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Development of Diversity-Enhanced Extracts of *Curcuma zedoaria* and Their New Sesquiterpene-like Compounds

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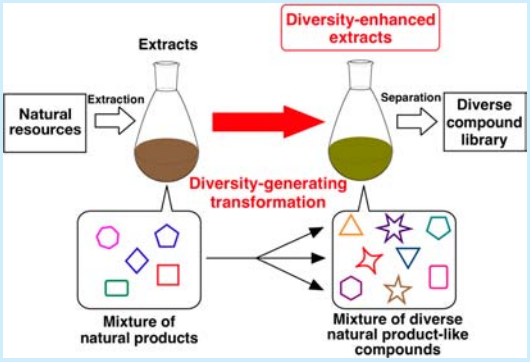
Supporting Information

ABSTRACT: Through the combination of natural products chemistry and diversity-oriented synthesis, a new approach, diversity-enhanced extracts, for increasing the diversity of natural product-like compounds is proposed. They are prepared from chemical reactions that remodel molecular scaffolds directly on extracts of natural resources. This method was applied to terpenes extracted from *Curcuma zedoaria*. Epoxidation and subsequent ring-opening reactions of epoxides were used to modify molecular skeletons. As a result, seven sesquiterpene-like compounds with some containing new molecular skeletons were obtained.



Natural products and their derivatives have been very useful in the search for biologically active compounds and for the development of new drugs because of their structural diversity. However, recently, pharmaceutical research into natural products has declined because of several factors, one of which is that it is becoming more difficult to collect novel compounds bearing "privileged structures".[1,2] Therefore, to retain their usefulness in the future, new approaches to increase the chemical diversity of natural products must be developed.

Diversity-oriented synthesis has recently emerged as an efficient methodology to construct complex and diverse compounds from simple and similar precursors.[3] In particular, there is an effective approach to accessing chemically diverse libraries by the use of natural products as starting scaffolds.[4] Thus, through the combination of natural products chemistry and diversity-oriented synthesis, we propose a new approach, diversity-enhanced extracts, for increasing the diversity of natural product-like compounds.

Diversity-enhanced extracts are prepared from chemical reactions that remodel molecular scaffolds directly on extracts of natural resources. The subsequent isolation of each compound produced from such reactions affords natural product-like compounds, whose chemical diversity is provided by both the strategy of diversity-enhanced reactions and the serendipitous discovery of natural product research (Figure 1). There have been several reports on similar methods that chemically convert natural extracts.[5,6] In these earlier reports, conversion of functional groups in original natural products was performed, but molecular skeletons were not altered. Diversity-enhanced natural extracts represents an unprecedented approach in terms of applying reactions to form new carbon−carbon bonds and modify molecular scaffolds.

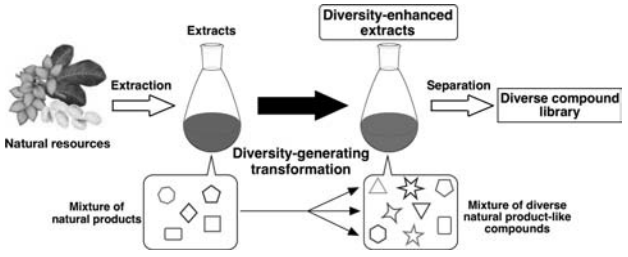


Figure 1. Schematic outline for the utilization of diversity-enhanced extracts.

In this paper, natural products from the traditional medicinal plant, *Curcuma zedoaria*, were diversified using this strategy, and seven new and diverse sesquiterpene-like compounds **1**−**7** with some containing new molecular skeletons were generated.

Curcuma zedoaria, or white turmeric, is a traditional medicinal plant used as an aromatic stomachic. Constituents of *C. zedoaria* have been extensively investigated since the 1960s,[7,8] and *C. zedoaria* includes several kinds of sesquiterpenes, which contain some carbonyl and olefin groups. Thus, we used ring-opening reactions of epoxides to prepare diversity-enhanced extracts of *C. zedoaria*. In some diversity-oriented synthetic studies based on natural products, transannulation caused by ring-opening reactions of epoxides contributed to the diversification of compounds.[4a,c]

To introduce epoxides into constituents of *C. zedoaria*, the extracts were treated with methyl(trifluoromethyl)dioxirane, which was produced from 1,1,1-trifluoroacetone and Oxone in situ[9] to yield the oxidized extracts. Use of such conditions

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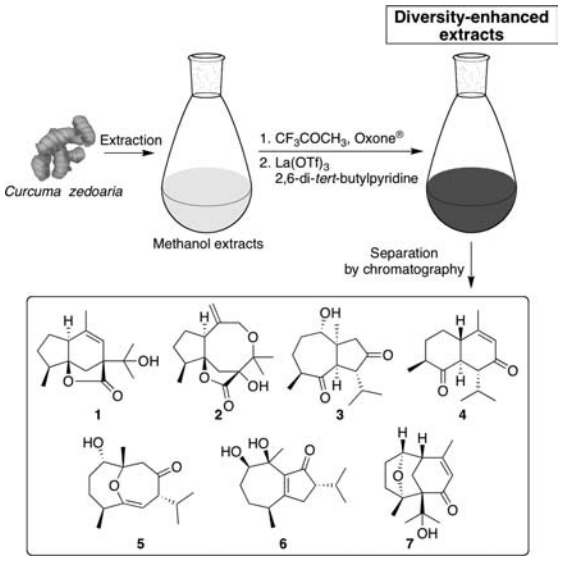
enabled the epoxidation of olefins present in the extract. Then, treatment with a Lewis acid catalyst caused ring-opening reactions of epoxides and afforded the diversity-enhanced extracts of *C. zedoaria* (Scheme 1). By comparison of the

Scheme 1. Preparation and Separation of Diversity-Enhanced Extract of *Curcuma zedoaria*



HPLC profiles of the extracts before and after the modification reactions (Figure S1, Supporting Information), major peaks in the original extract were disappeared and some new peaks emerged in the diversity-enhanced extract. Reproducibility of the diversity generating process was confirmed by the HPLC profiles of two independent experiments.

The diversity-enhanced extracts of *C. zedoaria* were separated by repeated column chromatography to yield eight unreported sesquiterpene-type compounds **1**–**7** with some containing unprecedented molecular skeletons (Scheme 1).

HRFABMS (*m*/*z* 251.1634 $[M + H]^+$) indicated the molecular formula of **1** as $C_{15}H_{22}O_3$. The correlational peaks of the $^1H-^1H$ COSY and HMBC spectra shown in Figure 2A

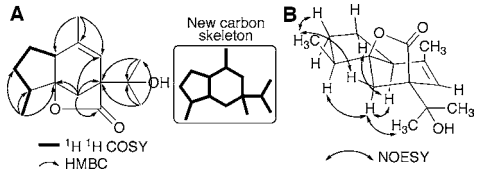
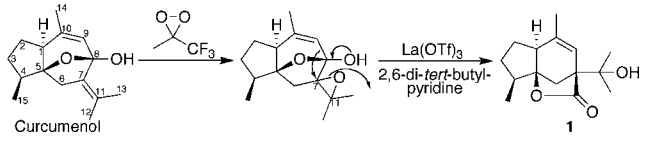


Figure 2. Structural elucidation of compound **1**: (A) planar structure; (B) relative structure.

revealed the planar structure of **1**. The relative stereochemistry of **1** was determined by its NOESY spectrum (Figure 2B). Compound **1** is assumed to be produced by sequential reactions depicted in Scheme 2. Curcumenol, a constituent of

Scheme 2. Plausible Synthetic Pathway of 1



C. zedoaria,[7c] is oxidized by a dioxirane to produce a 7,11-epoxy derivative. Then, quasi-Favorskii-type rearrangement associated with opening of the epoxide afforded a ring contraction product **1**. Because the stereochemistry of C-4 is retained through these reactions, the absolute configuration of C-4 in **1** is assumed to be *S*, which is the same as that of curcumenol. The carbon skeleton of **1**, 6-isopropyl-1,4,6-trimethyloctahydro-1*H*-indene, has not been reported in both synthetic and natural compounds.

The HRFABMS of **2** (*m*/*z* 267.1627 $[M+H]^+$) resulted in the molecular formula of $C_{15}H_{22}O_4$. Its $^1H-^1H$ COSY, HMBC, and NOESY spectrum revealed the structure of **2** bearing a 9,11-secoguaiane skeleton (Figure 3). The ring system of **2**,

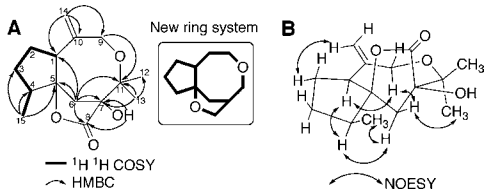


Figure 3. Structural elucidation of compound **2**: (A) planar structure; (B) relative structure.

8,12-dioxatricyclo[8.2.1.$0^{1,5}$]tridecane, is unprecedented in both synthetic and natural compounds. Compound **2** seems to be formed by cleavage of C-7/C-9 and an oxygen bridge between C-9/C-11 of **1**, whose origin is curcumenol. Thus, compound **2** could be produced from curcumenol or a more oxidized minor component of *C. zedoaria*, though the accurate synthetic pathway are uncertain.

Compounds **3**, **5**, and **6** have the same molecular formula, $C_{15}H_{24}O_3$, while the molecular formula of compound **4** is $C_{15}H_{22}O_2$, which differs from those of **3**, **5**, and **6** by a water unit. $^1H-^1H$ COSY, HMBC, and NOESY spectra revealed the structures of **3**–**6** including their relative stereochemistry (Figure 4). All compounds were isolated stereochemically pure, while compound **4** was isolated with 15% of its 1-epimer.

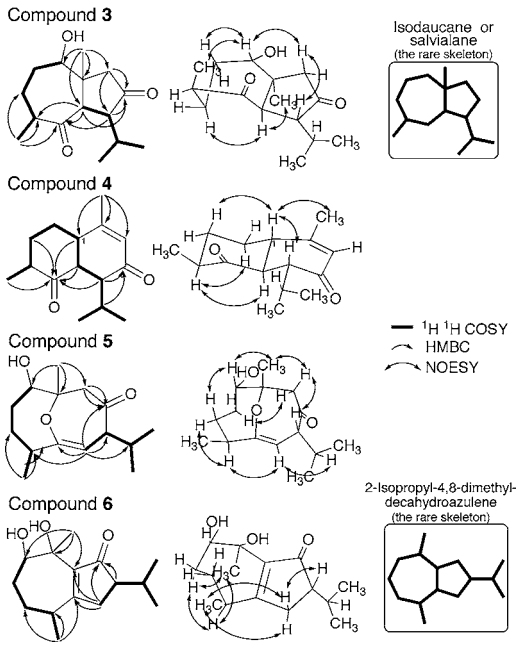


Figure 4. Structural elucidation of compounds **3**–**6**.

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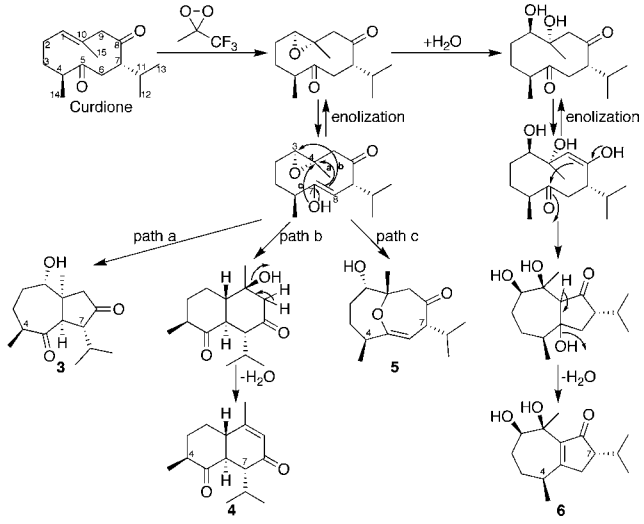
Compound **3** has an isodaucane or salvialane skeleton,[10] which is the rare group of sesquiterpenoids. Meanwhile, the carbon skeleton of **6** is 2-isopropyl-4,8-dimethyldecahydroazulene. Several sesquiterpenoids having this skeleton have been reported since the 1960s, and this skeleton was named as vetivane.[11] However, by some synthetic works, their structures turned out to be wrong.[12] Therefore, the compounds having a 2-isopropyl-4,8-dimethyldecahydroazulene skeleton represents a very rare group of sesquiterpenoids.[13]

These compounds could be produced from curdione, one of the constituents of *C. zedoaria* (Scheme 3).[7b] Epoxidation of

Scheme 3. Plausible Synthetic Pathway of 3-6



curdione followed by attack of the enol from C-9 (path a), opens the epoxide to generate C-8−C-4 bond in compound **3**. The nucleophilic attack from α-carbon atom on C-3 (path b) and subsequent dehydration afforded compound **4**. *O*-Alkylation of the enol with the C-4 epoxide carbon (path c) could yield compound **5**. On the other hand, hydration of an epoxide and aldol condensation between two carbonyl groups afforded compound **6**. Mimicking the biosynthetic diversification of sesquiterpenoids, ring-opening of epoxides by both *C*- and *O*-alkylation of enols further expanded skeletal diverisity. In addition, because the stereochemistry of C-4 and C-7 is retained through these reactions, both of the absolute configurations of C-4 and C-7 in compounds **3**−**6** are assumed to be *S*, which is the same as those of curdione.

HRFABMS (m/z 251.1614 $[M + H]^+$) indicated the molecular formula of **7** as $C_{15}H_{22}O_3$. Its $^1H-^1H$ COSY, HMBC, and NOESY spectrum revealed the structure of **7** bearing an 1-isopropyl-2,7-dimethylbicyclo[4.3.1]decane skeleton (Figure 5A, B), which is unprecedented in both synthetic and natural compounds. Although the origin of **7** is uncertain, its skeleton seems to be derived from curcumenone[14] (Figure 5C). Thus, compound **7** would be produced from a more oxidized curcumenone derivative contained in *C. zedoaria*.

Recently, we have focused on innate immunity,[15] and we established an *Att-Luc* assay by using *Drosophila* S2 cells, which were stably transfected with the *luciferase* reporter gene driven by an *attacin* promoter.[15d] Attacin is one of the antimicrobial peptides regulated by the *Drosophila* IMD pathway.[16] Thus, we evaluated the effect of compounds **1**−**7** on the innate immune response by the use of the *Att-Luc* assay. As a result,

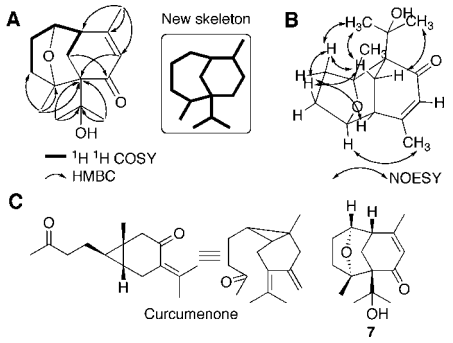


Figure 5. Structural elucidation of compound **7**: (A) planar structure; (B) relative structure; (C) structures of curcumenone and **7**.

compounds **3** and **6** inhibited innate immune responses to 47.5% and 57.6% of the control levels, respectively, at 100 μM, although other compounds showed no effect (Figure S3, Supporting Information). As compounds **3** and **6** showed no cytotoxicity on *Drosophila* S2 cells at 100 μM, these effects would be selective for the innate immune response.

Through the combination of natural products chemistry and diversity-oriented synthesis, we propose the utilization of diversity-enhanced extracts to collect diverse natural product-like compounds. Further, from the diversity-enhanced extract of *C. zedoaria*, new and diverse sesquiterpene-like compounds, some of which showed innate immune inhibitory activity, were obtained. It is noteworthy that the compounds containing new molecular skeletons can be obtained from *C. zedoaria,* which is a commercially available natural resource and whose constituents have been extensively explored.

On the other hand, unexpected compounds **2** and **7**, whose origins are uncertain, were obtained. They have unique and unprecedented skeletons or ring systems. Such compounds cannot be expectantly obtained by diversity-oriented synthesis based on isolated natural products, and this unexpected collection of compounds highlights one of the advantages of diversity-enhanced extracts.

There is a point to be improved. After diversity-generating reactions, the extracts become complex mixtures including remaining reagents. Such extracts are difficult to separate into each component and incompatible with any bioassays. To separate reagents, products, and unreacted compounds, any methods such as solid-phase synthesis and fluorous synthesis are necessary.

Methodology for diversity-enhanced extracts will be applied to several natural resources using various diversity-generating chemical reactions in order to create and discover new natural product-like compounds.

■ ASSOCIATED CONTENT

Supporting Information

Tables and experimental methods and NMR spectra for new compounds. These materials are available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Li, J. W.-H.; Vederas, J. C. *Science* **2009**, *325*, 161−165.
(2) Wolfender, J.-L.; Queiroz, E. F. *CHIMIA* **2012**, *66*, 324−329.
(3) (a) Schreiber, S. L. *Science* **2000**, *287*, 1964−1969. (b) Burke, M. D.; Berger, E. M.; Schreiber, S. L. *Science* **2003**, *302*, 613−618. (c) Burke, M. D.; Schreiber, S. L. *Angew. Chem., Int. Ed.* **2004**, *43*, 46−58.
(4) (a) Appendino, G.; Tron, G. C.; Jarevång, T.; Sterner, O. *Org. Lett.* **2001**, *3*, 1609−1612. (b) Kumar, N.; Kiuchi, M.; Tallarico, J. A.; Schreiber, S. L. *Org. Lett.* **2005**, *7*, 2535−2538. (c) Balthaser, B. R.; Maloney, M. C.; Beeler, A. B.; Porco, J. A., Jr.; Snyder, J. K. *Nat. Chem.* **2011**, *3*, 969−973. (d) Ignatenko, V. A.; Han, Y.; Tochtrop, G. P. *J. Org. Chem.* **2013**, *78*, 410−418.
(5) (a) López, S. N.; Ramallo, I. A.; Sierra, M. G.; Zacchino, S. A.; Furlan, R. L. E. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 441−444. (b) Salazar, M. O.; Ramallo, I. A.; Micheloni, O.; Sierra, M. G.; Furlan, R. L. E. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5067−5070. (c) Salazar, M. O.; Micheloni, O.; Escalante, A. M.; Furlan, R. L. E. *Mol. Diversity* **2011**, *15*, 713−719. (d) Ramallo, I. A.; Salazar, M. O.; Méndez, L.; Furlan, R. L. E. *Acc. Chem. Res.* **2011**, *44*, 241−250. (e) Méndez, L.; Salazar, M. O.; Ramallo, I. A.; Furlan, R. L. E. *ACS Comb. Sci.* **2011**, *13*, 200−204.
(6) Kawamura, T.; Matsubara, K.; Otaka, H.; Tashiro, E.; Shindo, K.; Yanagita, R. C.; Irie, K.; Imoto, M. *Bioorg. Med. Chem.* **2011**, *19*, 4377−4385.
(7) (a) Hikino, H.; Meguro, K.; Sakurai, Y.; Takemoto, T. *Chem. Pharm. Bull.* **1965**, *13*, 1484−1485. (b) Hikino, H.; Sakurai, Y.; Takahashi, H.; Takemoto, T. *Chem. Pharm. Bull.* **1967**, *15*, 1390−1394. (c) Hikino, H.; Sakurai, Y.; Numabe, S.; Takemoto, T. *Chem. Pharm. Bull.* **1968**, *16*, 39−42.
(8) Lobo, R.; Prabhu, K. S.; Shirwaikar, A.; Shirwaikar, A. *J. Pharm. Pharmacol.* **2009**, *61*, 13−21.
(9) Yang, D.; Wong, M.-K.; Yip, Y.-C. *J. Org. Chem.* **1995**, *60*, 3887−3889.
(10) (a) Tchuendem, M. H. K.; Mbah, J. A.; Tsopmo, A.; Ayafor, J. F.; Sterner, O.; Okunjic, C. C.; Iwu, M. M.; Schuster, B. M. *Phytochemistry* **1999**, *52*, 1095−1099. (b) Xia, W. J.; Li, D. R.; Shi, L.; Tu, Y. Q. *Tetrahedron: Asymmetry* **2001**, *12*, 1459−1462.
(11) (a) Yosioka, I.; Sasaki, Y.; Hikino, H. *Chem. Pharm. Bull.* **1961**, *9*, 84−85. (b) Yosioka, I.; Kimura, T. *Chem. Pharm. Bull.* **1965**, *13*, 1430−1434.
(12) (a) Marshall, J. A.; Andersen, N. H.; Johnson, P. C. *J. Am. Chem. Soc.* **1967**, *89*, 2748−2750. (b) Marshall, J. A.; Johnson, P. C. *J. Am. Chem. Soc.* **1967**, *89*, 2750−2751.
(13) (a) Bellesia, F.; Pagnoni, U. M.; Trave, R. *J. Chem. Soc., Chem. Commun.* **1976**, *65*, 34−35. (b) Orahovats, A. S.; Bozhkova, N. V.; Hilpert, H. *Tetrahedron Lett.* **1983**, *24*, 947−950.
(14) Shiobara, Y.; Asakawa, Y.; Kodama, M.; Yasuda, K.; Takemoto, T. *Phytochemistry* **1985**, *24*, 2629−2633.
(15) (a) Kikuchi, H.; Sekiya, M.; Katou, Y.; Ueda, K.; Kabeya, T.; Kurata, S.; Oshima, Y. *Org. Lett.* **2009**, *11*, 1693−1695. (b) Kikuchi, H.; Isobe, M.; Sekiya, M.; Abe, Y.; Hoshikawa, T.; Ueda, K.; Kurata, S.; Katou, Y.; Oshima, Y. *Org. Lett.* **2011**, *13*, 4624−4627. (c) Kikuchi, H.; Sato, Y.; Kurata, S.; Katou, Y.; Oshima, Y. *Tetrahedron* **2013**, *69*, 3536−3542. (d) Kikuchi, H.; Kubohara, Y.; Nguyen, V. H.; Katou, Y.; Oshima, Y. *Bioorg. Med. Chem.* **2013**, *21*, 4628−4633.
(16) Goto, A.; Matsushita, K.; Gesellchen, V.; Chamy, L. E.; Kuttenkeuler, D.; Takeuchi, O.; Hoffmann, J. A.; Akira, S.; Boutros, M.; Reichhart, J.-M. *Nat. Immunol.* **2008**, *9*, 97−104.