracts.

Compound **1**, a seed compound for small-molecule immune checkpoint inhibitors, was obtained by pharmacological screen-



DOI: 10.1021/acs.orglett.6b03057
*Org. Lett.* 2016, 18, 5948–5951

ing for iridoid-conjugated indole alkaloids. This fact indicates that the use of diversity-enhanced extracts is an effective methodology for constructing chemical libraries for screening biologically active compounds. To expand the chemical diversity of the compound library, more substituted indole alkaloid-like compounds will be obtained by using substituted tryptamines instead of tryptamine in the Pictet−Spengler reaction of iridoid-containing extracts. In addition, compounds bearing additional types of polycyclic scaffolds will be obtained by iso-Pictet−Spengler reactions[28] using several aminoalkylindoles with iridoid-containing extracts.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b03057.

Supplementary Figures, general experimental procedures, detailed experimental procedures, and characterization data for all new compounds (PDF)

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### Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

This work was supported in part by the Grants-in-Aid for Scientific Research (nos. 25350959 and 16H03279) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan; the Platform Project for Supporting in Drug Discovery and Life Science Research from Japan Agency for Medical Research and development (AMED); the Shorai Foundation for Science and Technology; Kobayashi International Scholarship Foundation; and the Takeda Science Foundation.

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DOI: 10.1021/acs.orglett.6b03057
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