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Endophyte inspired chemical diversity from *beta*-caryophyllene

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The natural product (–)-*β*-caryophyllene is considered as an ideal initiator to generate diverse scaffolds by transannular cyclization due to its macrocycle and abundant source in nature. An endophytic strain *Aspergillus tubingensis* KJ-9 in our lab was screened out to catalyse remodeling of *β*-caryophyllene skeleton. In the fermentation, macrocyclic *β*-caryophyllene was remodeled into a series of natural product-like polycyclic compounds with unusual diverse skeletons (**1**–**7**). Their structures, including absolute configuration, were elucidated by exhaustive spectra (1D & 2D NMR, HRMS, ECD and X-ray diffraction). Especially, scaffolds **1** and **3** were not found in the acid-catalysed products before. Our findings demonstrated the potential of the endophytic fungi for catalyzing macrocyclic compounds into diverse products.

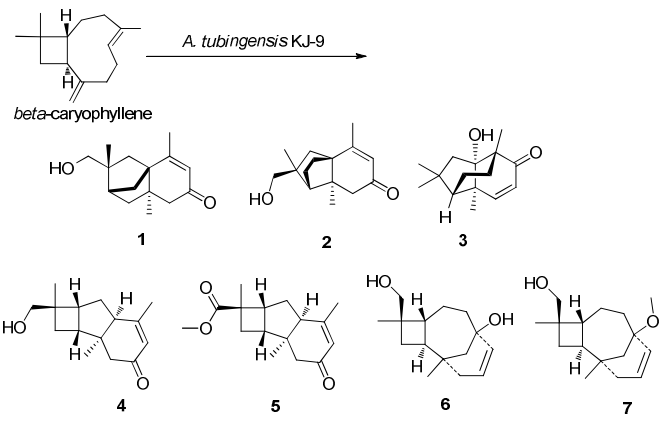
Introduction

Due to superb complexity and diversity, natural products (NP) become prime resource for drug leads.[1] But current collection of NP is limited by sophisticated isolation procedures or lengthy steps of total synthesis. Recently, diversity-oriented synthesis (DOS) has emerged as an efficient methodology to access chemically diverse libraries.[2–4] Especially, complex NP were selected as starting points to generate NP-like scaffolds.[5,6] On the other hand, transannular or Wagner-Meerwein rearrangements on terpenoids yield a wide variety of natural skeletons. Consequently, in our opinion, cascade transannular reaarangement on macrocyclic NP will be a very useful approach leading to high diverse skeletons.

The macrocyclic sesquiterpene, (–)-*β*-caryophyllene, is a major sesquiterpene component in the essential oils of plants (esp. in cloves)[7–9] and microorganisms[10–15]. Due to its strained cyclobutane, macrocyclic moiety and exocyclic olefin, *β*-caryophyllene acts as a key precursor in nature to form diverse tricyclic sesquiterpenes by transannular rearrangements.[16]

In our continuing work on NP-based biotransformation,[17–20] *β*-caryophyllene was selected as starting point to generate NP-like library with diverse skeletons. Especially, plant endophytes were considered as partners to biosynthesize of NPs. To further expand the structural diversity from caryophyllene and investigate the potential of employing endophytes as biocatalysts, an endophytic strain, *Aspergillus tubingensis* KJ-9, were screened out from our lab to catalyse transannular rearrangement of *β*-caryophyllene skeleton. HPLC profiles (Figure S1) indicated that the strain KJ-9 yields unusual metabolites when it was fermented with *β*-caryophyllene. The fermentations were then separated by repeated various column chromatography to yield seven previously unreported sesquiterpenes (**1**−**7**) with five different backbones, including an unprecedented skeleton **1** (**Scheme 1**).

Results and discussion



Scheme 1. *Aspergillus tubingensis catalysed DOS from* caryophyllene

Compound **1** was assigned a molecular formula (MF) of $C_{15}H_{22}O_2$ by HR ESIMS (*m/z* 257.1508 $[M+Na]^+$), due to five degrees of unsaturation. Its ^{13}C and DEPT NMR spectroscopic data (Figure S2) revealed the presence of an unsaturated ketone (δ_C 162.2, 127.0 and δ_C 199.8). Apart from those two degrees of unsaturated bonds, the remaining implied that **1** possesses three rings in the structure. Extensive analysis of the 2D NMR correlations (HSQC, $^1H-^1H$ COSY, and HMBC, as shown in **Fig. 1**a) established the whole planar structure. $^1H-^1H$ COSY data of **1** showed one isolated spin system of H_2-2/H-1/H_2-9.

HMBC correlations from H_3-15 to C-1, C-10, and C-14 indicated C-15, C-14, C-1 and C-10 linked up through the quaternary C-11. Correlations from H_3-12 to C-3, C-4 and C-5 confirmed the methyl C-12, C-3 and C-5 linked up at the olefinic C-4. The HMBC correlations of H_2-10/C-8, H_2-9/C-3, H_3-13/C-8 and H_3-13/C-9 revealed a bicyclo[2.2.1]heptane moiety. Furthermore, the correlations of H_3-13 to C-7 and C-8, H_2-7 to C-5 and C-6 indicated a cyclohex-2-en-1-one substructure. Thus, the whole planar structure of **1** was elucidated as a novel 5/5/6 sesquiterpene skeleton.

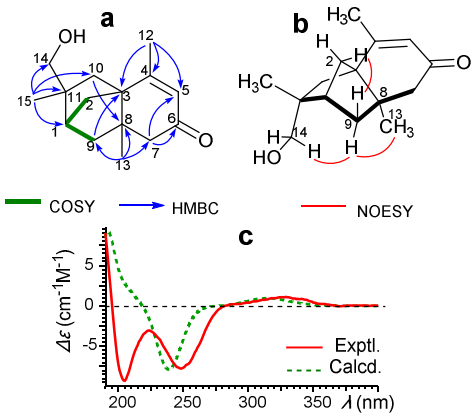


Fig. 1 Structure elucidation of **1**. **a**, Key correlations in COSY &HMBC; **b**, key correlations in NOESY; **c**, Experimental and theoretical CD spectra of (1*S*,3*S*,8*R*, 11*R*)-**1** (in MeCN).

The relative configuration (RC) of **1** was deduced from NOESY correlations (**Fig. 1**b). The NOE signals among H-14b, H-9α and H_3-13 indicated that C-14 and Me-13 are on the same side, which were arbitrarily assigned as α-orientation. A NOE correlation of H-9β/H-2 indicated that the bridge C-2 is on the β–orientation. Since all the products were derived from bioconversion of caryophyllene through complex arrangements, it is necessary to determine their absolute configuration (AC) on the basis of an unambiguous experimental proofs. Its experimental CD spectrum was compared with the theoretical curve calculated by TDDFT method.[21–23] The calculated CD curve for (1*S*,3*S*,8*R*,11*R*)-**1** (**Fig. 1**c) showed same positive Cotton effect as the experimental around 290 nm. Thus, its AC was established therein.

MF of **2**, $C_{15}H_{22}O_2$, was deduced from HR ESIMS (*m/z* 257.1504 $[M+Na]^+$), due to five degrees of unsaturation. Its ^{13}C NMR data indicate that a double bond and a carbonyl in its structure. The planar structure was elucidated on the basis of COSY and HMBC correlations, as shown in **Fig. 2**a, possessing a similar skeleton as that of α-neoclovene, a rearranged product from caryophyllene.[24,25] However, this is the first report of NMR data for this type of skeleton. Its RC was deduced by NOESY correlations as shown in **Fig. 2**b. The NOE correlations of H-15/H-1 hydroxymethyl C-14 and the bridge carbons C-1 and C-2 are on the same β side. The NOE cross peaks of H_3-14/H-10α/H_3-13 indicated that both methyls C-13 and C-14 are on the same α side. The theoretical CD curve of (3*R*,8*S*, 9*R*,11*S*)-**2**stereoisomer yield a similar curve as that of experimental as shown in **Fig. 2**c. Both calculated and experimental CD spectra showed negative Cotton effect around 225 nm and positive transition around 290 nm. Furthermore, the assigned AC is also consistent with the previous reported analogs.[24,25]

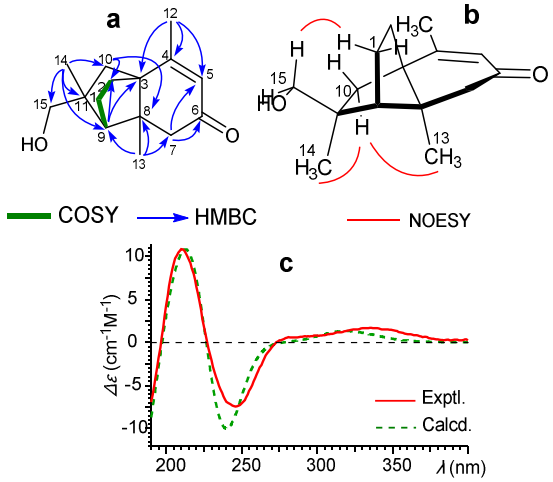


Fig. 2 Structure elucidation of **2**. **a**, COSY & HMBC; **b**, NOESY; **c**, Experimental and theoretical CD spectra of (3*R*,8*S*,9*R*,11*S*)-**2** (in MeCN).

Compound **3** had the same MF as that of **1** or **2**, as deduced by HR ESIMS (*m/z* 257.1505 $[M+Na]^+$). The ^1H and ^{13}C NMR data suggest an unsaturated ketone moiety in the molecule. Detailed analysis of the 2D NMR (**Fig. 3**a) revealed its core skeleton. The structure was similar to that of a product from H^+/MeCN,[26] except for the cyclohex-2-en-1-one substructure. Its RC was deduced from NOE correlations in NOESY spectrum (**Fig. 3**b). The Me-15 was arbitrarily assigned as β-orientation. The NOE correlations of H_3-15/H-2 indicated the carbonic bridge C-2/C-3 on β side. The NOE correlation of H_3-13/H-10α indicted the methyl C-13 at α side. Since the bridge C-2/C-3 connect C-1/C-4, respectively, the C-4 is forced on β-orientation. Therefore, the whole RC was assigned as the model in **Fig. 3**b. Its AC was determined by comparing its experimental CD curve with theoretical one. The calculated CD spectrum of (1*S*,4*R*,8*S*,9*S*)-**3** by TDDFT method has same positive Cotton effect around 220 nm to that of experimental CD curve (**Fig. 3**c). Thus, the AC of **3** was determined as 1*S*,4*R*,8*S*,9*S*.

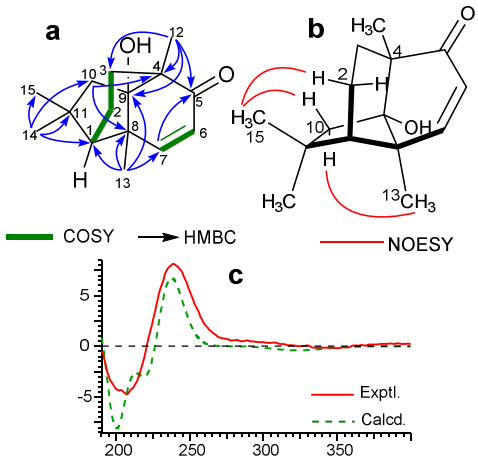


Fig. 3 Structure elucidation of **3**. **a**, COSY & HMBC; **b**, NOESY; **c**, Experimental and theoretical CD spectra of (1*S*,4*R*,8*S*,9*S*)-**3** (in MeCN).

1D NMR data (Figure S2) and 2D NMR correlations of compound **4** (**Fig. 4**a) revealed a 4/5/6 skeleton, same as the core skeletons of naematolins C and G from the fungus *Naematoloma fusciculure*.[27] Its RC was deduced by NOESY correlations (**Fig. 4**b). Compound **5** possesses similar ^{1}H and ^{13}C NMR data and 2D NMR correlations to those of **4** with only exception of a methoxyl ester group (δ_C53.1, 178.6). The group was assigned at C-15 based on the HMBC correlations of MeO/C-15 and H_3-14/C-15. Similar NOESY correlations and same Cotton effect around 215 nm (**Fig. 4**c) between **4** and **5** confirmed that they also possess the same AC. The calculated CD spectrum of (1*R*,3*S*,8*S*,9*R*,11*R*)-**5** stereoisomer displayed same negative Cotton effects with that of the experimental one (**Fig. 4**c). Thus, their ACs were assigned therein.

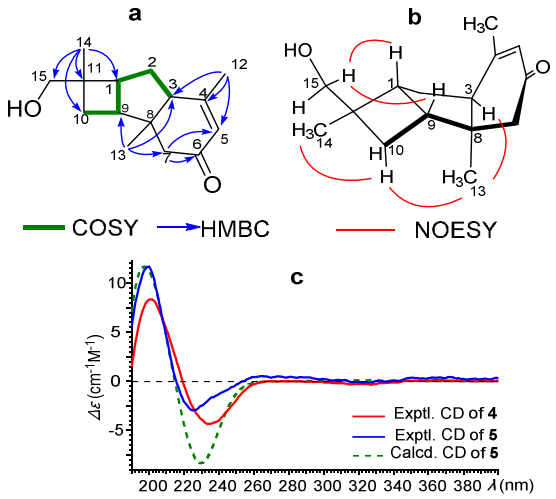


Fig. 4 Structure elucidation of **4**. **a**, COSY & HMBC; **b**, NOESY; **c**, Experimental and theoretical CD spectra of (1*R*,3*S*,8*S*,9*R*,11*R*)-**5** (in MeCN).

The 2D NMR correlations (HSQC, COSY and HMBC) as shown in **Fig. 5**a of compound **6** revealed a 4/7/6 skeleton. Its planar structure and RC were further confirmed by X-ray diffraction (**Fig. 5**b, CCDC No. 1413642). Since the compound does not contain any chromophore, its CD cannot give rise to reliable Cotton effect to assign its AC. However, the stereochemistry of two chiral centers C-1 and C-9 reserved in arrangement. As a result, the configurations of C-4 and C-8 can be deduced from X-ray analysis. X-ray model indicated that C-12 bridge and C-15 are on β side, same as that of H-1. Therefore, the AC of **6** was assigned as 1*R*,4*R*,8*S*,9*S*,11*R*.

Compound **7** have very close chemical shifts in ^{1}H and ^{13}C NMR spectra to those of **6**, besides a methoxyl substitution in **7**. Its MF of $C_{16}H_{26}O_2$, deduced from HR EIMS, also indicated **7** has one more MeO group. HMBC correlations of **7** from the MeO to C-4 indicated the position of the MeO group attached at C-4.

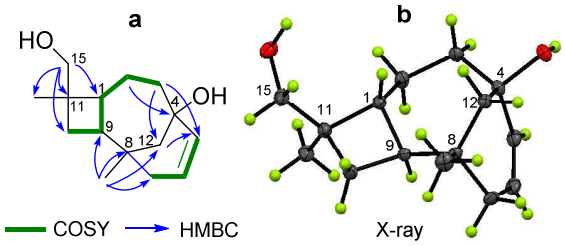
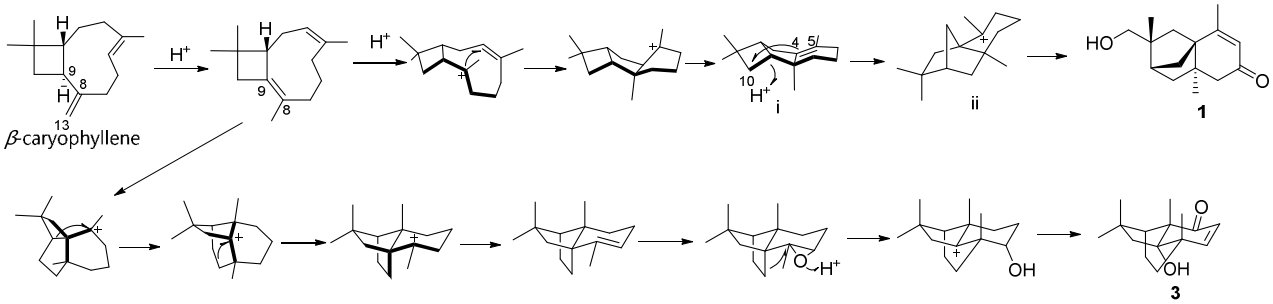


Fig. 5 Selected 2D NMR correlations and X-ray diffraction of **6**

In the formation of those rearranged scaffolds, the double bond at C-8/C-13 shifted to C-8/C-9. The transannularcyclizations of β-caryophyllene initiated by electrophilic attack on the shifted double bond (**Scheme 2**). In the formation of **1**, the cyclobutane moiety open to yield carbonium ion at C-10 (**i**), which then attacked to the double bond at C-4/C-5 to form the skeleton of **1**. Then, elimination and oxidation reactions on the carbonium ion **ii** gave the product **1**. The formation of **3** also achieved *via* transannular rearrangement, elimination and oxidation reactions.The other scaffolds (**2** and **4**–**7**) are typical skeletons derived from caryophyllene. Their formatations can be referred to a previous review.[16]

Conclusions

Our findings demonstrated the potential of the endophytic fungi catalysing macrocyclic sesquiterpenoid β-caryophyllene to afford NP-like polycyclic compounds containing novel skeletons. Among them, skeleton **4** was also found in nature. Notably, scaffolds of **1** and **3** have not acquired by acid catalysis yet. Compared to catalysis of acid or Lewis acid, the fungus KJ-9 promoted chemical diversity even more by oxidizing on *gem*-dimethyls and allylic positions on the arranged skeletons. In addition, β-caryophyllene is very plentiful in natural source, so it can be served as a synthon to construct complex cyclic scaffolds covering unknown chemical space or bioactive space. On the basis of the current findings, combination of caryophyllene with other naturally bioactive blockers will produce more diverse products with NP-like scaffolds.



Scheme 2 Plausible pathway to **1** and **3**

In fact, nature always knows the best choice. For example, a medicinal plant *Psidium guajava* (guava) selects *β*-caryophyllene and another bioactive block to generate an *α*-glucosidase inhibitor, a novel caryophyllene-based meroterpenoid, guajadial, with a higher activity than that of the drug acarbose.[28–31] In our lab, a biomimic synthesis is being carried out to combine *β*-caryophyllene and other NP pharmacophore, which will generate novel chemical library by rearrangement in order to cover unknown chemical space with high bioactivities.

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Notes and references

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Electronic Supplementary Information (ESI) available: experimental and calculation procedures, spectra data, NMR, IR and HRMS spectra of compounds **1**-**7**.,. See DOI: 10.1039/b000000x/

1. J. W.-H. Li and J. C. Vederas, *Science*, 2009, **325**, 161.
2. V. A. Ignatenko, Y. Han, and G. P. Tochtrop, *J. Org. Chem.*, 2013, **78**, 410.
3. B. R. Balthaser, M. C. Maloney, A. B. Beeler, J. A. Porco, and J. K. Snyder, *Nat. Chem.*, 2011, **3**, 969.
4. N. Kumar, M. Kiuchi, J. A. Tallarico, and S. L. Schreiber, *Org. Lett.*, 2005, **7**, 2535.
5. H. Kikuchi, K. Sakurai, and Y. Oshima, *Org. Lett.*, 2014, **16**, 1916.
6. G. Appendino, G. C. Tron, T. Jarevång, and O. Sterner, *Org. Lett.*, 2001, **3**, 1609.
7. J. T. Knudsen, L. Tollsten, and L. G. Bergström, *Phytochemistry*, 1993, **33**, 253.
8. I. Kubo, S. K. Chaudhuri, Y. Kubo, Y. Sanchez, T. Ogura, T. Saito, H. Ishikawa, and H. Haraguchi, *Planta Med*, 1996, **62**, 427.
9. J. R. Hanson, *Nat. Prod. Rep.*, 2001, **18**, 607.
10. M. Wichlacz, W. A. Ayer, L. S. Trifonov, P. Chakravarty, and D. Khasa, *J. Nat. Prod.*, 1999, **62**, 484.
11. S.-W. Yang, T.-M. Chan, J. Terracciano, E. Boehm, R. Patel, G. Chen, D. Loebenberg, M. Patel, V. Gullo, B. Pramanik, and M. Chu, *J. Nat. Prod.*, 2009, **72**, 484.
12. W.-J. Wang, D.-Y. Li, Y.-C. Li, H.-M. Hua, E.-L. Ma, and Z.-L. Li, *J. Nat. Prod.*, 2014, **77**, 1367.
13. M. Pulici, F. Sugawara, H. Koshino, J. Uzawa, S. Yoshida, E. Lobkovsky, and J. Clardy, *J. Org. Chem.*, 1996, **61**, 2122.
14. Y. Li, S. Niu, B. Sun, S. Liu, X. Liu, and Y. Che, *Org. Lett.*, 2010, **12**, 3144.
15. Q.-Y. Qi, L. Bao, J.-W. Ren, J.-J. Han, Z.-Y. Zhang, Y. Li, Y.-J. Yao, R. Cao, and H.-W. Liu, *Org. Lett.*, 2014, **16**, 5092.
16. I. G. Collado, J. R. Hanson, and A. J. Macías-Sánchez, *Nat. Prod. Rep.*, 1998, **15**, 187.
17. G.-W. Wu, J.-M. Gao, X.-W. Shi, Q. Zhang, S.-P. Wei, and K. Ding, *J. Nat. Prod.*, 2011, **74**, 2095.
18. G. W. Wu, X. J. Li, Y. Gao, Y. Jia, and J. M. Gao, *Chin. Chem. Lett.*, 2010, **21**, 446.
19. T. Dong, G.-W. Wu, X.-N. Wang, J.-M. Gao, J.-G. Chen, and S.-S. Lee, *J. Mol. Catal. B: Enzym.*, 2010, **67**, 251.
20. C. Yang, H. Fan, Y. Yuan, and J. Gao, *Chin. J. Chem.*, 2013, **31**, 127.
21. M. Xue, Q. Zhang, J.-M. Gao, H. Li, J.-M. Tian, and G. Pescitelli, *Chirality*, 2012, **24**, 668.
22. J.-M. Gao, J.-C. Qin, G. Pescitelli, S. Di Pietro, Y.-T. Ma, and A.-L. Zhang, *Org. Biomol. Chem.*, 2010, **8**, 3543.
23. S.-X. Yang, J.-M. Gao, H. Laatsch, J.-M. Tian, and G. Pescitelli, *Chirality*, 2012, **24**, 621.
24. W. Parker, R. A. Raphael, and J. S. Roberts, *Tetrahedron Lett.*, 1965, **6**, 2313.
25. H. Iwabuchi, M. Yoshikura, Y. Ikawa, and W. Kamisako, *Chem. Pharm. Bull.*, 1987, **35**, 1975.
26. O. I. Yarovaya, D. V. Korchagina, T. V. Rybalova, Y. V. Gatilov, M. P. Polovinka, and V. A. Barkhash, *Russ. J. Org. Chem.*, 2004, **40**, 1593.
27. K. Doi, T. Shibata, N. Yokoyama, H. Terasawa, O. Matsuda, and S. Kashino, *J. Chem. Soc., Chem. Commun.*, 1990, 725.
28. X.-L. Yang, K.-L. Hsieh, and J.-K. Liu, *Org. Lett.*, 2007, **9**, 5135.
29. M. Shao, Y. Wang, Z. Liu, D.-M. Zhang, H.-H. Cao, R.-W. Jiang, C.-L. Fan, X.-Q. Zhang, H.-R. Chen, X.-S. Yao, and W.-C. Ye, *Org. Lett.*, 2010, **12**, 5040.
30. Y. Ou, X. Zhu, R. Liu, X. Liu, L. Su, L. Xie, and Y. Cao, *J. Hunan Univ. CM*, 2014, **34**, 17.
31. H.-Z. Fu, Y.-M. Luo, C.-J. Li, J.-Z. Yang, and D.-M. Zhang, *Org. Lett.*, 2010, **12**, 656.