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# Chemo-enzymatic syntheses of drimane-type sesquiterpenes and the fundamental core of hongoquercin meroterpenoid by recombinant squalene-hopene cyclase<sup>†</sup>

Yukie Yonemura, Takuro Ohyama and Tsutomu Hoshino\*

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Squalene–hopene cyclase (SHC) converts squalene ( $C_{30}$ ) into pentacyclic triterpenes of hopene and hopanol. A linear sesquiterpene, (6E, 10E)-2, 6, 10-trimethyldodeca-2, 6, 10-triene, underwent cyclization catalyzed by SHC, affording the following six bicyclic sesquiterpenes (drimane skeleton) in relatively high yield (68%): drim-7(8)-ene, drim-8(12)-ene, drim-8(9)-ene, driman-8 $\alpha$ -ol, driman-8 $\beta$ -ol, and the novel sesquiterpene, named quasiclerodane, the skeleton of which is analogous to that of clerodane diterpene. To extend the scope of the enzymatic syntheses, acyclic sesquiterpenes to which a phenol moiety was appended were subjected to the enzymatic reaction catalyzed by SHC. The cyclic meroterpene core present in hongoquercins A and B was successfully prepared. The formation mechanisms of drimane-type sesquiterpenes and the cyclic meroterpene core of hongoquercins A and B are discussed.

# Introduction

Enzymatic cyclization reactions of polyolefin substrates have fascinated chemists for over half a century. The polycyclization reactions of squalene 1 and (3S)-2,3-oxidosqualene, each having C<sub>30</sub>, proceed with complete regio- and stereochemical specificity to furnish the remarkable structural diversity of triterpene scaffolds.<sup>1</sup> We have reported the enzymatic characterization of squalenehopene cyclase (SHC) from Alicyclobacillus acidocaldarius, which yields pentacyclic hopene 2 and hopanol 3 (diplopterol) via a hopanyl cation (Scheme 1A), through mutagenesis and substrate analog experiments.1a Remarkably, this enzyme is highly tolerant to truncated and elongated substrates with carbon numbers of C15- $C_{35}$ .<sup>2-4</sup> Farnesol ( $C_{15}$ , 4), for example, proved to be an excellent substrate to afford cyclic sesquiterpenes in high yield (64%).<sup>2</sup> We succeeded in synthesizing drimane-type sequiterpenes 5–7 by incubating farnesol 4 with SHC (Scheme 1B). Drimenol 5<sup>5</sup> was isolated from Bazzania trilobata<sup>5</sup> and Diplophylum serrulatum,<sup>6</sup> and used as the starting material for the syntheses of cytotoxic 8-epipuupehedione<sup>7</sup> and  $\gamma$ -polypodatetraene.<sup>8</sup> Albicanol **6** was isolated from the liverworts of Diplophyllum albicans,9 Diplophylum serrulatum<sup>6</sup> and Bazzania japonica,<sup>10</sup> and employed for the syntheses of  $\alpha$ -polypodatetraene<sup>8</sup> and cryptoporic acid A methyl ester<sup>11</sup> with anti-tumor promotion activity. Driman-8,11-diol 7

was found in the volatile extracts of Greek tobacco.<sup>12</sup> Compound **8** has not yet been found in nature. Therefore, SHC holds great potential for the syntheses of drimane-type sesquiterpenes from the readily available farnesol  $4^2$ .

To extend the scope of enzymatic syntheses of sesquiterpenes by SHC, substrate 9 (6E,10E)-2,6,10-trimethyldodeca-2,6,10-triene), lacking the hydroxyl group of 4, was prepared and incubated with SHC in the effort to synthesize 15-17, which are analogous to 5-7, but lack a hydroxyl group at the 11-position. Interestingly, in addition to 15-17, novel sesquiterpenes 18 and 20 and naturally occurring 19 were prepared. In addition, other acyclic sesquiterpene analogs 13 and 14 containing phenol moiety were also synthesized and subjected to the enzymatic reaction in order to synthesize a cyclic meroterpene core, such as hongoquercin A 10 and hongoquercin B 11, and BE-40644 12 (Scheme 1C). The C(8)-stereochemistry (S) of 12 is opposite to those of 10 and 11 (R), but their basic cores are the same. Meroterpenoids are hybrid natural products composed of both terpenoid and polyketide-derived structures.13 Hongoquercins A and B are bioactive compounds with antibacterial activity,14 and BE-40664 has a human thioredoxin system inhibitory activity.<sup>15</sup> Herein, we report the first chemo-enzymatic syntheses of drimane-type terpenes 15–20 from 9 and the cyclic meroterpene cores 21–25 from 13 and 14, in which a phenol moiety is appended to 9.

# **Results and discussion**

# Enzymatic reaction of 9 with recombinant SHC

Incubation of 9, prepared from farnesol 4 (ESI, page S3 $\dagger$ ), with SHC was conducted as follows. Compound 9 (1 mg), emulsified with Triton X-100 (20 mg), was incubated with the cell free extract

Department of Applied Biological Chemistry, Faculty of Agriculture, and Graduate School of Science and Technology, Niigata University, Nishi-ku, Ikarashi 2-8050, Niigata, 950-2181, Japan. E-mail: hoshitsu@agr.niigatau.ac.jp

 <sup>&</sup>lt;sup>†</sup> Electronic supplementary information (ESI) available: synthetic methods of compounds 9, 13, and 14; GC trace of reaction mixture of compound 14; and spectroscopic data (EIMS and NMR) of the enzymatic products 15–25. See DOI: 10.1039/c1ob06419c



Scheme 1 (A) Cyclization cascade of squalene 1 into hopene 2 and hopanol 3 mediated by squalene–hopene cyclase (SHC). (B) Enzymatic products<sup>2</sup> of farnesol 4 and the substrate structure 9 to be tested. (C) Structures of hongoquercins A and B and BE-40644 (cyclic meroterpenes), and those of substrates 13 and 14 to be tested.

(2 ml) as the enzyme source at pH 6.0 and 60 °C for 20 h with a total reaction volume of 5 ml. Then, 15% KOH/MeOH was added to the reaction mixture, and the products were extracted with hexane. Triton X-100 included in the extracted solution was removed with a short SiO<sub>2</sub> column chromatography eluting with a mixture of hexane and EtOAc (100:20). Fig. 1A shows the GC

profile of the product distribution pattern. A full conversion was not attained -32% of **9** remained unreacted - but 68% of **9** was successfully converted into the enzymatic products. A large-scale incubation of **9** (100 mg) was conducted in order to isolate the enzymatic products. Non-polar and polar factions were crudely separated by SiO<sub>2</sub> column chromatography; the non-polar fraction



**Fig. 1** (A) GC trace of the reaction mixture obtained by incubating **9** with the native SHC. The asterisk mark shows the impurity. GC conditions: column temp., 80–250 °C; rate, 5.0 °C min<sup>-1</sup>, injection temp., 290 °C; carrier gas (N<sub>2</sub>), 0.75 kg cm<sup>-2</sup>.(B) GC-chromatogram of the non-polar fraction, which was separated by SiO<sub>2</sub> column chromatography. GC conditions: column temp., 140 °C; injection temp., 290 °C; carrier gas (N<sub>2</sub>), 0.2 kg cm<sup>-2</sup>.

was collected by eluting with hexane, and polar fraction was eluted with a mixture of hexane and EtOAc (100:10). Four enzymatic products (**15**, **16**, **19**, and **20**) were included in the non-polar fraction (Fig. 1B). These compounds were successfully separated and purified using a 5% AgNO<sub>3</sub>-impregnated SiO<sub>2</sub> column eluting with hexane containing a trace of EtOAc. Two products (**17** and **18**) included in the polar fraction were isolated by a SiO<sub>2</sub> column (hexane : EtOAc = 100:5). Fig. 1A and 1B revealed the following product distribution: 10% for **15**, 4% for **16**, 16% for **17**, 14% for **18**, 4% for **19** and 20% for **20**.

Structures of all the enzymatic products were determined by <sup>1</sup>H- and <sup>13</sup>C NMR spectral analyses, including DEPTs, <sup>1</sup>H-<sup>1</sup>H COSY, NOESY, HMOC and HMBC spectra. The molecular formula of 15, 16, 19 and 20 was determined to be  $C_{15}H_{26}$  by HREIMS, indicating the unsaturation number of 3. Each of these products had one double bond, which was revealed by observing the following chemical shifts in C<sub>6</sub>D<sub>6</sub>:  $\delta_{\rm C}$  121.9 (d) and 135.2 (s) for 15; 106.3 (t) and 151.5 (s) for 16; 124.3 (s) and 136.2 (s) for 19: and 121.0 (d) and 143.8 (s) for 20, indicating that bicyclic skeletons should be assigned to these products. In the <sup>1</sup>H NMR spectrum of 15, one doublet Me (3H,  $\delta_{\rm H}$  0.958, J = 7.2 Hz, Me-11) and one allylic Me (3H,  $\delta_{\rm H}$  1.75, br s, Me-12) were found. Me-11 had HMBC correlations with C-9 ( $\delta_{\rm C}$  49.03, d), C-10 ( $\delta_{\rm C}$ 36.02, s) and C-8 ( $\delta_c$  135.3, s). Me-12 had HMBC cross peaks with C-9, C-8 and C-7 (121.9, d); the detailed HMBC analyses are shown in ESI (page S6).<sup>†</sup> The  $\alpha$ -oriented H-9 was confirmed from clear NOEs of H-9/Hax-5/Hax-1/Hax-3. These data allowed us to propose the structure of drim-7(8)-ene for 15 (Scheme 2). For 16, the presence of one doublet Me (3H,  $\delta_{\rm H}$  1.04, J = 6.8 Hz, Me-11) and one vinylidene residue (1H,  $\delta_{\rm H}$  4.98, brs and 1H,  $\delta_{\rm H}$ 4.79, brs,  $CH_2$ -12) was confirmed. The clear HMBC correlations of Me-11/C-9, Me-11/C-10 and Me-11/C-8, as well as those of H-12/C-9 and H-12/C-7, established the structure of 16 to be drim-8(12)-ene as depicted in Scheme 2. H-9 was determined to be  $\alpha$ -oriented by the unambiguous NOE between H-5 and H-9. In the <sup>1</sup>H NMR spectrum of **19**, two allylic Me groups (Me-11 and Me-12) were found at  $\delta_{\rm H}$  1.67 (6H, brs). No doublet Me was detected. The clear HMBC cross peaks of Me-15 ( $\delta_{\rm H}$  1.12, 3H, s)/C-9 ( $\delta_c$  136.2, s), Me-11/C-10 ( $\delta_c$  38.50, s), Me-12/C-7 ( $\delta_{\rm C}$  34.05, t) and Me-12/C-8 (( $\delta_{\rm C}$  124.3, s) demonstrated that the double bond is located at C-8 and C-9. A strong NOE was observed between Me-14 ( $\delta_{\rm H}$  0.985, 3H, s) and Me-15. Thus, the structure of 19 was determined as shown in Scheme 2. Products 17 and 18 are both polar compounds; alcoholic carbons were found at C-8 ( $\delta_{\rm C}$  72.25 (s) for 17 and  $\delta_{\rm C}$  71.84 (s) for 18). For 17, a strong NOE between Me-15 ( $\delta_{\rm H}$  0.800, 3H, s) and Me-12 ( $\delta_{\rm H}$ 1.13, 3H, s) was found, but no corresponding NOE was found for 18, indicating that the C8-stereochemistry is opposite between



Scheme 2 Enzymatic products 15–20 prepared by incubating 9 with the native SHC. The deprotonation reactions from intermediate 26 gave 15, 16 and 19. A water molecule attack to 26 afforded 17 and 18. 1,2-Shifts of hydrides and methyl groups in antiparallel fashion according to the curved arrows shown in 26 generated novel sesquterpene 20, named quasiclerodane.

**17** and **18**. The structures of **17** and **18** were determined to be driman-8α-ol and driman-8β-ol, respectively (Scheme 2). For **20**, one allylic Me (Me-13,  $\delta_{\rm H}$  1.72, 3H, d, J = 1.2 Hz) was found that had HMBC correlations with C-3 ( $\delta_{\rm C}$  121.0, d), C-4 ( $\delta_{\rm C}$  143.9, s) and C-5 ( $\delta_{\rm C}$  38.48, s). One doublet Me (Me-12,  $\delta_{\rm H}$  0.963, 3H, d, J = 6.8 Hz) had a cross peak with C-9 ( $\delta_{\rm C}$  36.55, s), which was further correlated with Me-11 ( $\delta_{\rm H}$  1.01, 3H, s) and Me-15 ( $\delta_{\rm H}$  0.809, 3H, s) in the HMBC spectrum. Further detailed NOE and HMBC analyses (ESI, pages S14, S18, S20 and S21†) revealed an unnatural novel bicyclic skeleton (SciFinder), as shown in Scheme 2. This sesquiterpene skeleton is analogous to that of clerodane diterpene. We propose the name quasiclerodane for **20**. It should be noted that novel product **20** was generated in a highest yield (20%) among all of the products **15–20**.

#### Enzymatic reactions of 13 and 14 with recombinant SHC

Substrates 13 and 14, in which an *o*-hydroxylphenyl ring is appended to a farnesyl moiety, were prepared (ESI, page S3–S5†) in order to construct the fundamental skeletons (21 and 24) of hongoquercins A and B. The incubation conditions of 13 and 14 were the same as that of 9. As shown in Fig. 2, GC analysis of the incubation mixture of 13 with SHC afforded three product peaks (with conversion yield of 10%). The yields of products 21, 22 and 23 were 6%, 2.5% and 1.5%, respectively.



Fig. 2 GC trace of the enzymatic reaction obtained by incubating substrate 13 with SHC. Triton X-100 was removed by a short SiO<sub>2</sub> column. GC conditions: column temp., 190 °C; injection temp., 280 °C; carrier gas  $(N_2)$ , 1.0 kg cm<sup>-2</sup>.

Separation by a SiO<sub>2</sub> column (hexane : EtOAc = 100 : 0.05) afforded two fractions, one containing **21** and the other containing a mixture of **22** and **23**. Products **22** and **23** were separated by a 5% AgNO<sub>3</sub>-SiO<sub>2</sub> column (hexane : EtOAc = 100 : 8–100 : 20). Products **21–23** were finally purified *via* normal phase HPLC. The structure of product **21** was confirmed by NMR analyses, including 1D and 2D NMR spectral data. In addition to a phenolic carbon ( $\delta_c$  154, s), an alcoholic carbon ( $\delta_c$  76.77, s, C-8) appeared, indicating that an ether linkage was present between C-13 and C-8 of **21**. The position of the alcoholic carbon (C-8) was confirmed by the HMBC cross peak between Me-21 ( $\delta_H$  1.22, 3H, s) and C-8. The allylic methyl protons, which are present in substrate **13**, were not found, indicating that the complete polycyclization reaction

occurred. The detailed analyses of the HMBC data (ESI, page 22<sup>†</sup>) led to the proposal of the 6/6/6/6-fused tetracyclic structure of 21. The molecular formula of products 22 and 23 was  $C_{21}H_{30}O$ with an unsaturation number of 7. In addition to a benzene ring (unsaturation number of 4), one double bond was involved in 22 and 23:  $\delta_{\rm C}$  122.4 (d) and 135.8 (s) for 22;  $\delta_{\rm C}$  107.5 (t) and 149.0 (s) for 23, suggesting that 22 and 23 are both tricyclic compounds containing a benzene ring. In the HMBC spectrum of 22, Me-20  $(\delta_{\rm H} 1.04, 3H, s)$  and allylic Me-21  $(\delta_{\rm H} 1.77, 3H, s)$  had a cross peak with C-9 ( $\delta_{\rm C}$  54.51, d), and H-5 ( $\delta_{\rm H}$  1.41, m) and H-9 ( $\delta_{\rm H}$  2.56, m) showed a strong NOE between them. The allylic Me-21 had definitive HMBC cross peaks with C-7 ( $\delta_{\rm C}$  122.4, d) and C-8 ( $\delta_{\rm C}$ 135.8, s), demonstrating that the double bond is located at C-7 and C-8. Thus, the whole structure of 22 was determined as shown in Scheme 3. For 23, protons of a vinylidene moiety were found at  $\delta_{\rm H}$ 4.82 (1H, s) and  $\delta_{\rm H}$  4.71 (1H, s), which were correlated with C-21 ( $\delta_{\rm C}$  107.5, t) in the HMQC spectrum. The methylene protons at 21-position and Me-20 ( $\delta_{\rm H}$  0.820, 3H, s) had definitive HMBC correlations with C-9 ( $\delta_c$  56.08, d). Detailed analyses of the HMBC and NOESY spectra allowed us to propose the structure of 23, as shown in Scheme 2. SHC accepts 2,3-oxidosqualene as well as squalene,<sup>16</sup> and so we examined whether 14 also is acceptable as the substrate. GC analysis of the reaction mixture showed that only one product was produced (14:24 = ca. 1:1, see ESI, pageS5<sup>†</sup>). Purification by a SiO<sub>2</sub> column (hexane: EtOAc = 100:05) and subsequent HPLC (hexane: 2-PrOH = 100:0.05) afforded pure product 24, which was then acetylated with Ac<sub>2</sub>O/py and subjected to NMR analyses. An ether bridge was found between C-8 ( $\delta_c$  76.43, s) and C-13 ( $\delta_c$  154, s). The assignment of C-8 was confirmed by the HMBC correlation between Me-21 and C-8. H-3 showed the following splitting pattern (1H,  $\delta_{\rm H}$  4.70, dd, J = 12.0, 4.8 Hz), indicating axial-orientation of H-3 and equatorial-arrangement of OH. An acetyl group ( $\delta_{\rm C}$  169.8, s;  $\delta_{\rm H}$  1.87, 3H, s) was determined to be at C-3 ( $\delta_{\rm C}$  80.06, s) by confirming the HMBC correlation of H-3 with the acetyl carbonyl carbon ( $\delta_{\rm C}$  169.8, s). No allylic methyl group was found in the <sup>1</sup>H NMR spectrum, indicating that a complete cyclization reaction had occurred as shown in the structure of 24 in Scheme 3. The  $\beta$ -oriented hydroxyl group at C-3 of 24 indicates that the (10S)-10,11-epoxy derivative 14 was stereoselectively converted, but that the10*R*-isomer underwent no reaction. Products 21 and 25 (acetate of 24) are fundamental cores of (+)-hongoquercin A and (+)hongoquercin B, respectively. Thus, we succeeded in the syntheses of these cyclic meroterpene cores by using recombinant SHC.

All the enzymatic products from **9** are listed in Scheme 2. Drim-8(12)-ene **16** was isolated from *Hedychium acuminatum*,<sup>17</sup> *Cistus* L.<sup>18</sup> *Eupatorium odoratum*,<sup>19</sup> rhizomes of *Hedychium spicatum*,<sup>20</sup> *Silphium* L.<sup>21</sup> and so forth. Driman-8 $\alpha$ -ol **17** was found in Greek tobacco and plant culture of *Nicotiana silvestris*,<sup>22,23</sup> and from *Nicotiana tabacum*.<sup>24</sup> Drim-8(9)-ene **19** is a natural product isolated from Colorado shale oil.<sup>25</sup> Product **20** is a novel compound. Neither drim-7(8)-ene **15** nor driman-8 $\beta$ -ol **18** have been isolated as natural products (SciFinder). Substrate **9** could undergo cyclization reactions to give an intermediary bicyclic C8cation **26**. The subsequent deprotonation reactions of H-7, H-12 and H-9 could afford **15**, **16** and **19**, respectively. The *re*-face attack of a water molecule on the C8-cation gave **17**, while the *si*-face attack yielded **18**. A series of 1,2-shifts of hydrides and methyl groups in an antiparallel fashion could generate novel compound



Scheme 3 Enzymatic products 21-25 obtained by the incubations of 13 and 14 with SHC. The tricyclic intermediate 27 could be produced. The deprotonation reactions from H-7 and H-21 gave 22 and 23, respectively. A nucleophilic attack of phenolic OH to C-8 cation afforded 21 that is a fundamental skeleton of hongoquercin A and B.

20: H-9  $\rightarrow$  C-8; Me-11  $\rightarrow$  C-9; H-5  $\rightarrow$  C-10; Me-14  $\rightarrow$  C-5 and the final elimination reaction of H-3. The skeletons of products 18-20 were not found in the products obtained by the cyclization of farnesol 4. This finding suggests that the OH group of 4 interacts with certain amino acid(s) via hydrogen bonding inside the SHC cavity, but 9 cannot form the hydrogen bonding. Therefore, the location of 9 inside the cavity may be different from that of 4, possibly contributing to the production of 18-20.

SHC could convert substrate 13 to the bicyclic C-8 cation 27. Phenolic OH group could attack to this cation as a nucleophile, successfully affording the fundamental core 21 of hongoquercin A (6/6/6/6-fused tetracycle, where ring C is a tetrahydropyran) in 6% yield. The proton eliminations of H-7 and Me-21 from 27 gave tricyclic products 22 (2.5%) and 23 (1.5%), respectively. The total conversion yield was significantly smaller (10%) than that of phenol-lacking substrate 9 (68%). Presumably, the bulky phenol moiety may have hindered access to the DXDD motif <sup>1b</sup> that enables initiation of the polycyclization reaction by proton attack on the terminal double bond and further prevented the access of the phenol ring to the C-8 cation of 27, and thus, the cyclization reaction terminated at the bicyclic stage to yield 22 and 23. The conversion ratio of epoxide 14 was significantly higher than that of 13. Furthermore, the S-form of 14 was exclusively cyclized to give 24 in a high yield (almost quantitative yield, see the GC trace shown in ESI, page S5<sup>†</sup>), but the alternative *R*-stereoisomer underwent no reaction (no detectable amount of product with  $\alpha$ oriented OH). The acetate of 24, *i.e.* 25, forms the fundamental framework of hongoquercin B. At present, it is not clear why the conversion yield of 14 was higher than that of 13, and the reason for the enantioselective preference is not yet understood. The more facile access to the DXDD sequence of (10S)-14, compared to that of 13, may have caused the higher yield of (10S)-14. In other words, a more favorable orientation of (10S)-14 in the catalytic cavity may

have afforded the higher conversion. The epoxide ring of (10S)-14, folded in a chair conformation,<sup>26</sup> could have accessed the acidic DXDD motif, but that of the (10R)-14, folded in a boat form,<sup>26</sup> could not gain proximity to the DXDD motif, resulting in no reaction. Previously, we reported that a squalene analog with  $C_{20}$ 28 was cyclized in 33% yield to give complete cyclization products 29 and 30 (Scheme 4).<sup>2</sup> The structure of 28 (C<sub>20</sub>) has one additional isoprene unit than is found in 9 ( $C_{15}$ ). The conversion yield of 9 (68%) was approximately two times that (33%) of 28, indicating that C<sub>15</sub> was more efficiently cyclized than C<sub>20</sub>, but the reason for this is not clear at present. Tanaka et al. reported the enzymatic reaction of squalene analogs (C20) containing an indole ring 3127 or a pyrrole ring 32.<sup>28</sup> They succeeded in the syntheses of indoleor pyrrole-containing cyclic polyprenoid compounds by SHC, but in very small yields (7.5% for indole-containing polyprenoid;<sup>27</sup> and 1.1% for pyrrole-containing polyprenoid<sup>28</sup>). The enzymatic products (36-39) of substrates 31 and 32, prepared by SHC,<sup>27,28</sup> are described in the ESI (page S30).<sup>†</sup> The cyclization yield of 28 is significantly higher than those of 31 and 32. As was discussed in the comparison of 9 with 13, the low enzymatic yields of substrates 31 and 32 can be explained by 1) limited access of the terminal



 28: R=H, complete cyclized product (33% yield)
 31: R=indole, 6/6/5-fused tricyclic+ indole 36 and 6/6-fused-bicyclic + indole 37 (7.5% yield) 32: R=pyrrole, 6/6/5-fused tricyclic + pyrrole 38 and 6/6-fused-bicycli + pyrole 39 (1.1% yield)

Scheme 4 Structures of polyprenoids 28, 31 and 32 with acyclic  $C_{20}$ isoprenoid chain length. Indole and pyrrole rings are substituted in the case of 31 and 32, respectively. 28 undergoes a complete cyclization reaction to give fully cyclized products 29 and 30,<sup>2</sup> but the enzymatic reactions of 31 and 32 give incomplete cyclization products (see ESI, page S30<sup>†</sup>).<sup>27,28</sup>

double bond of **31** and **32** to the DXDD motif, and 2) improper orientation of **31** and **32** in the reaction cavity caused by the bulky size of the indole and pyrrole rings; these impediments halted the polycyclization reactions at the partially cyclized stage (ESI, page S30†). Thus, the fully cyclized petromindole skeleton **40**,<sup>29</sup> in which the indole ring is fused to the 6/6/6/6-fused carbotetracycle (ESI, page S30†), was not produced.<sup>27</sup>

In conclusion, we succeeded in the chemo-enzymatic syntheses of unnatural novel sesquiterpenes 15, 18 and 20 in relatively high yields, and also of the basic cores of hongoquercins A and B (21 and 25, respectively) by recombinant SHC. As demonstrated here, the enzymatic reactions of 9, 13 and 14 proceeded also with regioand stereochemical specificity as well as that of squalene molecule. SHC has a great plasticity to tolerate a variety of squalene analogs. Native or mutated SHC is a promising tool for obtaining further novel cyclic terpenoid derivatives by chemo-enzymatic syntheses.

# **Experimental section**

#### Analytical methods

NMR spectra were mainly recorded in  $C_6D_6$  on a Bruker DMX 600 or DPX 400 spectrometer, the chemical shifts being relative to the solvent peak  $\delta_{\rm H}$  7.28 and  $\delta_{\rm C}$  128.0 ppm as the internal reference for <sup>1</sup>H- and <sup>13</sup>C NMR spectra, respectively. Some synthetic intermediates were measured in CDCl<sub>3</sub>. The chemical shifts in CDCl<sub>3</sub> solution were given according to the internal solvent peaks of  $\delta_{\rm H}$  7.26 and  $\delta_{\rm C}$  77.0 ppm. To further validate the proposed structures, the NMR data of some enzymic products were measured in acetone- $d_6$ , the solvent peak being referred to be  $\delta_{\rm H}$  2.04,  $\delta_{\rm C}$  29.8 ppm. GC analyses were done on a Shimadzu GC-8A chromatograph equipped with a flame ionization detector (DB-1 capillary column  $(0.53 \text{ mm} \times 30 \text{ m})$ . GC-MS spectra were on a JEOL SX 100 spectrometer under electronic impact at 70 eV with a DB-1 capillary column ( $0.32 \text{ mm} \times 30 \text{ m}$ ), the oven temperature being elevated according to the methods described in the legends to Fig. 1 and 2. HR-EIMS was measured by direct inlet system. Specific rotation values were measured at 25 °C with a Horiba SEPA-300 polarimeter.

#### Incubation condition and purification of enzymatic products

Standard incubation conditions were performed according to the published protocols.<sup>26,30</sup> The cell-free extracts were prepared as follows. A one liter culture of E. coli encoding the native SHC was harvested by centrifugation and to the collected pellets was added 50 cm<sup>3</sup> of citrate buffer solution (pH 6.0), and then subjected to ultrasonication to disrupt the cells. The supernatant was used for the incubations after removing the cell debris by centrifugation. One ml of the supernatant contains ca. 200 µg of the pure SHC. The products and the unreacted substrate analogs were extracted three times with a mixture of hexane and EtOAc (100:10). An excess of Triton X-100 detergent was removed by passing a short  $SiO_2$  column eluting with hexane/EtOAc (100:20). Each product was purified by SiO<sub>2</sub> column chromatography and normal phase HPLC, as described in the text. It is likely that the enzymatic products of 15, 16, 19 and 20 vaporize under reduced pressure. Therefore, the organic solvents dissolving the sesquiterpene products were evaporated at room temperature without handling them at reduced pressure.

Syntheses of substrates 9, 13 and 14. The details are described in the ESI.<sup>†</sup>

Spectroscopic data of enzymatic products 15–25. Products 16, 17 and 19 are known compounds. The spectroscopic data are described in the ESI.<sup>†</sup>

**Product 15.** <sup>1</sup>H NMR (C<sub>6</sub>D<sub>6</sub>, 400 MHz) δ 0.917 (3H, s, Me-15), 0.958 (d, J = 7.2 Hz, Me-11), 0.977(3H, s, Me-13), 0.98 (m, H-1), 1.01 (3H, s, Me-14), 1.25 (ddd, 13.0, 12.8, 3.6 Hz, H-3), 1.29 (dd, J = 11.8, 5.2 Hz, H-5), 1.50 (m, H-2), 1.54 (m, H-3), 1.63 (m, H-2), 1.75 (3H, br s, Me-12), 1.86 (m, H-1), 1.94 (m, H-9), 1.95 (m, H-6); 2.05(m, H-6), 5.58 (brs, H-7); <sup>13</sup>C NMR (C<sub>6</sub>D<sub>6</sub>, 100.6 MHz) δ 11.58 (q, C-11), 13.47 (q, C-15), 19.29 (t, C-2), 21.98 (q, C-12), 22.12 (q, C-14), 24.10 (t, C-6), 33.03 (s, C-4), 33.47 (q, C-13), 36.02 (s, C-10), 39.81 (t, C-1), 42.55 (t, C-3), 49.03 (d, C-9), 50.35 (d, C-5), 121.9 (d, C-7), 135.3 (s, C-8), the assignments of C-12 and C-14 may be interchangeable due to the close values; GCMS (EI) *m/z* 206 (M<sup>+</sup>, 18), 191 (13), 124 (33), 109 (100), 82 (58); HREIMS *m/z* 206.2067 (calcd for C<sub>15</sub>H<sub>26</sub>, 206.2035); [α]<sub>D</sub><sup>25</sup> -12.5 (*c* 0.12, C<sub>6</sub>H<sub>6</sub>).

**Product 18.** <sup>1</sup>H NMR (acetone  $d_6$ , 400 MHz)  $\delta$  0.82 (m, H-1), 0.846 (3H, s, Me-14), 0.862 (3H, s, Me-13), 0.87 (m, H-5), 0.888 (3H, d, J = 6.8 Hz, Me-11), 0.974(3H, s, Me-15), 1.01(1H, q, J = 7.2 Hz, H-9), 1.08(3H, s, Me-12), 1.14 (ddd, J = 13.6, 13.6, 4.4 Hz, H-3), 1.35 (m, H-2), 1.36 (m, H-3), 1.42 (m, H-6), 1.43(m, H-7), 1.59 (m, H-2), 1.59 (m, H-6), 1.68 (m, H-1), 1.76(m, H-7), 2.69 (br s, OH); <sup>13</sup>C NMR (acetone d<sub>6</sub>, 100.6 MHz) δ 7.88 (q, C-11), 14.88 (q, C-15), 19.25 (t, C-6), 19.31 (t, C-2), 22.19 (q, C-14), 31.41 (q, C-12), 33.88 (s, C-4), 34.00 (q, C-13), 38.65 (s, C-10), 40.82 (t, C-1), 42.77 (t, C-3), 43.62 (t, C-7), 53.58 (d, C-9), 56.89 (d, C-5), 71.84 (s, C-8), the assignments of C-2 and C-6 may be interchangeable due to the close values; GCMS (EI) m/z 224 (M<sup>+</sup>, 3), 206 (18), 191 (38), 137 (28), 109 (100), 95 (37), 82 (43), 81 (37), 67 (35); HREIMS m/z 206.2022 (M<sup>+</sup>-H<sub>2</sub>O, calcd for C<sub>15</sub>H<sub>26</sub>, 206.2035);  $[\alpha]_{D}^{25}$  -32.1 (c 0.037, C<sub>6</sub>D<sub>6</sub>), cf. lit.  $[\alpha]_{D}$  -12.3 (CHCl<sub>3</sub>, concentr. not described<sup>31</sup>);  $R_f$  value on SiO<sub>2</sub> plate = 0.54, when developed with hexane : EtOAc (100/20).

**Product 20.** <sup>1</sup>H NMR (C<sub>6</sub>D<sub>6</sub>, 400 MHz) δ 0.809 (3H, s, Me-15), 0.963 (3H, d, J = 6.8 Hz, Me-12), 1.011 (3H, s, Me-11), 1.107 (3H, s, Me-14), 1.24 (m, H-8), 1.27 (dd, 12.0,1.8 Hz, H-10), 1.27 (m, H-6), 1.28 (m, H-7), 1.42(m, H-7), 1.56 (m, H-1), 1.72 (3H, d, J = 1.2 Hz, Me-13), 1.72 (m, H-6), 1.75 (m, H-1), 2.14 (2H, m, H-2), 5.36 (br s, H-3); <sup>13</sup>C NMR (C<sub>6</sub>D<sub>6</sub>, 100.6 MHz) δ 16.64 (q, C-12), 16.72 (q, C-15), 18.27 (q, C-13),, 19.28 ((t, C-1), 19.62 (q, C-14), 27.59 (t, C-2), 28.04 (t, C-7), 29.30 (q, C-11), 30.03 (t, C-6), 36.55 (s, C-9), 38.48 (s, C-5), 42.69 (d, C-8), 52.91 (d, C-10), 121.0 (d, C-3), 143.8 (s, C-4), the carbon signals of C-12 and C-15 may be interchangeable due to the close values; GCMS (EI) m/z 206 (M<sup>+</sup>, 23), 191 (37), 163 (100), 136 (39), 123 (62), 107 (97), 95 (85), 81 (62), 69 (58); HREIMS m/z 206.2040 (calcd for C<sub>15</sub>H<sub>26</sub>, 206.2035); [α]<sub>D</sub><sup>25</sup> -10.7 (*c* 0.14, C<sub>6</sub>H<sub>6</sub>).

**Product 21.** <sup>1</sup>H NMR (C<sub>6</sub>D<sub>6</sub>, 600 MHz) δ 0.74 (m, H-1), 0.757 (3H,s, Me-20), 0.863 (3H,s, Me-19), 0.89 (m, H-5), 0.914 (3H,s, Me-18), 1.14 (m, H-3), 1.216 (3H,s, Me-21), 1.23 (m, H-6), 1.41 (m, H-3), 1.44 (m, H-2), 1.52 (bd, J = 12.2 Hz, H-1), 1.59 (m, H-9), 1.60 (m, H-6), 1.63 (m, H-2), 1.86 (ddd, J = 13.0, 13.0, 4.0

Hz, H-7); 2.16 (ddd. J = 12.5, 3.2, 3.2 Hz, H-7), 2.54 (2H,d,9.0 Hz, H-11), 6.99 (m, H-14), 7.16 (bd.J = 7.6 Hz, H-17) 7.18 (2H, m, H-16 & H-15);<sup>13</sup>C NMR (C<sub>6</sub>D<sub>6</sub>, 150.9 MHz)  $\delta$  14.89 (q, C-20), 18.73 (t, C-6), 19.87 (t, C-2), 20.95 (q, C-21), 21.66 (q, C-19), 22.55 (t, C-11), 33.15 (s, C-4), 33.45 (q, C-18), 36.77 (s, C-10), 39.07 (t, C-1), 41.48 (t, C-7), 41.99 (t, C-3), 52.12 (d, C-9), 55.97 (d, C-5), 76.77 (s, C-8), 117.5 (d, C-15), 119.9 (d, C-14), 122.4 (s, C-12), 127.6 (d, C-16), 130.1 (d, C-17), 154.0 (s, C-13). EIMS *m/z* 298 (M<sup>+</sup>, 53), 191 (62), 107 (84), 69 (100); HREIMS *m/z* 298.2286 (calcd for C<sub>21</sub>H<sub>30</sub>O 298.2297). [ $\alpha$ ]<sub>25</sub><sup>25</sup> + 49.76 (c = 0.4, CHCl<sub>3</sub>).

**Product 22.** <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  0.998(3H, s, Me-19), 1.01 (3H, s, Me-18), 1.04 (3H, s, Me-20), 1.17(ddd, J =3.6,13.2,13.2 Hz, H-1), 1.30 (m, H-2), 1.31(m, H-3), 1.41 (m, H-5), 1.53 (m, H-3), 1.60 (m, H-2), 1.77(3H, s, Me-21), 1.94(bd, J = 11.6 Hz, H-1), 2.08 (2H, m, H-6), 2.56 (bd, J = 8.0 Hz, H-9), 2.76 (d,15.2 Hz, H-11), 2.93 (m, H-11), 5.57 (bs, H-7), 6.39 (m, H-14), 6.98 (t, J = 7.6 Hz, H-16), 7.08 (t, J = 7.6 Hz, H-15), 7.32 (d, J = 7.6 Hz, H-17);<sup>13</sup>C NMR (CDCl<sub>3</sub>, 100.6 MHz) δ 14.09 (q, C-20), 19.30 (t, C-2), 22.13 (q, C-19), 22.69 (q, C-21), 24.97 (t, C-6), 26.49 (t, C-11), 33.16 (s, C-4), 33.47 (q, C-18), 37.17 (s, C-10), 39.72 (t, C-1), 42.58 (t, C-3), 50.45 (d, C-5), 54.51 (d, C-9), 115.3 (d, C-14), 120.7 (d, C-16), 122.4 (d, C-7), 127.7 (d, C-15), 130.2 (d, C-17), 130.3 (s, C-12), 135.8 (s, C-8), 153.8 (s, C-13); EIMS *m*/*z* 298 33), 191 (100), 109 (72); HREIMS m/z 298.2292 (calcd for C<sub>21</sub>H<sub>30</sub>O 298.2297). Specific optical rotation was not determined due to the very small amount available.

**Product 23.** <sup>1</sup>H NMR (CDCl<sub>3</sub>, 600 MHz)  $\delta$  0.820 (3H, s Me-20), 0.834 (3H, s, Me-19), 0.885 (3H, s, Me-18), 1.17 (m, H-1), 1.19 (m, H-3), 1.20 (m, H-5), 1.37 (m, H-6), 1.41 (d, *J* = 12.0 Hz, H-3), 1.52 (m, H-2), 1.63 (m, H-2), 1.76 (m, H-6), 1.91(d, J = 12.0 Hz, H-1), 2.03 (ddd, J = 4.2, 12.7, 12.7 Hz), 2.22 (br m, H-9), 2.38 (d, J = 12.7 Hz. H-7), 2.75 (2H, d, J = 8.8 Hz, H-11), 4.71 (s, H-21), 4.82 (s, H-21), 6.72 (d, J = 7.5 Hz, H-14), 6.83 (t, J = 7.5 Hz, H-16), 7.03 (t, J = 7.51 Hz, H-15), 7.10 (d, J = 7.5 Hz, H-17); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 150.9 MHz) δ 19.47 (t, C-2), 21.76 (q, C-19), 23.71 (t, C-11), 24.48 (t, C-6), 33.64 (q, C-18), 33.67 (s, C-4), 38.29 (t, C-7), 39.20 (t, C-1), 40.29 (s, C-10), 42.21 (t, C-3), 55.74 (d, C-5), 56.08 (d, C-9), 107.5 (t, C-21), 115.2 (d, C-14), 120.5 (d, C-16), 126.6 (d, C-15), 128.3 (C-12), 129.9 (d, C-17), 14.50 (q, C-20), 149.0 (s, C-8), 153.6 (s, C-13); GCMS (EI) m/z 298 (M<sup>+</sup>, 50), 191 (65), 137 (48), 107 (100), 81 (50), 69 (62); HREIMS m/z 298.2292 (calcd for  $C_{21}H_{30}O$  298.2297). Specific optical rotation was not determined due to a very small amount available.

**Product 24 acetate (25).** <sup>1</sup>H NMR (C<sub>6</sub>D<sub>6</sub>, 400 MHz) δ 0.687 (3H, s, Me-20), 0.83 (m, H-1), 0.86(m, H-5), 0.931(6H, s, Me-18 & Me-19)), 1.167 (3H, s, Me-21), 1.37 (m, H-1), 1.47(m, H-9), 1.52 (2H, m, H-6), 1.64 (m, H-2), 1.79 (m, H-7), 1.82(m, H-2), 1.87 (3H, s, Me-23), 2.13(m, H-7), 2.44 (2H, m, H-11), 4.70 (dd, J = 12.0, 4.8 Hz, H-3), 6.99(m, H-14), 7.15(bd. J = 8.0 Hz, H-17), 7.18(2H, m, H-15 & H-16); <sup>13</sup>C NMR (C<sub>6</sub>D<sub>6</sub>, 100.6 MHz) δ 14.88 (q, C-20), 16.78 (q, C-19), 19.36 (t, C-6), 20.77 (q, C-21), 20.81 (q, C-23), 22.49 (t, C-11), 23.84 (t, C-2), 28.02 (q, C-18), 36.30 (s, C-10), 36.80 (t, C-1), 37.76 (s, C-4), 41.22 (t, C-7), 51.59 (d, C-9), 54.80 (d, C-5), 76.43 (s, C-8), 80.06 (d, C-3), 117.5 (d, C-15), 120.0 (d, C-14), 122.4 (s, C-12), 127.6 (d, C-16), 130.0 (d, C-17), 154.0 (s, C-13), 169.8 (s, C-22): the assignments of C-21 and C-23 may be exchangeable due to the close values. EIMS *m/z* 356 (100),

281 (33), 189 (77), 107 (33); HREIMS m/z 356.2357 (calcd for  $C_{23}H_{32}O_3$  356.2351).  $[\alpha]_D^{25} + 25.5$  (c = 0.05, CHCl<sub>3</sub>),

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