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PAPER

Chemo-enzymatic syntheses of drimane-type sesquiterpenes and the fundamental core of hongoquercin meroterpenoid by recombinant squalene–hopene cyclase†

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Squalene–hopene cyclase (SHC) converts squalene (C_{30}) into pentacyclic triterpenes of hopene and hopanol. A linear sesquiterpene, (6*E*,10*E*)-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-8α-ol, driman-8β-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 (3*S*)-2,3-oxidosqualene, each having C_{30}, proceed with complete regio- and stereochemical specificity to furnish the remarkable structural diversity of triterpene scaffolds.[1] We have reported the enzymatic characterization of squalene–hopene 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 C_{15}–C_{35}.[2–4] Farnesol (C_{15}, **4**), for example, proved to be an excellent substrate to afford cyclic sesquiterpenes in high yield (64%).[2] We succeeded in synthesizing drimane-type sequiterpenes **5–7** by incubating farnesol **4** with SHC (Scheme 1B). Drimenol **5**[5] was isolated from *Bazzania trilobata*[5] and *Diplophylum serrulatum*,[6] and used as the starting material for the syntheses of cytotoxic 8-epipuupehedione[7] and γ-polypodatetraene.[8] Albicanol **6** was isolated from the liverworts of *Diplophyllum albicans*,[9] *Diplophylum serrulatum*[6] and *Bazzania japonica*,[10] and employed for the syntheses of α-polypodatetraene[8] and cryptoporic acid A methyl ester[11] with anti-tumor promotion activity. Driman-8,11-diol **7** was found in the volatile extracts of Greek tobacco.[12] 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** (6*E*,10*E*)-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.[15] 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†), with SHC was conducted as follows. Compound **9** (1 mg), emulsified with Triton X-100 (20 mg), was incubated with the cell free extract

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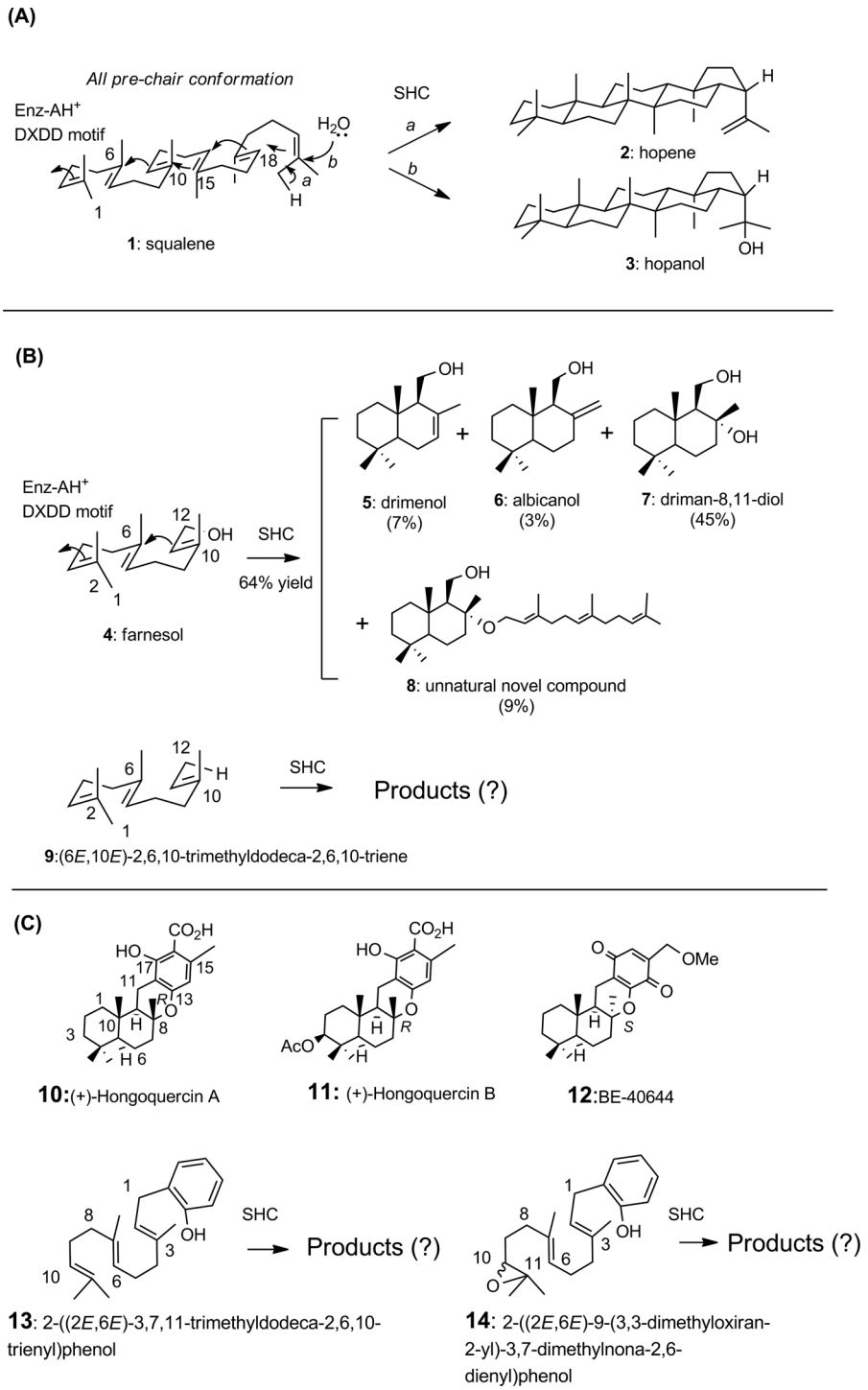
† Electronic supplementary information (ESI) available: synthetic methods of compounds **9**, **13**, and **14**; GC trace of reaction mixture of compound **14**; and spectroscopic data (EIMS and NMR) of the enzymatic products **15–25**. See DOI: 10.1039/c1ob06419c



Scheme 1 (A) Cyclization cascade of squalene **1** into hopene **2** and hopanol **3** mediated by squalene–hopene cyclase (SHC). (B) Enzymatic products[2] 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_2 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_2 column chromatography; the non-polar fraction

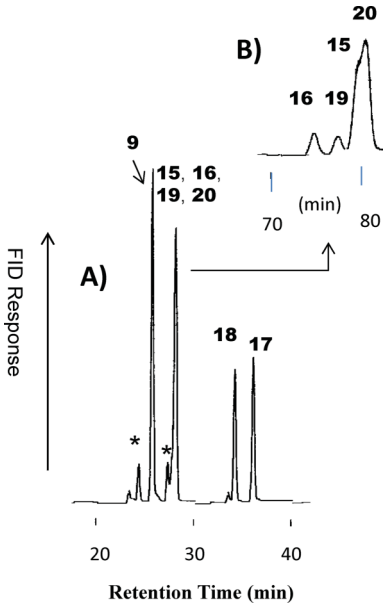
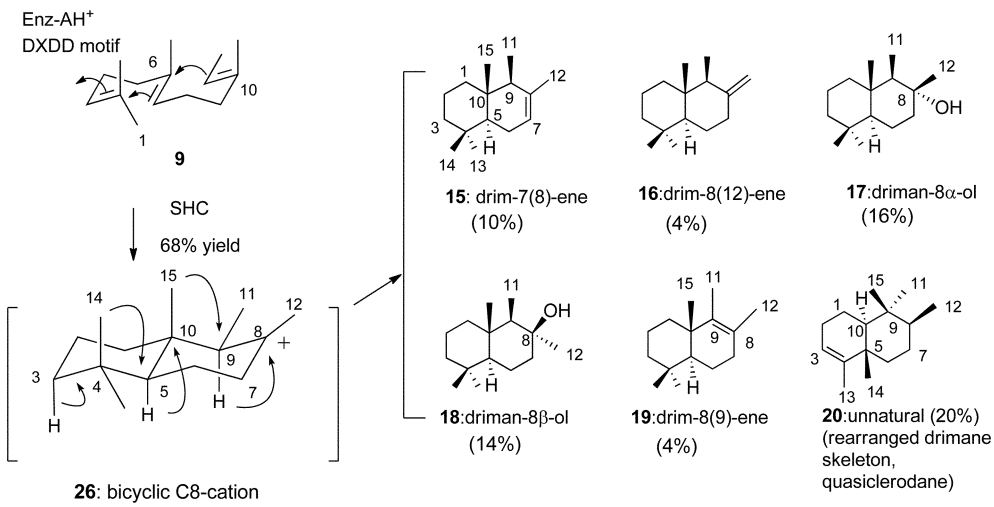


Fig. 1 (A) GC trace of the reaction mixture obtained by incubating **9** with the native SHC. The asterisk mark shows the impurity. GC conditions: column temp., 80–250 °C; rate, 5.0 °C min^{-1}, injection temp., 290 °C; carrier gas (N_2), 0.75 kg cm^{-2}.(B) GC-chromatogram of the non-polar fraction, which was separated by SiO_2 column chromatography. GC conditions: column temp., 140 °C; injection temp., 290 °C; carrier gas (N_2), 0.2 kg cm^{-2}.

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% $AgNO_3$-impregnated SiO_2 column eluting with hexane containing a trace of EtOAc. Two products (**17** and **18**) included in the polar fraction were isolated by a SiO_2 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 ^{1}H- and ^{13}C NMR spectral analyses, including DEPTs, ^{1}H–^{1}H COSY, NOESY, HMQC 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_6D_6: δ_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 ^{1}H NMR spectrum of **15**, one doublet Me (3H, δ_H 0.958, J = 7.2 Hz, Me-11) and one allylic Me (3H, δ_H 1.75, br s, Me-12) were found. Me-11 had HMBC correlations with C-9 (δ_C 49.03, d), C-10 (δ_C 36.02, s) and C-8 (δ_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).† The α-oriented H-9 was confirmed from clear NOEs of H-9/H_{ax}-5/H_{ax}-1/H_{ax}-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, δ_H 1.04, J = 6.8 Hz, Me-11) and one vinylidene residue (1H, δ_H 4.98, brs and 1H, δ_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 α-oriented by the unambiguous NOE between H-5 and H-9. In the ^{1}H NMR spectrum of **19**, two allylic Me groups (Me-11 and Me-12) were found at δ_H 1.67 (6H, brs). No doublet Me was detected. The clear HMBC cross peaks of Me-15 (δ_H 1.12, 3H, s)/C-9 (δ_C 136.2, s), Me-11/C-10 (δ_C 38.50, s), Me-12/C-7 (δ_C 34.05, t) and Me-12/C-8 ((δ_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 (δ_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 (δ_C 72.25 (s) for **17** and δ_C 71.84 (s) for **18**). For **17**, a strong NOE between Me-15 (δ_H 0.800, 3H, s) and Me-12 (δ_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, δ_H 1.72, 3H, d, J = 1.2 Hz) was found that had HMBC correlations with C-3 (δ_C 121.0, d), C-4 (δ_C 143.9, s) and C-5 (δ_C 38.48, s). One doublet Me (Me-12, δ_H 0.963, 3H, d, J = 6.8 Hz) had a cross peak with C-9 (δ_C 36.55, s), which was further correlated with Me-11 (δ_H 1.01, 3H, s) and Me-15 (δ_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.

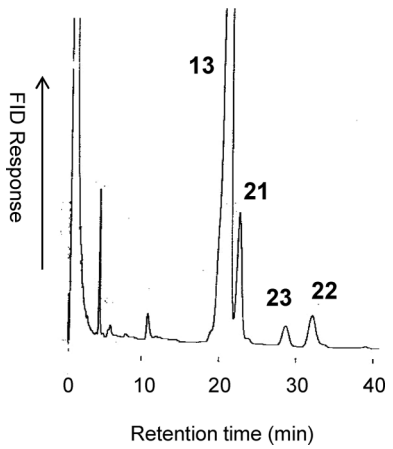
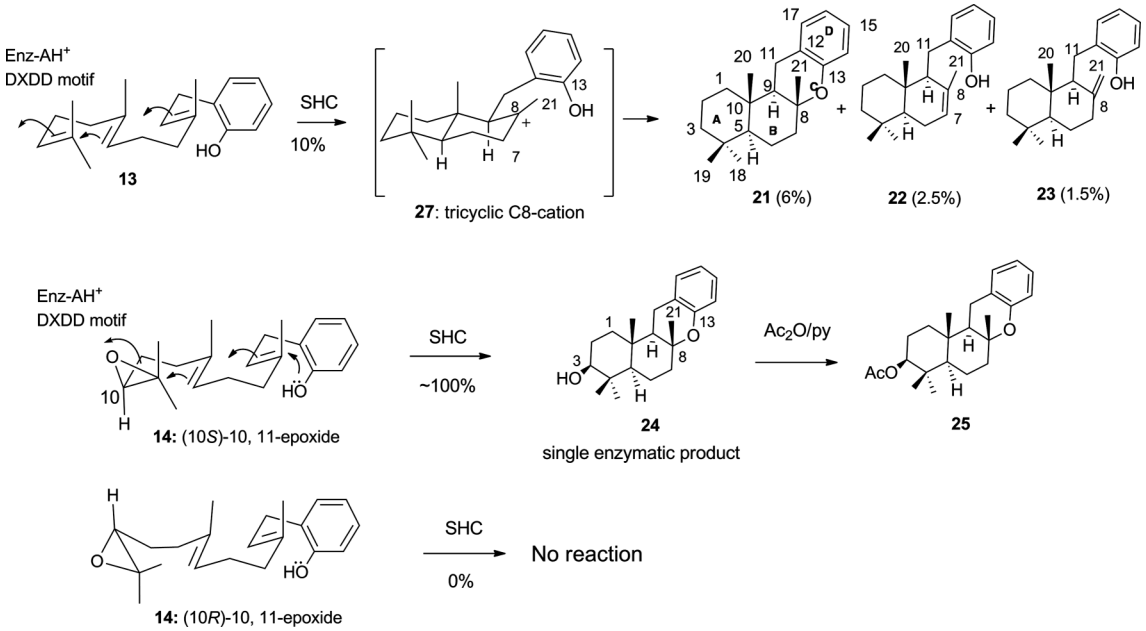


Fig. 2 GC trace of the enzymatic reaction obtained by incubating substrate **13** with SHC. Triton X-100 was removed by a short SiO_2 column. GC conditions: column temp., 190 °C; injection temp., 280 °C; carrier gas (N_2), 1.0 kg cm^{-2}.

Separation by a SiO_2 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% $AgNO_3$-SiO_2 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 (δ_C 154, s), an alcoholic carbon (δ_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 (δ_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†) 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**: δ_C 122.4 (d) and 135.8 (s) for **22**; δ_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 (δ_H 1.04, 3H, s) and allylic Me-21 (δ_H 1.77, 3H, s) had a cross peak with C-9 (δ_C 54.51, d), and H-5 (δ_H 1.41, m) and H-9 (δ_H 2.56, m) showed a strong NOE between them. The allylic Me-21 had definitive HMBC cross peaks with C-7 (δ_C 122.4, d) and C-8 (δ_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 δ_H 4.82 (1H, s) and δ_H 4.71 (1H, s), which were correlated with C-21 (δ_C 107.5, t) in the HMQC spectrum. The methylene protons at 21-position and Me-20 (δ_H 0.820, 3H, s) had definitive HMBC correlations with C-9 (δ_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,[16] 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, page S5†). Purification by a SiO_2 column (hexane : EtOAc = 100 : 05) and subsequent HPLC (hexane : 2-PrOH = 100 : 0.05) afforded pure product **24**, which was then acetylated with Ac_2O/py and subjected to NMR analyses. An ether bridge was found between C-8 (δ_C 76.43, s) and C-13 (δ_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, δ_H 4.70, dd, J = 12.0, 4.8 Hz), indicating axial-orientation of H-3 and equatorial-arrangement of OH. An acetyl group (δ_C 169.8, s; δ_H 1.87, 3H, s) was determined to be at C-3 (δ_C 80.06, s) by confirming the HMBC correlation of H-3 with the acetyl carbonyl carbon (δ_C 169.8, s). No allylic methyl group was found in the ^{1}H NMR spectrum, indicating that a complete cyclization reaction had occurred as shown in the structure of **24** in Scheme 3. The β-oriented hydroxyl group at C-3 of **24** indicates that the (10*S*)-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.

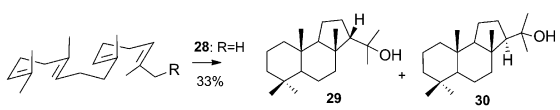
All the enzymatic products from **9** are listed in Scheme 2. Drim-8(12)-ene **16** was isolated from *Hedychium acuminatum*,[17] *Cistus* L.[18] *Eupatorium odoratum*,[19] rhizomes of *Hedychium spicatum*,[20] *Silphium* L.[21] and so forth. Driman-8α-ol **17** was found in Greek tobacco and plant culture of *Nicotiana silvestris*,[22,23] and from *Nicotiana tabacum*.[24] Drim-8(9)-ene **19** is a natural product isolated from Colorado shale oil.[25] Product **20** is a novel compound. Neither drim-7(8)-ene **15** nor driman-8β-ol **18** have been isolated as natural products (SciFinder). Substrate **9** could undergo cyclization reactions to give an intermediary bicyclic C8-cation **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 → C-8; Me-11 → C-9; H-5 → C-10; Me-14 → 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 [1b] 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†), but the alternative *R*-stereoisomer underwent no reaction (no detectable amount of product with α-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 (10*S*)-**14**, compared to that of **13**, may have caused the higher yield of (10*S*)-**14**. In other words, a more favorable orientation of (10*S*)-**14** in the catalytic cavity may have afforded the higher conversion. The epoxide ring of (10*S*)-**14**, folded in a chair conformation,[26] could have accessed the acidic DXDD motif, but that of the (10*R*)-**14**, folded in a boat form,[26] 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).[2] The structure of **28** (C_{20}) 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_{15} was more efficiently cyclized than C_{20}, but the reason for this is not clear at present. Tanaka *et al.* reported the enzymatic reaction of squalene analogs (C_{20}) containing an indole ring **31**[27] or a pyrrole ring **32**.[28] They succeeded in the syntheses of indole- or pyrrole-containing cyclic polyprenoid compounds by SHC, but in very small yields (7.5% for indole-containing polyprenoid;[27] and 1.1% for pyrrole-containing polyprenoid[28]). The enzymatic products (**36–39**) of substrates **31** and **32**, prepared by SHC,[27,28] are described in the ESI (page S30).† 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**,[2] but the enzymatic reactions of **31** and **32** give incomplete cyclization products (see ESI, page S30†).[27,28]

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**,[29] in which the indole ring is fused to the 6/6/6/6-fused carbotetracycle (ESI, page S30†), was not produced.[27]

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 regio- and 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 δ_H 7.28 and δ_C128.0 ppm as the internal reference for ^{1}H- and ^{13}C NMR spectra, respectively. Some synthetic intermediates were measured in $CDCl_3$. The chemical shifts in $CDCl_3$ solution were given according to the internal solvent peaks of δ_H 7.26 and δ_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 δ_H 2.04, δ_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 mm × 30 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 mm × 30 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.[26,30] 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^3 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_2 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.†

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

Product 15. ^{1}H NMR (C_6D_6, 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); ^{13}C NMR (C_6D_6, 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$^+$, 18), 191 (13), 124 (33), 109 (100), 82 (58); HREIMS m/z 206.2067 (calcd for $C_{15}H_{26}$, 206.2035); $[\alpha]_D^{25}$ −12.5 (c 0.12, C_6H_6).

Product 18. ^{1}H NMR (acetone d_6, 400 MHz) δ 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, O*H*); ^{13}C NMR (acetone d_6, 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$^+$, 3), 206 (18), 191 (38), 137 (28), 109 (100), 95 (37), 82 (43), 81 (37), 67 (35); HREIMS m/z 206.2022 (M$^+$–H_2O, calcd for $C_{15}H_{26}$, 206.2035); $[\alpha]_D^{25}$ −32.1 (c 0.037, C_6D_6), *cf.* lit. $[\alpha]_D$ −12.3 ($CHCl_3$, concentr. not described[31]); R_f value on SiO_2 plate = 0.54, when developed with hexane : EtOAc (100/20).

Product 20. ^{1}H NMR (C_6D_6, 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); ^{13}C NMR (C_6D_6, 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$^+$, 23), 191 (37), 163 (100), 136 (39), 123 (62), 107 (97), 95 (85), 81 (62), 69 (58); HREIMS m/z 206.2040 (calcd for $C_{15}H_{26}$, 206.2035); $[\alpha]_D^{25}$ −10.7 (c 0.14, C_6H_6).

Product 21. ^{1}H NMR (C_6D_6, 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);^{13}C NMR (C_6D_6, 150.9 MHz) δ 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^+, 53), 191 (62), 107 (84), 69 (100); HREIMS m/z 298.2286 (calcd for $C_{21}H_{30}O$ 298.2297). $[\alpha]_D^{25}$ + 49.76 (c = 0.4, $CHCl_3$).

Product 22. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 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);^{13}C NMR ($CDCl_3$, 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 $C_{21}H_{30}O$ 298.2297). Specific optical rotation was not determined due to the very small amount available.

Product 23. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 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); ^{13}C NMR ($CDCl_3$, 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^+, 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). ^{1}H NMR (C_6D_6, 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); ^{13}C NMR (C_6D_6, 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, $CHCl_3$),

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