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Letter

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Removal of the *E*-Olefin Barrier of Humulene Leading to Unnatural Terpenoid-like Skeletons

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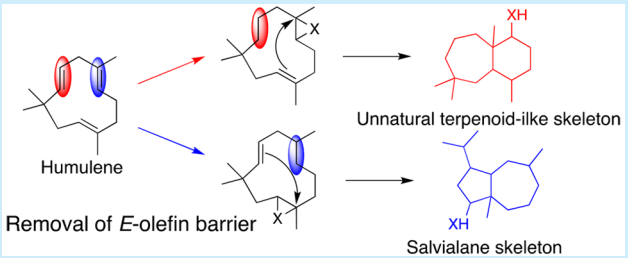
Supporting Information

ABSTRACT: An unnatural terpenoid scaffold containing a bicyclo[5.4.0]undecane moiety, as well as a salvialane skeleton based on an intramolecular C−C bond formation strategy were synthesized. Such a strategy was made possible by the removal of strained *E*-olefin conformations of the humulene skeleton. Some compounds were identified to show PPARα antagonist activity.



The diversity and high three-dimensionality of natural products have made these products useful in developing new drugs. However, because natural products have been explored for a long time, it is becoming difficult to discover structurally diverse compounds.[1,2] Diversity-oriented synthesis based on natural products is an effective strategy for constructing chemical libraries bearing diverse structures.[3−8] There have been some successful examples of the construction of useful compound libraries based on natural products. The "ring-distortion strategy,"[5] proposed by Hergenrother et al., produced compounds that possess a higher molecular complexity and diversity than those from standard screening collections by systematically altering the core ring structures of the natural products[9,10] via chemoselective reactions that distort the ring system. Terpenoids bearing a medium-sized ring system are used in the biosynthesis of sesquiterpenoids.[11] They are also useful precursors for constructing diverse ring systems[7,8] because these systems may be afforded by controlling the cyclization reactions of this process. The structural transformation of compounds containing medium-sized rings is another effective strategy for the construction of diverse and sp^3-rich natural product-like chemical libraries that are useful for drug discovery. Baran et al. used epoxy-germacrenol as a core skeleton and constructed a variety of sesquiterpenoid frameworks by acid-mediated transannular cyclization reactions.[7]

Humulene is commonly found in nature in sources such as hops, or *Humulus lupulus*, and contains an 11-membered ring structure with three *E*-configured double bonds.[12] In other words, humulene is a suitable starting point for the construction of a chemical library based on natural products. We have previously constructed a humulene-derived terpenoid alkaloid-like compound library containing diverse structures that is useful for drug discovery via Lewis acid catalyzed intramolecular C−O bond formation followed by a ring-rearrangement metathesis reaction.[4] On the other hand, intramolecular C−C bond formation based on the humulene skeleton would also be a useful strategy for fabricating of diverse natural product-like compounds.[13−15] However, the treatment of tetracyanoethylene with humulene epoxide II affords only bicyclo[8.1.0]undecane (**1**) and tricyclo-[7.2.0.$0^{2,4}$]undecane (**2**) moieties because of the highly strained structure provided by three *E*-olefins, which limits the possible reactions that may occur under nonenzymatic conditions (Figure 1A).[14] Recently, Appendino et al. have constructed isoprenoid compounds based on zerumbone under conditions permitting Nazarov's reaction. In this case, reactions began from the C6−C10 bond formation of zerumbone because the carbonyl group at the C8 position acted as a trigger of transannular cyclization, permitting the breach of the *E*-olefin barrier (Figure 1B).[16] Thus, we attempted to overcome this barrier using a different strategy, in which we removed the strained *E*-olefin conformation to construct new ring systems. In this letter, we used humulene epoxide II as a starting point and constructed rare and non-natural terpenoid scaffolds by intramolecular electrophilic C−C bond formation.

The Lewis acid-mediated ring-opening reaction of epoxides and electrophilic olefin cyclization is a useful strategy for the construction of terpenoid scaffolds from well-designed precursors.[17,18] Because the humulene skeleton contains three olefins, it can be converted into various other skeletons depending on the combination of electron-rich olefins and nucleophilic epoxides that are used. For example, as shown in Figure 1C, 6,7-epoxy-9,10-saturated humulene derivative **3** and 2,3-epoxy-6,7-saturated humulene derivative **5** would be converted into unnatural terpenoid skeletons **4** and **6**,

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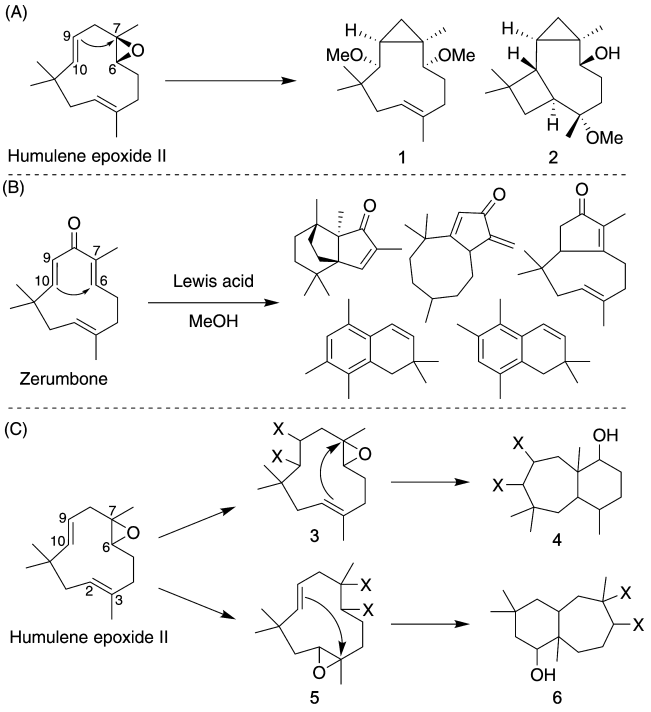

Figure 1. (A,B) Transannulation reactions based on a humulene skeleton. (C) Our strategy for production unreported terpenoid-like structures.

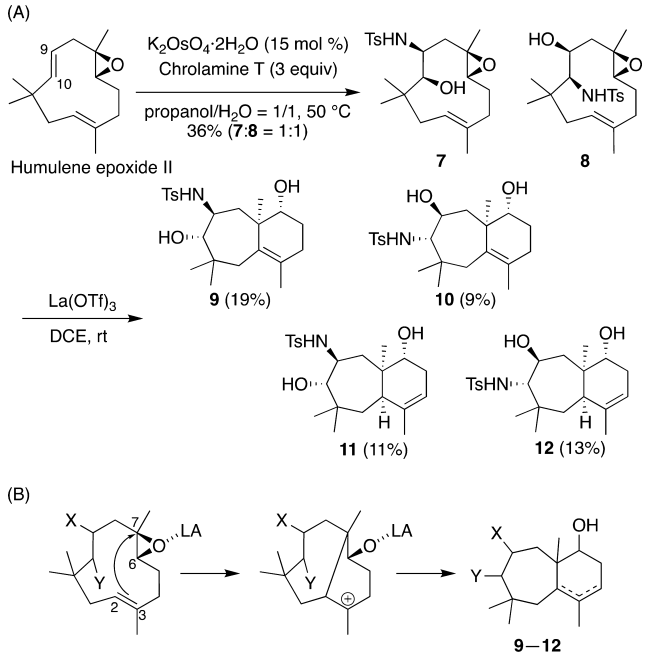

Figure 2. (A) Synthesis of unnatural terpenoid-like compounds **9**–**12**. (B) Predicted reaction mechanism of **9**–**12**.

respectively, which are not biosynthetically constructed in nature.

To remove a strained *E*-double bond of humulene epoxide II, hydroxyamination with the nitrogen source chloramine T was chosen.[19] The introduction of a nitrogen atom, which acts as a hydrogen bonding donor or acceptor, is of value when synthesizing compounds useful for drug discovery.[20,21] The hydroxyamination of humulene epoxide II afforded 6,7-epoxy-9,10-saturated humulene derivatives **7** and **8** by the site-selective and facial selective reaction of the C9−C10 double bond; osmium(VIII) oxide could not approach the sterically hindered C2−C3 olefin and C9−C10 double bond from the opposite face of epoxide because of the strained humulene skeleton. By the treatment of a mixture of compounds **7** and **8** with lanthanum(III) triflate, compounds **9**−**12** were obtained (Figure 2A). Using Lewis acid activation, we used the remaining olefin in the electrophilic C−C bond formation between C2 and C7 because of the stability of the carbocation at C3 and the fact that C7 is more electrophilic than is C6 under Lewis acidic conditions, which produced the bicyclo[5.4.0]undecane moiety (Figure 2B). This terpenoid-like structure is not found in nature and has not yet been synthetically derived. These terpenoid-like compounds are expected to show unique bioactivities. Here, we used chloramine T as a nitrogen sourse. The *tert*-butyl carbamate- or benzyl carbamate-based hydroxyamination would afford *N*-Boc or *N*-Cbz products, which can be used as interemediates for further deritives.[22]

On the other hand, aziridination has a great advantage in terms of the introduction of nitrogen; like epoxide, this functional group is expected to act as a nucleophile in electrophilic cyclization. We expected that the aziridination of the C2−C3 double bond and ring-opening of C6−C7 epoxide would afford a 2,3-imino-6,7-saturated scaffold, which would be a precursor for the unnatural terpenoid moiety **6**. The aziridination of humulene epoxide II with a rhodium catalyst and *O*-(2,4-dinitrophenyl)hydroxylamine predominantly afforded compound **13**, which was obtained with diastereomers of 2,3- or 9,10-aziridinated products.[23] Treatment with CSA in MeOH afforded an epoxide ring-opened product without aziridine cleavage. This was followed by the *N*-protection of aziridine with a nosyl group to provide compound **14** for the activation of the aziridine. Treatment of **14** with lanthanum(III) triflate afforded cyclized compounds **15**−**17** bearing a bicyclo[5.3.0]decane ring system, denoted as a salvialane skeleton,[24] which was not expected from bicyclo[5.4.0]-undecane ring system. The treatment of compounds **15** and **16** with thiophenol and potassium carbonate afforded denosylated products **18** and **19**, respectively (Figure 3A). A possible reaction mechanism is shown in Figure 3B. At first, as was expected and as shown in Figure 1C, a bicyclo[5.4.0]-undecane system was constructed by the formation of a C3−C9 bond via the similar mechanism that permitted the formation of compounds **9**−**12**. Wagner−Meerwein rearrangement then afforded a salvialane skeleton, generating a more stable carbocation by 1,2-alkyl shift not methyl group migration. This rearrangement was also proposed in Vietmeyer's and Ohe and Uemura's reports.[13,25] To our best knowledge, the salvialane moiety has only been reported for 15 natural products[26−35] and has only been constructed in six synthetic reports.[6,13,24,36−38] Because the salvialane skeleton is relevant in biosynthesis and is synthetically derived from a germacrene-type precursor by a ring expansion reaction, our approach provides a new efficient method for the selective construction of a salvialane moiety.

We synthesized unnatural terpenoid-like (**9**−**12**) and salvialane-type compounds (**15**−**19**). To determine whether these compounds are useful for drug discovery, we screened the synthesized terpenoid-like compounds for peroxisome proliferator-activated receptor α (PPARα) activity (Figure 4). Because humulene derived compound which we previously

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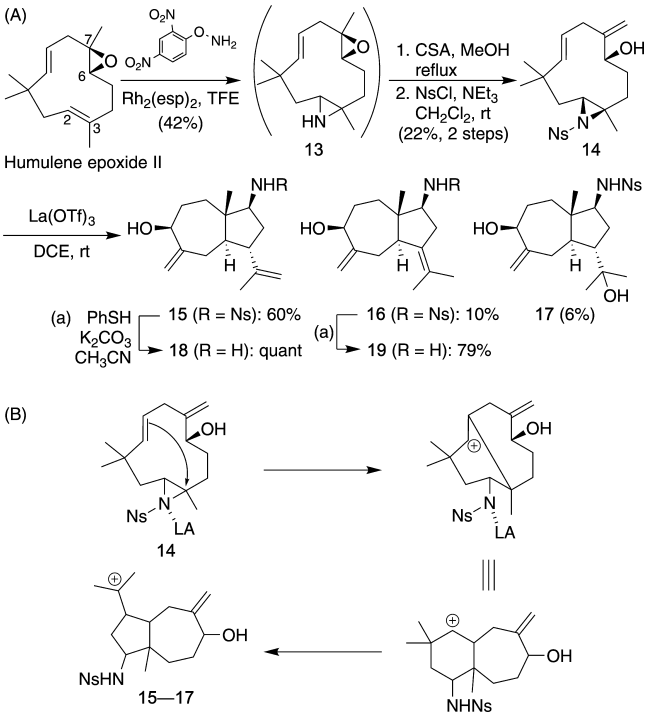

Figure 3. (A) Synthesis of salvialane-type compounds **15**–**17**. (B) Predicted reaction mechanism for **15**–**17**.

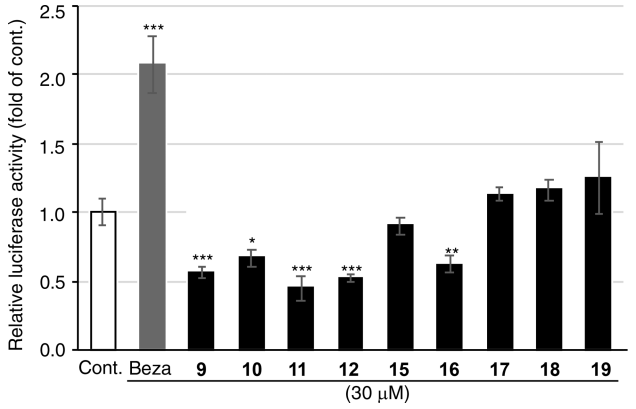

Figure 4. Effects on PPARα activity as determined by luciferase reporter assay using the Gal4/PPAR chimera system.[47] A pGL4.35 luc vector and pBIND-hPPARα were transfected into HepG2 cells. At 24 h after transfection, the cells were treated with each compound for 5 h. Control cells (Cont.) received the vehicle (DMSO) at a final concentration of 0.3% in the media. Bezafibrate (Beza) was used as a positive control. Data are expressed relative to the mean value of the control cells. Bars indicate the standard error of three independent experiments. The statistical significance of the differences was determined by Dunnett's test. *p < 0.05**p < 0.01, ***p < 0.001 vs Cont.

synthesized enhanced PPARα gene expression,[4] we expected compounds constructed in this work to show similar activities. As a result, the bicyclo[5.4.0]undecane series (**9**–**12**) and **16** were found to inhibit PPARα activity. In particular, compound **11** inhibited 45% of this activity compared with a control at 30 μM. PPARα plays an important role in controlling the gene expression of lipid metabolism.[39−42] In the liver, PPARα acts as a mediator that maintains the rates of β-oxidation and ketogenesis during a state of fasting. On the other hand, it is suggested that chronic hepatitis C virus infections are associated with sustained PPARα gene expression, which increases intracellular H_2O_2 and causes DNA damage and thus leads to liver cancer.[43] Therefore, a PPARα antagonist could be used for novel cancer therapy.[44] However, a limited number of PPARα antagonists have been disclosed, such as GW6471[45] and MK886.[46] For these reasons, compound **11** would be a seed compound for downregulating PPARα in patients with hepatitis and may have application in cancer therapy.

In summary, we synthesized unreported bicyclo[5.4.0]-undecane terpenoid-like (**9**–**12**) and salvialane-type compounds (**15**–**19**) by the removal of a *E*-configured double bond of humulene epoxide II, allowing us to overcome the *E*-barrier of the humulene skeleton. The former compound type has never been found in nature or fabricated in previous humulene transannulation studies. The construction of the latter type provided a basis for the efficient formation of a salvialane moiety. Biological screening illustrated that bicyclo[5.4.0]undecane-containing compounds **9**–**12** and **16** inhibited PPARα activity, demonstrating that the most potent compound **11** would be a seed compound in prevention of liver cancer for the patients with hepatitis. In our previous work, diverse ring structures were constructed by new C−O bond formation, in contrast to the C−C bond formation strategy explored in this study. These results support the usefulness of a medium-sized ring containing natural products for constructing diverse ring systems. We will expand upon our strategy to the use of other medium sized-ring containing natural products, such as germacrene and some macrolides, as well as screen for various bioactivities in future studies.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.8b03259.

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.

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