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Preparation of regio- and stereoisomeric di- and tetrahydrogeranylgeraniols and identification of esterifying groups in natural (bacterio)chlorophylls

Hitoshi Tamiaki *, Kota Nomura, Tadashi Mizoguchi

Graduate School of Life Sciences, Ritsumeikan University, Kusatsu, Shiga 525-8577, Japan

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ABSTRACT

All regioisomeric di- and tetrahydrogeranylgeraniols possessing the C2=C3 double bond were prepared as authentic samples. The synthetic C_{20}-isoprenoid alcohols were separated well by gas chromatography. Based on the chromatographic analysis, the enzymatic reduction pathway of a geranylgeranyl group was investigated to identify the last stage of (bacterio)chlorophyll biosynthesis in phototrophs. The geranylgeranyl group was triply reduced to the phytyl group through the first regio- and stereospecific hydrogenation of C10=C11 to C10H—C11(*S*)H, the second of C6=C7 to C6H—C7(*S*)H, and the third of C14=C15 to C14H—C15H. The identification of the reduction sequence completes the biosynthetic pathways for naturally occurring chlorophyll-*a* and bacteriochlorophyll-*a* bearing a phytyl group as the esterifying moiety in the 17-propionate residues.

1. Introduction

Isoprenoids, called also terpenes and terpenoids, are natural compounds and consisted of an isoprene unit, its oligomers, and their derivatives through oxidation, reduction, and cyclization.[1] Photosynthetic organisms utilize a variety of isoprenoids as bioactive components. For example, geranylgeranyl (GG) diphosphate, one of the C_{20}-isoprenoids (diterpenoids), is transformed directly into tocotrienols and geranylgeranylated (bacterio)chlorophylls [(B)Chls], and it dimerizes and successively dehydrogenates to give carotenoids with π-conjugated polyene chromophores often after further modifications. GG diphosphate is sequentially hydrogenated by a GG reductase to afford phytyl diphosphate via dihydrogeranylgeranyl (DHGG) and tetrahydrogeranylgeranyl (THGG) diphosphates (see Fig. 1, XOH = diphosphoric acid). The resulting phytyl diphosphate is enzymatically reacted with homogentidate, 1,4-dihydroxy-2-naphthoate, and (bacterio)chlorophyllides to give tocopherols (vitamin E), phylloquinone (vitamin K_1), and phytylated (B)Chls, respectively. Phytylated (B)Chls are alternatively biosynthesized by the triply hydrogenation of geranylgeranylated (B)Chls [Fig. 1, XOH = (bacterio)chlorophyllide].[2] In the last stage of (B)Chl biosynthesis, the following two pathways are possible.[3] 1) GG diphosphate is transformed into phytyl diphosphate by GG reductases, ChlP and BchP for oxygenic and anoxygenic phototrophs, respectively. Phytyl diphosphate is then reacted with chlorophyllides and bacteriochlorophyllides by Chl and BChl synthases (ChlG and BchG), respectively, to give (B)Chls possessing a phytyl group. 2) Chlorophyllides and bacteriochlorophyllides are first esterified with GG diphosphate by ChlG and BchG, and the GG group of the resultant Chls and BChls is hydrogenated by ChlP and BchP to afford (B)Chls bearing a phytyl group.

Although GG reductases stereospecifically hydrogenate the double bonds of (DH/TH)GG substrates to give chiral (7*S*)- and (11*S*)-configurations, their regioselectivity has not been completely determined yet (see Fig. 1).[3,4] A previous investigation of Chl pigments in etiolated oat seedlings showed that a 6,7,10,11-THGG group was biosynthesized during the GG reduction, but a regioisomeric DHGG moiety was not confirmed: both 6,7- and 10,11-DHGG were possible as the intermediate.[5] One of the purple photosynthetic bacteria, a *Rhodopseudomonas* sp. Rits strain, accumulated a large amount of BChls-*a* possessing GG, DHGG, and THGG groups in the 17-propionate residue as well as a mature phytyl group (Fig. 2, middle). Spectroscopic analyses indicated that the isolated BChl-*a* intermediates were esterified with 6,7-DHGG and 6,7,10,11-THGG groups.[6] This is the first report for the determination of a regioisomeric DHGG group, where the GG reduction in BChl-*a* biosynthesis was speculated to occur in the order of GG

* Corresponding author.
E-mail address: tamiaki@fc.ritsumei.ac.jp (H. Tamiaki).

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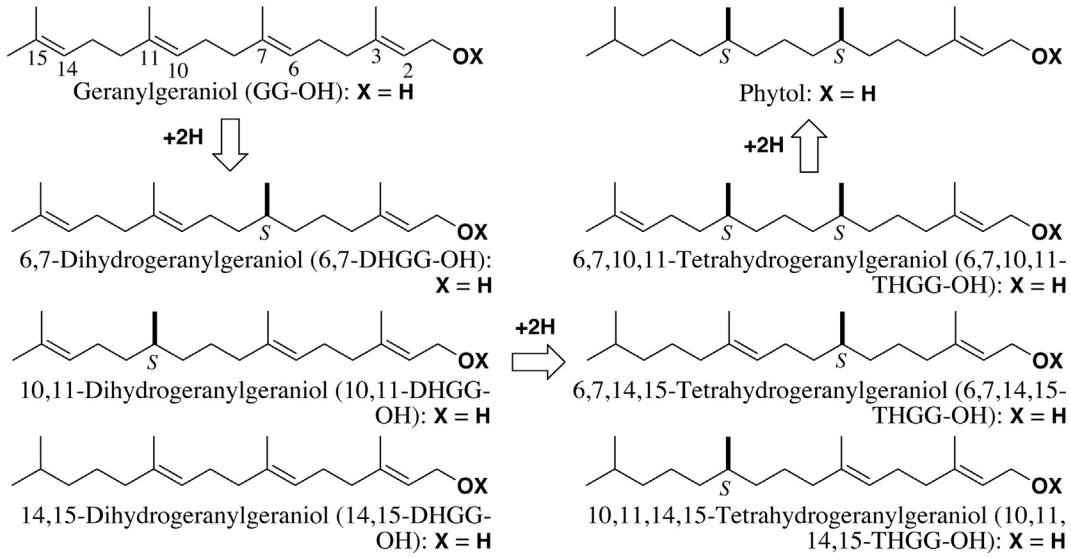


Fig. 1. Sequential hydrogenation of geranylgeranyl (GG) to phytyl moiety via di- and tetrahydrogeranylgeranyl (DHGG and THGG) moieties in a stereoselective manner: ROX = C_{20}-isoprenoid alcohols (X = H), diphosphates (XOH = diphosphoric acid), and (bacterio)chlorophylls [XOH = (bacterio)chlorophyllide].

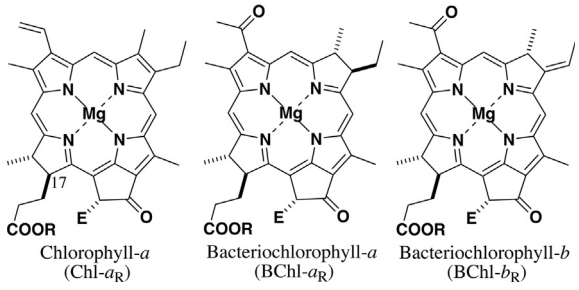


Fig. 2. Molecular structures of naturally occurring chlorophylls possessing a series of geranylgeranyl and its hydrogenated moieties as the esterifying group (R) in the 17-propionate residue: E = $COOCH_3$.

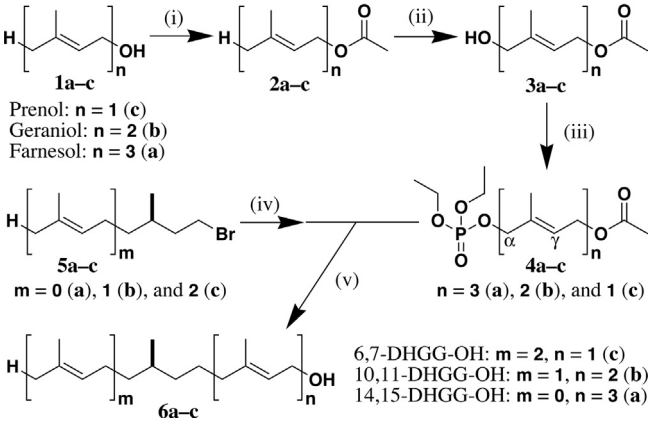
→ 6,7-DHGG → 6,7,10,11-THGG → phytyl (6,7,10,11,14,15-hexahydrogeranylgeranyl). Recently, it was reported that commercially available diatom (*Chaetoceros calcitrans*) cultures contained Chls-*a* bearing 10,11-DHGG and 6,7,10,11-THGG groups (Fig. 2, left) from their NMR spectroscopic analyses.[7] This observation shows another possible route for the GG reduction pathway in Chl-*a* biosynthesis: C10=C11 → C6=C7 → C14=C15. It is noteworthy that all the sequential reduction pathways employ the hydrogenation of the C14=C15 double bonds at the last step.

The greening processes in the etiolated leaves of angiosperm are useful for determining regiospecific GG reduction pathways.[5,8–10] The temporally produced Chl intermediates are present in low amounts and their physical properties are little differentiated. Therefore, the following technique is effective for their regioisomeric determination. Chls possessing DHGG and THGG groups are first isolated from greening leaves, then cleaved to chlorophyllides and DHGG- and THGG-OHs (see Fig. 1, X = H), which are analyzed by gas chromatography (GC). Here, we report regio- and stereospecific synthesis of 6,7-, 10,11-, and 14,15-DHGG-OHs as authentic samples and preparation of 6,7,10,11-, 6,7,14,15-, and 10,11,14,15-THGG-OHs from naturally occurring (B)Chls. The present GC analysis of DHGG- and THGG-OHs confirmed the sequential reduction pathway of a GG to a phytyl group in phototrophs including barley, radish sprouts, diatom, and purple bacteria. Moreover, some photosynthetic bacteria biosynthesize (B)Chls specifically esterified with a THGG group.[11–14] We note their regiospecific reduction pathways from GG to THGG via DHGG.

2. Results and discussion

2.1. Synthesis of dihydrogeranylgeraniols

14,15-Dihydrogeranylgeraniol (14,15-DHGG-OH, **6a**) was prepared as shown in Scheme 1. Commercially available farnesol (**1a**) was esterified with acetic anhydride in the presence of 4-(*N*,*N*-dimethylamino)pyridine (DMAP) to give farnesyl acetate (**2a**) in a 76% yield [step (i) in Scheme 1]. The resulting ester **2a** was treated using *tert*-butyl peroxide with selenium oxide as a catalyst in the presence of salicylic acid at room temperature for 2 days [step (ii)].[15] The oxidation proceeded at the allyl positions and the less sterically hindered terminal methyl group was primarily oxidized to give **3a** as the desired product, whereas the other allylic positions were partially oxidized and a small amount of aldehyde was obtained by the further oxidation of **3a**. After separation from the starting **2a** (ca. 30%) and the by-products with flash column chromatography (FCC), analytically pure **3a** (27%) was isolated as the regio- and stereoselectively oxidized product. Primary alcohol **3a** was esterified by diethyl chlorophosphate with DMAP to yield the corresponding phosphate **4a** (64%) [step (iii)]. The coupling



Scheme 1. Synthesis of 14,15- and (11*S*)-10,11-dihydrogeranylgeraniols (**6a** and **6b**): (i) Ac_2O, DMAP/CH_2Cl_2; (ii) tBuOOH, Se_2O, *o*-HOC_6H_4COOH/CH_2Cl_2; (iii) $(EtO)_2$-POCl, DMAP/CH_2Cl_2; (iv) Mg, I_2/THF; (v) THF.

reaction[16] of **4a** with isopentylmagnesium bromide prepared freshly from **5a** [step (iv)] accompanying the cleavage of the acetate ester by the Grignard reagent afforded 14,15-DHGG-OH **6a** (28%) as the desired α-attacked product after separation from the γ-attacked by-product by reverse-phase (RP) HPLC [step (v)].

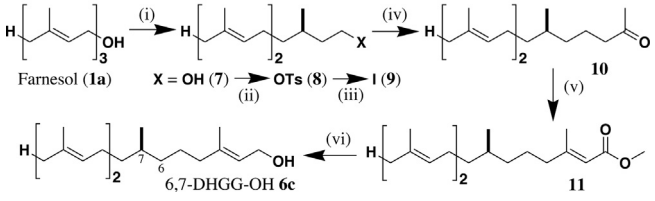
Similar to the synthesis of 14,15-DHGG-OH **6a**, enantiomerically pure (11*S*)-10,11-dihydrogeranylgeraniol (10,11-DHGG-OH, **6b**) was successfully prepared from geraniol **1b** and (*R*)-citronellyl bromide **5b**: **1b** → **2b** (71%) → **3b** (46%) → **4b** (64%) + **5b** → **6b** (15%). To obtain (7*S*)-6,7-dihydrogeranylgeraniol (6,7-DHGG-OH, **6c**), the coupling of phosphate **4c** from prenol **1c** with the Grignard reagent from **5c** was applied: **5c** was obtained by the bromination of the corresponding tosylate (see below). The Grignard reaction yielded no desired product **6c**, which was ascribable to the low reactivity of the sterically demanded Grignard reagent. Therefore, the other pathway to **6c** was examined as follows.

According to reported procedures,[17] farnesol **1a** was stereo- and regioselectively hydrogenated with a chiral ruthenium catalyst to give 2,3-dihydrofarnesol **7** [step (i) in Scheme 2]. The product was a 4:1 mixture of (3*R*) and (3*S*)-enantiomers from the ^{1}H NMR analysis of its diastereomeric ester with (*R*)-(-)-α-methoxyphenylacetic acid (see Fig. S1). The isolated yield (80%) and stereoselectivity (60% ee) were comparable to the reported values (65% and 81% ee).[17] The resulting alcohol **7** was converted into the corresponding iodide **9** via tosylate **8** by conventional procedures [steps (ii) and (iii)]: **7** → **8** (84%) → **9** (70%). 2,3-Dihydrofarnesyl iodide **9** [(3*R*):(3*S*) = 4:1] was reacted with ethyl acetoacetate in the presence of sodium methoxide, followed by hydrolysis and pyrolysis [step (iv)].[18] The acetoacetic ester synthesis gave methyl ketone **10** (32%). The Horner-Wadsworth-Emmons reaction[18] of **10** with ethyl 2-(diethoxyphosphoryl)acetate in the presence of sodium methoxide afforded α,β-unsaturated methyl ester **11** (64%) [step (v)], which was reduced by Vitride reagent[18] to yield (7*S*)-6,7-DHGG-OH **6c** (80%, 60% ee) [step (vi)].

2.2. Synthesis of tetrahydrogeranylgeraniols

Three regio- and stereoisomeric tetrahydrogeranylgeraniols, 6,7,10,11-, 6,7,14,15-, and 10,11,14,15-THGG-OHs, were prepared by the methanolysis of naturally occurring (B)Chls. The specific (B)Chls esterified with one of the THGG-OHs were isolated from cultured phototrophs and the ester bonds in their 17-propionate residues were cleaved under basic conditions to give the desired THGG-OHs.

Green sulfur bacteria have Chl-*a* species as a primary electron-acceptor in their reaction centers. The specific Chl-*a* molecule was esterified with a 10,11,14,15-THGG group (see the left drawing of Fig. 2, R = 10,11,14,15-THGG).[12] *Chlorobaculum tepidum*,[19] one of the green sulfur bacterial species, was cultured and the harvested cells were extracted with acetone and methanol (1:1). This species produces a large amount of BChls-*a*/*c*, where the desired Chl-*a* is a minor pigment. To roughly separate the more polar BChl pigments,



Scheme 2. Synthesis of (7*S*)-6,7-dihydrogeranylgeraniol (**6c**): (i) L*Ru(II)Cl$_2$(*p*-MeC$_6$H$_4$iPr), KOH/iPrOH, reflux; (ii) TsCl, DMAP/CH$_2$Cl$_2$, rt; (iii) NaI/THF, reflux; (iv) AcCH$_2$COOEt, MeONa–MeOH/THF, rt, then aq. KOH/iPrOH, 80 °C; (v) (EtO)$_2$POCH$_2$COOEt, NaOMe/C$_6$H$_{14}$, rt; (vi) NaAl(OCH$_2$CH$_2$OMe)$_2$H$_2$–PhMe, rt. L* = (*S*)-2,2′-[(*p*-MePh)$_2$P]$_2$–1,1′-binaphtyl.

the above extract was treated with hexane in which the less polar Chl-*a* was highly dissolved. The hexane phase was purified with RP- and successive normal phase (NP)-HPLC to give a pure sample of 10,11,14,15-tetrahydrogeranylgeranylated Chl-*a*. The separated Chl-*a* molecule was treated with potassium hydroxide in methanol[20] to give 10,11,14,15-THGG-OH after purification by RP-HPLC.

Halorhodospira halochloris is one of the purple bacteria species and primarily produces BChl-*b* esterified with a 6,7,14,15-THGG group (Fig. 2, right, R = 6,7,14,15-THGG).[11,21] After culturing this species, the harvested cells were extracted with acetone and methanol. The extract was treated with petroleum ether, diethyl ether, and distilled water at 0 °C. The separated ethereal phase was purified with RP-HPLC to give 6,7,14,15-tetrahydrogeranylgeranylated BChl-*b* as the major fraction. The isolated BChl-*b* molecule was methanolyzed (vide supra) to give 6,7,14,15-THGG-OH. In the above procedures, strictly dark operation was required for avoiding the decomposition of chemically fragile BChl-*b*.

The *Rhodopseudomonas* sp. Rits strain is a purple bacterium and biosynthesizes BChls-*a* with a variety of GG and its hydrogenated groups in the cells (Fig. 2, middle).[6,22–24] The hydrogenation positions in the THGG group have been already determined to be at C6=C7 and C10=C11 double bonds.[6] Similar to the extraction and purification of the above BChl-*b* molecule, BChls-*a* were extracted from the cultured cells, then 6,7,10,11-tetrahydrogeranylgeranylated BChl-*a* was separated from non-, di-, and hexahydrogeranylgeranylated (phytylated) BChls-*a* and methanolyzed to afford 6,7,10,11-THGG-OH.

2.3. Identification of regioisomeric di- and tetrahydrogeranylgeraniols

The discrimination of three regioisomeric DHGG-OHs possessing the C2=C3 double bond was examined. The ^{1}H NMR spectrum of 14,15-DHGG-OH showed a characteristic doublet peak for the terminal methyl protons [C15H(C$\underline{H}_3$)$_2$] and was different from those of 6,7- and 10,11-DHGG-OHs (see Fig. S2). The latter two spectra resembled each other, so their distinction was difficult from the ^{1}H NMR spectral analysis. The ^{13}C NMR spectra of the three regioisomers were partly different in CD$_3$OD (see Fig. S3). Especially, their three tertiary alkenyl carbons (—CH=) gave regioisomerically dependent peaks at around 125 ppm. A relatively large amount of the samples was necessary for such NMR spectral measurements. The authentic samples of the three DHGG-OH regioisomers were submitted to GC and clearly separated as shown in the left profiles of Fig. 3. For the GC analysis, a less volume of their solutions was enough, then the present GC performance was useful for the identification of the regioisomers. Regioisomeric 14,15-, 11,10-, and 6,7-DHGG-OHs were eluted in the order. The hydrogenation of a double bond near the terminus position of the GG group shortened the retention times (t_R) of the resulting DHGG-OHs.

Under the same conditions, the three regioisomeric THGG-OHs were separated by the present GC (Fig. 3, right). Similarly as in DHGG-OHs, the presence of a double bond near the THGG terminus lengthened their t_R. This observation is consistent with their GC analysis reported previously.[25] Geranylgeraniol and phytol eluted at 14.4 and 8.4 min, respectively (see Fig. S4 and Table S1). The successive hydrogenation of GG-OH shortened the t_R.

2.4. Characterization of esterifying groups in the 17-propionate residue of chlorophyll-a

Recently, we reported that commercially available cultures of *Chaetoceros calcitrans*, one of the diatoms, contained a variety of Chls-*a* esterified with GG, 10,11-DHGG, and 6,7,10,11-THGG moi-

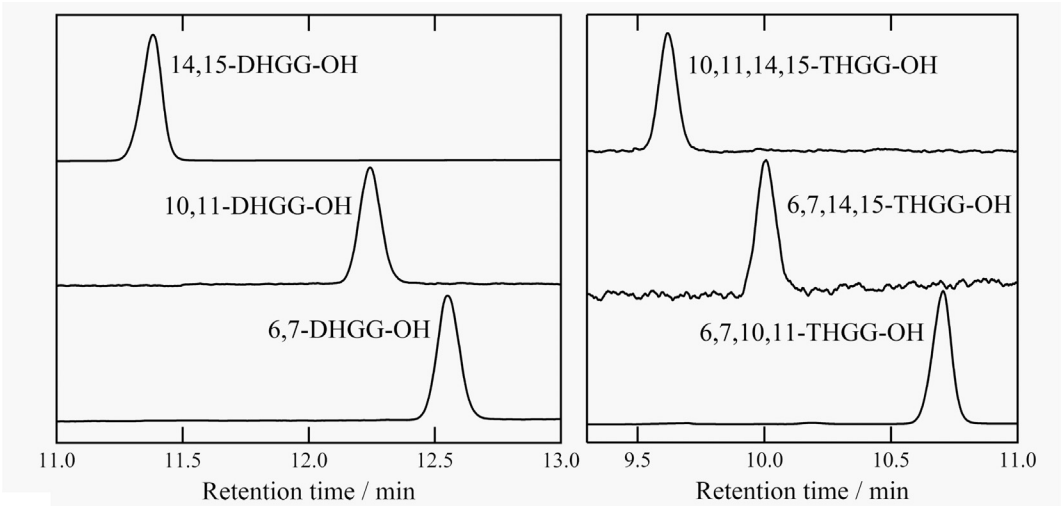


Fig. 3. GC profiles of authentic di- (left) and tetrahydrogeranylgeraniol regioisomers (right): see the analytical conditions in Section 4.4.

eties as well as a phytyl group from their spectral analyses and the former three species were also photosynthetically active in the cells.[7] Their molecular structures were reconfirmed as follows. From the *Chaetoceros calcitrans* cultures, Chl-*a* species were extracted and analyzed by LC-MS. The first, second, third, and fourth fractions (#1–#4 in Fig. 4, top) were assigned to Chls-*a* esterified with GG, DHGG, THGG, and phytyl groups, respectively. The second and third RP-HPLC fractions were separated and both the isolated Chls-*a* were methanolyzed under the basic conditions (vide supra). The resulting C_{20}-isoprenoid alcohols, DHGG-OH and THGG-OH, were analyzed by GC (Fig. 5, top). Regioisomeric 10,11-DHGG-OH and 6,7,10,11-THGG-OH were exclusively detected, so Chl-*a* species possessing sole 10,11-DHGG and 6,7,10,11-THGG groups were present in the *Chaetoceros calcitrans* cultures. This suggested that the GG group was biosynthetically reduced in the hydrogenation order of the C10=C11, C6=C7, and C14=C15 double bonds in the oxygenic phototroph primarily producing Chl-*a* with a phytyl group.

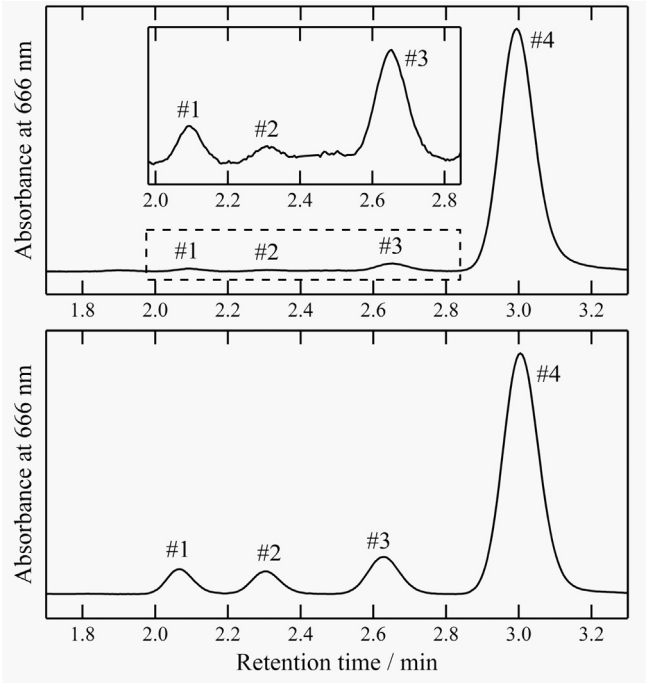


Fig. 4. RP-HPLC profiles of Chl-*a* species isolated from commercially available *Chaetoceros calcitrans* cultures (top) and the greening leaves of *Hordeum vulgare* (bottom, 25-min illumination): Cosmosil 5C_{18}-AR-II 3.0 × 150 mm, methanol: acetonitrile = 37:3, 1.0 ml/min.

During the greening in *Hordeum vulgare*, barley, substantial amounts of three Chl-*a* species esterified with GG, DHGG, and THGG moieties were temporally found as the precursors of mature Chl-*a* with a phytyl group.[10] The Chl-*a* pigment extract gave four species as shown in the bottom panel of Fig. 4. After illumination for 25 min to the etiolated leaves, the HPLC-isolated fractions #2 and #3 were chemically cleaved at the 17-propionate residue to give DHGG-OH and THGG-OH, respectively. Their GC analyses indicated the production of sole 10,11-DHGG-OH and 6,7,10,11-THGG-OH (Fig. 5, upper middle). Their exclusive detection showed that a GG group was hydrogenated during the Chl-*a* biosynthesis in barley through C10=C11 → C6=C7 → C14=C15, similarly as in the diatom.

Prolonged illumination for 6 h gave a small amount of regioisomeric 6,7-DHGG-OH as well as 10,11-DHGG-OH, but no regioisomers were detected other than 6,7,10,11-THGG-OH (Fig. 5, lower middle). The observation can be rationalized by the following two enzymatic pathways. 1) The C6=C7 double bond of a GG moiety might be initially reduced in the late stage of the greening process in *Hordeum vulgare*. 2) The 6,7,10,11-THGG moiety would be reversibly dehydrogenated at the C10H—C11H position to give 6,7-DHGG-OH. The latter route is likely due to the favorable enzymatic regioselectivity at the C10=C11 over C6=C7, an increasing amount of the THGG moiety, and an accumulation of $NADP^+$ in the late stage. Throughout the greening in *Raphanus sativus*, white radish sprouts, 10,11-DHGG-OH and 6,7,10,11-THGG-OH were exclusively detected after the modification of Chl-*a* intermediates with dehydrogenated GG moieties (Fig. 5, bottom). Therefore, a GG moiety is enzymatically reduced to give phytylated Chl-*a* via 10,11-DHGG and 6,7,10,11-THGG species in *Raphanus sativus*.

2.5. Characterization of esterifying groups in the 17-propionate residue of bacteriochlorophylls

In full growth cultures of many purple bacteria, BChls esterified with GG, DHGG, and THGG groups were observed as well as standard phytylated BChls.[6,22–24] They work photosynthetically in the light-harvesting and reaction center apparatuses. *Rhodobacter sphaeroides*, one of the purple photosynthetic bacteria, produced

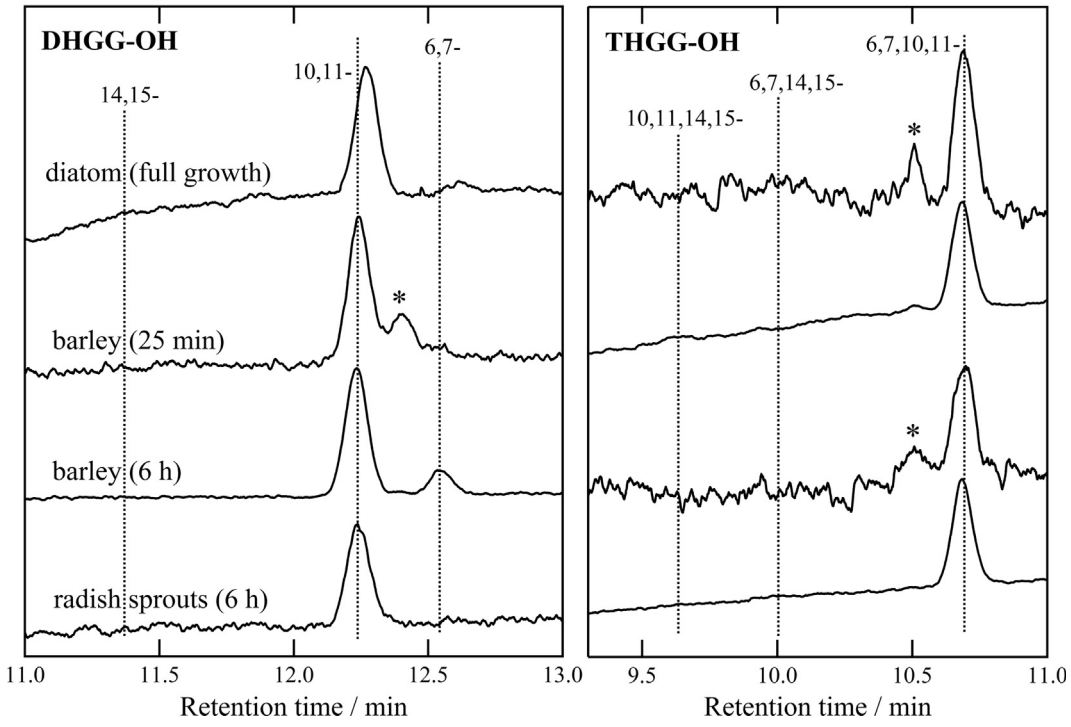


Fig. 5. GC profiles of di- (left) and tetrahydrogeranylgeraniol regioisomers (right) derived from Chl-*a* in commercially available *Chaetoceros calcitrans* cultures (top) as well as the greening leaves of *Hordeum vulgare* (upper and lower middle after illumination for 25 min and 6 h, respectively) and *Raphanus sativus* (bottom, 6-h illumination): see the analytical conditions in Section 4.4. Peaks at the asterisk are ascribable to undetermined impurities.

BChls-*a* with GG, DHGG, THGG, and phytyl groups in the first, second, third, and fourth eluted fractions, respectively in RP-HPLC (Fig. 6, top).[22,24] The structures of the DHGG and THGG moieties

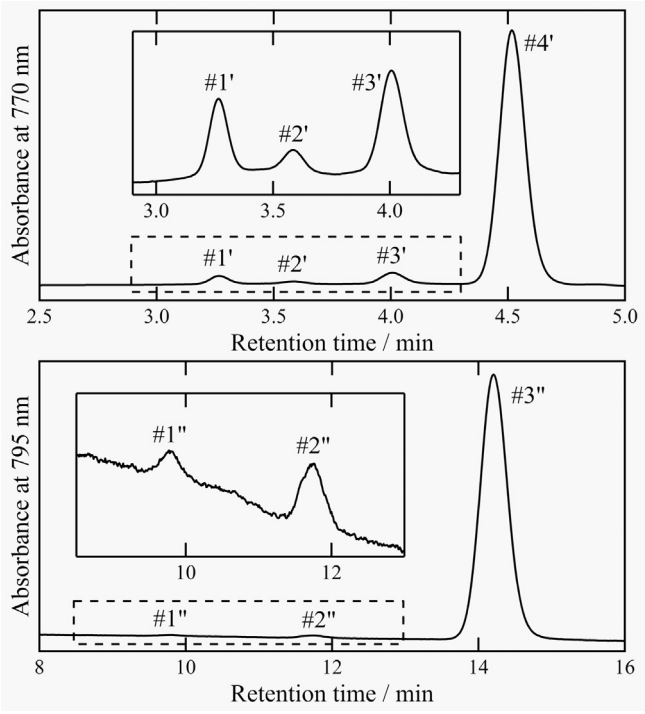


Fig. 6. RP-HPLC profiles of BChl-*a* isolated from *Rhodobacter sphaeroides* (top) and BChl-*b* from *Halorhodospira halochloris* (bottom): Cosmosil $5C_{18}$-AR-II 4.6 × 150 mm, methanol:acetonitrile = 37:3 (top) and methanol:water = 19:1 (bottom), 1.0 ml/min.

were determined by HPLC-isolation of the BChl-*a* fractions #2′/#3′, methanolysis to their alcohols, and successive GC analyses. The top GC profiles of Fig. 7 proved the production of sole 10,11-DHGG-OH and 6,7,10,11-THGG-OH. Therefore, phytylated BChl-*a* in the purple bacterium was biosynthesized through sequential enzymatic reduction of the C10=C11, C6=C7, and C14=C15 of a GG moiety.

For the *Rhodopseudomonas* sp. Rits strain, another purple bacterium, larger amounts of BChl-*a* intermediates were accumulated in its cultured cells.[6,22–24] Based on the GC analysis (Fig. 7, middle), the culture gave also the same BChl-*a* intermediates with the 10,11-DHGG and 6,7,10,11-THGG groups as in the above *Rhodobacter sphaeroides*. The observation is partially different from our previous report[6] that a specific strain produced BChls-*a* bearing 6,7-DHGG and 6,7,10,11-THGG groups (see Introduction section). Their molecular structures had been characterized by their ^{1}H NMR spectra. The structural conflict in the DHGG moiety may be dependent on the culturing conditions. BChl-*a* esterified with a 6,7-DHGG group might be produced by similar dehydrogenation of the 6,7,10,11-THGG group as in the greening in *Hordeum vulgare* (vide supra). From various stages of the cultured strain, no more 6,7-DHGG-OH could be detected in the present GC analysis. The reason for the inconsistency is being pursued and will be reported anywhere if fixed.

Halorhodospira halochloris, one of the thermophilic purple bacteria, produced predominantly BChl-*b* esterified with a 6,7,14,15-THGG group.[11,21] Based on LC-MS analyses, the cultured cells contained small amounts of BChls-*b* with GG and DHGG moieties as the first and second fractions, respectively (see Fig. 6, bottom), without phytylated BChl-*b*.[21] GC analyses of their esterifying alcohols (Fig. 7, bottom) clearly indicated that the cells biosynthesized BChls-*b* with 6,7-DHGG and 6,7,14,15-THGG moieties. As a result, the GG moiety in *Halorhodospira halochloris* was first reduced at the C6=C7, followed by hydrogenation at the C14=C15. The reduc-

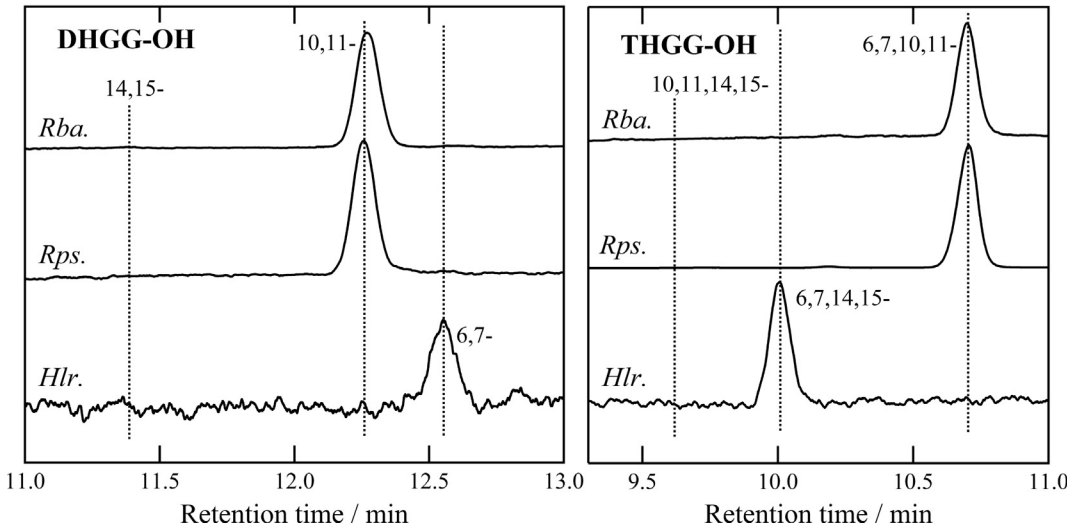


Fig. 7. GC profiles of di- (left) and tetrahydrogeranylgeraniol regioisomers (right) derived from BChl-*a* in *Rhodobacter sphaeroides* (top) and *Rhodopseudomonas* sp. (Rits strain, middle) as well as BChl-*b* in *Halorhodospira halochloris* (bottom): see the analytical conditions in Section 4.4.

tion sequence was the same as those described above, although the initial hydrogenation at the C10═C11 observed in the other phototrophs was inhibited. The C10═C11 double bond of a GG substrate would be fully protected from the enzymatic reduction in *Halorhodospira halochloris* cells. It cannot be ruled out that the enzymatic active site is different from the others.

3. Conclusions

To elucidate the hydrogenation (reduction) pathway from a GG group to THGG and phytyl groups in the last stage of (B)Chl biosynthesis, three possible DHGG-OH regioisomers were synthesized through C—C couplings. The authentic DHGG-OHs as well as three THGG-OHs obtained from naturally occurring and structurally determined (B)Chls were analyzed by GC, showing well-resolved peaks for the six C_{20}-isoprenoid alcohols. Trace amounts of (B)Chl intermediates produced temporally in phototrophs were isolated by HPLC and successively methanolyzed to give DHGG- and THGG-OHs. The resulting alcohols were characterized by the above GC analyses and the molecular structures of the biosynthetic (B)Chl precursors were identified.

During the greening processes in etiolated leaves of barley and radish sprouts, 10,11-DHGG-OH and 6,7,10,11-THGG-OH were observed from the derivatization of Chls-*a* isolated in the initial stage and comparison of their GC peaks. The regioselectivity indicated the following hydrogenation sequence. A GG moiety as the esterifying group in the 17-propionate residue of Chl-*a* was first reduced at the C10═C11 double bond, followed by the hydrogenation of the C6═C7, and transformed to a phytyl moiety by the final reduction of the C14═C15. The first hydrogenation position of the GG group in the oxygenic phototrophs was confirmed by the present work to be its C10═C11 and the biosynthetic pathway for Chl-*a* was fully determined here. In addition, the same pathway was proposed in a diatom, which was consistent with the recent report investigated by spectroscopic analyses.[7]

Based on the aforementioned GC analysis, both the *Rhodobacter sphaeroides* and *Rhodopseudomonas palustris* strains produced BChls-*a* esterified with 10,11-DHGG and 6,7,10,11-THGG groups in their full-growth cultures. Purple photosynthetic bacteria would biosynthesize phytylated BChl-*a* through the same sequential reduction of GG moiety as in the above angiosperm. *Halorhodospira halochloris*, another purple bacterium, specifically produced BChl-*b* possessing the 6,7,14,15-THGG group and the biosynthetic route was proposed to be the order of GG → 6,7-DHGG → 6,7,14,15-THGG. This sequence is consistent with that in the above standard purple bacteria, except for the complete suppression of the C10═C11 reduction. Based on the final reduction at the C14═C15, Chl-*a* bearing the 10,11,14,15-THGG group found in green sulfur bacteria might be produced through the successive reduction of C10═C11 → C14═C15 of a GG moiety, where the C6═C7 reduction is fully inhibited.[12]

4. Experimental

4.1. General

^{1}H NMR spectra were recorded on a JEOL AL-400 (400 MHz) or ECA-600 (600 MHz) spectrometer; residual $CHCl_3$ (δ_H = 7.26 ppm) or CD_2HOD (δ_H = 3.31 ppm) was used as an internal reference. ^{13}C NMR spectra were recorded on an ECA-600 (151 MHz) spectrometer; residual $^{13}CD_3OD$ (δ_C = 49.00 ppm) was used as an internal reference. High resolution mass spectra (HRMS) were recorded on a Bruker micrOTOF II spectrometer: atmospheric pressure chemical ionization (APCI) and positive mode in an acetonitrile solution. FCC was performed with silica gel (Merck, Kieselgel 60, 40–63 μm, 230–400 mesh). HPLC was performed by a Shimadzu LC-10AD$_{VP}$ pump, SPD-M10A$_{VP}$ diode-array detector, and SCL-10A$_{VP}$ system controller using a packed ODS column (Nacalai Tesque Cosmosil 5C$_{18}$-AR-II) for RP or a packed silica gel column (Cosmosil 5SL-II) for NP. LC-MS was obtained on a Shimadzu LCMS-2010EV chromatograph with APCI and positive mode. GC was done with a Shimadzu GC-2010 plus high-end gas chromatograph with an AOC-20i auto-injector and an FID-2010 plus flame ionization detector.

*Chaetoceros calcitran*s cultures were purchased from Marintech Co Ltd, and the *Rhodobacter sphaeroides* ATH2.4.1, *Rhodopseudomonas* sp. Rits, and *Chlorobaculum tepidum* ATCC49652 strains were in our collection. All the reaction reagents were obtained from commercial suppliers and utilized as supplied. Commercially available solvents (Nacalai Tesque) were used for the synthetic procedures and distilled water was prepared by a Yamato AutoStill WG250 system. All HPLC solvents except distilled water were obtained as HPLC grade from Nacalai Tesque and the mixed solvents were degassed before use.

4.2. Synthesis of dihydrogeranylgeraniols

4.2.1. 6,7-Dihydrogeranylgeraniol (**6c**)

To a solution of farnesol (**1a**, 50.0 mg, 225 mmol) in 2-propanol (22.5 ml, dried over molecular sieves 3A for 1 day) were chloro[(*S*)-(-)-2,2′-bis(di-*p*-tolyl-phosphino)-1,1′-binaphytyl] (*p*-cymene) ruthenium(II) chloride (22 mg, 24 μmol) and potassium hydroxide (85%, 3.0 mg, 45 μmol) and the mixture was refluxed under nitrogen for 2 days. The reaction mixture was cooled down to room temperature, to which were added distilled water and hexane with stirring. The separated organic phase was washed twice with distilled water and brine and dried over sodium sulfate. After evaporation of the solvent, the residue was purified with FCC (hexane:ethyl acetate = 4:1) to give 2,3-dihydrofarnesol (**7**, 40.4 mg, 180 mmol) in 80% isolated yield (lit.[17] 65%) as a 4:1 mixture of (3*R*)- and (3*S*)-enantiomers (60% ee, see below; lit.[17] 81% ee): yellow oil; ^{1}H NMR ($CDCl_3$, 600 MHz) δ = 5.12–5.08 (2H, m, 6-, 10-H), 3.73–3.64 (2H, m, CH_2O), 2.08–1.95 (6H, m, 4-, 7-, 8-CH_2), 1.68 (3H, s, 11-CH_3 *trans* to C10—CH_2), 1.60 (6H, s, 7-CH_3, 11-CH_3 *cis* to C10—CH_2), 1.42–1.15 (5H, m, 1-CH_2CHCH_2), 0.91 (3H, d, J = 7 Hz, 3-CH_3) [The 1-OH was invisible.]; see the spectral data of its racemic mixture in Ref. 18.

The above alcohol **7** (22 mg, 98 μmol) and (*R*)-(-)-α-methoxyphenylacetic acid (18 mg, 110 μmol) were dissolved in dichloromethane (0.5 ml), to which *N*,*N*′-diisopropylcarbodiimide (13.8 mg, 109 μmol) and DMAP (6 mg, 50 μmol) were added. The mixture was stirred at room temperature under argon for 12 h. After addition of diethyl ether to the reaction mixture, the ethereal phase was washed with aq. 1% hydrogen chloride, aq. 4% sodium hydrogen carbonate, distilled water, and brine and dried over sodium sulfate. All the solvents were evaporated and the residue was purified with FCC (hexane:ethyl acetate = 10:1) to give 2,3-dihydrofarnesyl (*R*)-α-methoxyphenylacetate (20 mg, 54 mmol) in 55% yield with 60% de: colorless oil; ^{1}H NMR ($CDCl_3$, 600 MHz) δ (3*R*/3*S* = 4/1) = 7.33–7.44 (5H, m, 1-OCOCC_6H_5), 5.11–5.04 (2H, m, 6-, 10-H), 4.75 (1H, s, 1-OCOCH), 4.21–4.10 (2H, m, CH_2O), 3.41 (2H, s, OCH_3), 2.09–1.85 (6H, m, 4-, 7-, 8-CH_2), 1.68 (3H, s, 11-CH_3 *trans* to C10—CH_2), 1.60 (3H, s, 11-CH_3 *cis* to C10—CH_2) 1.58 (3H, s, 7-CH_3), 1.43–1.08 (5H, m, 1-CH_2CHCH_2), 0.81/0.84 (3H, d, J = 7 Hz, 3-CH_3).

To a solution of (3*R*)-2,3-dihydrofarnesol (**7**, 60% ee, 159 mg, 709 μmol) in dichloromethane (10 ml) were added *p*-tosyl chloride (200 mg, 1050 μmol) and DMAP (88 mg, 720 μmol). The mixture was stirred at room temperature under nitrogen for 16 h and the reaction was quenched with distilled water. After extraction with diethyl ether, the organic phase was washed twice with aq. 1% hydrogen chloride, aq. 4% sodium hydrogen carbonate, distilled water, and brine and dried over sodium sulfate. After evaporation of the solvent, the residue was purified with FCC (hexane:ethyl acetate = 9:1) to give 2,3-dihydrofarnesyl *p*-tosylate (**8**, 225.1 mg, 595 μmol) in 84% yield: colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 7.93 (2H, d, J = 8 Hz, *o*-H of Ts), 7.34 (2H, d, J = 8 Hz, *m*-H of Ts), 5.10–5.04 (2H, m, 6-, 10-H), 4.10–4.04 (2H, m, CH_2O), 2.45 (3H, s, *p*-CH_3 of Ts), 2.09–1.86 (6H, m, 4-, 7-, 8-CH_2), 1.68 (3H, s, 11-CH_3 *trans* to C10—CH_2), 1.60 (3H, s, 11-CH_3 *cis* to C10—CH_2), 1.57 (3H, s, 7-CH_3), 1.48–1.07 (5H, m, 1-CH_2CHCH_2), 0.82 (3H, d, J = 7 Hz, 3-CH_3); see the spectral data of its racemic mixture in Ref. 26.

A THF solution (20 ml) of the above tosylate **8** (665 mg, 1.76 mmol) and sodium iodide (3.0 g, 20 mmol) was refluxed under argon for 2 h and cooled down to room temperature, followed by the addition of distilled water. After extraction with diethyl ether, the organic phase was washed twice with aq. 10% sodium thiosulfate, distilled water and brine and dried over sodium sulfate. After evaporation of the solvents, the residue was purified with FCC (hexane) to give 2,3-dihydrofarnesyl iodide (**9**, 410 mg, 1.23 mmol) in 70% yield: colorless oil; ^{1}H NMR ($CDCl_3$, 600 MHz) δ = 5.11–5.08 (2H, m, 6-, 10-H), 3.27–3.15 (2H, m, CH_2I), 2.09–1.86 (6H, m, 4-, 7-, 8-CH_2), 1.68 (3H, s, 11-CH_3 *trans* to C10—CH_2), 1.60 (6H, s, 7-CH_3, 11-CH_3 *cis* to C10—CH_2), 1.38–1.15 (5H, m, 1-CH_2CHCH_2), 0.89 (3H, d, J = 7 Hz, 3-CH_3); see also the spectral data of its racemic mixture in Ref. 18.

The above iodide **9** (218 mg, 0.652 mmol) was dissolved in THF (5 ml), to which were added 28% sodium methoxide in methanol (0.3 ml, 1.5 mmol) and ethyl acetoacetate (441 μl, 450 mg, 3.46 mmol). The mixture was stirred at room temperature under nitrogen for 20 h, diluted with distilled water, and extracted with diethyl ether. The ethereal phase was washed twice with distilled water and brine and dried over sodium sulfate. After evaporation of the solvents, the residue was dissolved in 2-propanol (2 ml), to which was added aq. 50% sodium hydroxide. The mixture was heated at 80 °C under nitrogen for 1 h, cooled down to room temperature, acidified with aq. 0.1 M hydrogen chloride, and extracted with diethyl ether. The ethereal phase was washed twice with aq. 0.1 M hydrogen chloride, aq. 4% sodium hydrogen carbonate, distilled water, and brine and dried over sodium sulfate. After evaporation of the solvents, the residue was purified with FCC (hexane: ethyl acetate = 9:1) to give 6,10,14-trimethylpentadeca-9,13-dien-2-one (**10**, 55 mg, 0.208 mmol) in 32% yield: colorless oil; ^{1}H NMR ($CDCl_3$, 600 MHz) δ = 5.11–5.08 (2H, m, 9-, 13-H), 2.41–2.39 (2H, m, 1-$COCH_2$), 2.13 (3H, s, $COCH_3$), 2.08–1.91 (6H, m, 7-, 10-, 11-CH_2), 1.68 (3H, s, 14-CH_3 *trans* to C13—CH_2), 1.62–1.50 (2H, m, 3-CH_2), 1.60 (3H, s, 14-CH_3 *cis* to C13—CH_2), 1.59 (3H, s, 10-CH_3), 1.43–1.11 (5H, m, 4-CH_2CHCH_2), 0.87 (3H, d, J = 7 Hz, 6-CH_3); see also the spectral data of its racemic mixture in Ref. 18.

To a solution of the above ketone **10** (50 mg, 189 μmol) in hexane (1 ml) were added sodium methoxide (80 mg, 1480 μmol) and ethyl 2-(diethoxyphosphoryl)acetate (80 mg, 357 μmol). The mixture was stirred at room temperature under nitrogen for 2 h and the reaction was quenched with distilled water. After extraction with hexane, the organic phase was washed with twice with aq. 60% methanol and brine and dried over sodium sulfate. After evaporation of the solvent, the residue was purified with FCC (hexane: ethyl acetate = 19:1) to give methyl 3,7,11,15-tetramethylhexadeca-2,10,14-trienoate (**11**, 39.0 mg, 122 μmol) in 64% yield: colorless oil; ^{1}H NMR ($CDCl_3$, 600 MHz) δ = 5.67–5.65 (1H, m, 2-H), 5.12–5.08 (2H, m, 10-, 14-H), 3.68 (3H, s, $COOCH_3$), 2.15 (3H, s, 3-CH_3), 2.13–1.92 (8H, m, 3-, 8-, 11-, 12-CH_2), 1.68 (3H, s, 15-CH_3 *trans* to C13—CH_2), 1.60 (3H, s, 15-CH_3 *cis* to C13—CH_2), 1.59 (3H, s, 11-CH_3), 1.43–1.08 (7H, m, 4-$CH_2CH_2CHCH_2$), 0.87 (3H, d, J = 7 Hz, 7-CH_3); see also the spectral data of its racemic mixture in Ref. 18.

To a hexane solution (1 ml) of the above ester **11** (30 mg, 94 μmol) was added a toluene solution of sodium bis(2-methoxyethoxy)aluminum hydride (Red-Al, ≈70%, 118 μl, 120 mg, ≈420 μmol) and stirred at room temperature under nitrogen for 2 h. A small amount of acetone and aq. 50% acetic acid (1 ml) were added to the reaction mixture and further stirred at room temperature under nitrogen for 1 h. After extraction with diethyl ether, the organic phase was washed with aq. 4% sodium hydrogen carbonate and brine and dried over sodium sulfate. The solvents were evaporated and the residue was purified with FCC (hexane:ethyl acetate = 6:1) to give 6,7-DHGG-OH **6c** (22 mg, 75 μmol) in 80% yield: colorless oil; ^{1}H NMR (CD_3OD, 600 MHz) δ = 5.35 (1H, tq, J = 7, 2 Hz, 2-H), 5.10, 5.09 (each 1H, br-t, J = 7 Hz, 10-, 14-H), 4.08 (2H, d, J = 7 Hz, CH_2O), 2.08 (2H, q, J = 7 Hz, 12-CH_2), 2.05–1.94 (6H, m, 3-, 8-, 11-CH_2), 1.67 (3H, s, 15-CH_3 *trans* to C13—CH_2), 1.66 (3H, s, 3-CH_3), 1.60 (6H, s, 11-CH_3, 15-CH_3 *cis* to C13—CH_2), 1.49–1.28, 1.18–1.08 (5H + 2H, m, 4-$CH_2CH_2CHCH_2$), 0.88 (3H, d, J = 7 Hz, 7-CH_3) [The 1-OH was invisible.]; ^{13}C NMR (CD_3OD, 151 MHz) δ = 139.73, 135.62, 132.05, 126.11, 125.46, 124.79, 59.42, 40.93, 40.87, 38.19, 37.67, 33.32, 27.73, 26.38,

26.28, 25.89, 20.05, 17.74, 16.13, 16.03; HRMS (APCI) found: *m*/*z* 291.2682, calcd for $C_{20}H_{35}O$: $[M-H]^+$, 291.2682; see also the spectral data of its racemic mixture in Ref. 18.

*4.2.2. 10,11-Dihydrogeranylgeraniol (**6b**)*

To a solution of geraniol (**1b**, 4.45 g, 28.8 mmol) in dichloromethane (15 ml) were added DMAP (175 mg, 1.43 mmol) and acetic anhydride (6.0 ml, 63 mmol) and the mixture was stirred at room temperature under nitrogen for 1 h. After addition of methanol (10 ml), the reaction mixture was further stirred for 1 h, diluted with diethyl ether, washed twice with aq. 4% sodium hydrogen carbonate, distilled water, and brine, and dried over sodium sulfate. The solvents were evaporated and the residue was purified with FCC (hexane:ethyl acetate = 20:1) to give geranyl acetate (**2b**, 4.04 g, 20.6 mmol) in 71% yield: colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 5.34 (1H, t, *J* = 7 Hz, 2-H), 5.08 (1H, t, *J* = 7 Hz, 6-H), 4.58 (2H, d, *J* = 7 Hz, CH_2O), 2.13–2.03 (4H, m, 3-CH_2CH_2), 2.06 (3H, s, 1-$OCOCH_3$), 1.70 (3H, s, 3-CH_3), 1.68 (3H, s, 7-CH_3 *trans* to C6—CH_2), 1.60 (3H, s, 7-CH_3 *cis* to C6—CH_2); see also its spectral data in Ref. 15.

The above acetate **2b** (1.02 g, 5.20 mmol) was dissolved in dichloromethane (10 ml) and cooled down to 0 °C, to which were added an aqueous 70% *tert*-butyl hydroperoxide solution (1.8 ml, 13 mmol), salicylic acid (88 mg, 0.64 mmol), and selenium oxide (30 mg, 0.27 mmol). The mixture was stirred at room temperature for 2 days and the reaction was quenched with distilled water. After extraction with diethyl ether, the organic phase was washed twice with distilled water and brine and dried over sodium sulfate. All the solvents were evaporated and the residue was purified with FCC (hexane:ethyl acetate = 4:1) to give 8-hydroxy-farnesyl acetate (**3b**, 505 mg, 2.38 mmol) in 46% isolated yield: colorless oil; ^{1}H NMR ($CDCl_3$, 600 MHz) δ = 5.38–5.33 (2H, m, 2-, 6-H), 4.58 (2H, d, *J* = 7 Hz, CH_2OAc), 3.99 (3H, s, 7-CH_2), 2.19–2.08 (4H, m, 3-CH_2CH_2), 2.05 (3H, s, 1-$OCOCH_3$), 1.71 (3H, s, 7-CH_3), 1.66 (3H, s, 3-CH_3) [The 8-OH was invisible.]; see also its spectral data in Ref. 15.

The above alcohol **3b** (60 mg, 283 μmol) was dissolved in dichloromethane (2 ml), to which were added diethyl chlorophosphate (103.2 mg, 598 μmol) and DMAP (60 mg, 491 μmol). The ice-cooled mixture was stirred under nitrogen for 6 h and the reaction was quenched with distilled water. After extraction with diethyl ether, the organic phase was washed twice with aq. 0.1 M hydrogen chloride, distilled water, and brine and dried over sodium sulfate. All the solvents were evaporated and the residue was purified with FCC (hexane:ethyl acetate:methanol = 50:50:1) to give 8-diethoxyphosphoryloxy-geranyl acetate (**4b**, 63 mg, 181 μmol) in 64% yield: colorless oil; ^{1}H NMR ($CDCl_3$, 600 MHz) δ = 5.48 (1H, m, 6-H), 5.34 (1H, m, 2-H), 4.58 (2H, d, *J* = 7 Hz, CH_2OAc), 4.39 (2H, m, 7-CH_2O), 4.15–4.07 (4H, m, $POCH_2 \times 2$), 2.20–2.07 (4H, m, 3-CH_2CH_2), 2.06 (3H, s, 1-$OCOCH_3$), 1.70 (3H, s, 3-CH_3), 1.69 (3H, s, 7-CH_3), 1.33 (6H, t, *J* = 7 Hz, $POCCH_3 \times 2$).

Dry THF (15 ml) was added to magnesium (170 mg, 7.0 mmol) and a piece of iodine under argon. (*R*)-Citronellyl bromide (**5b**, 1.5 g, 6.8 mmol) was slowly dropped into the stirred mixture at room temperature and THF (30 ml) was further added with stirring for 1 h. To the THF solution of the resulting Grignard reagent was gradually added the above phosphate (636 mg, 1.83 mmol). After stirring for 4 h at room temperature, the reaction was quenched with aq. 1% ammonium chloride and the mixture was extracted with diethyl ether. The ethereal phase was washed with distilled water and dried over sodium sulfate. All the solvents were evaporated and the residue was purified with FCC (hexane:ethyl acetate = 6:1) and RP-HPLC (acetonitrile) to give (11*S*)-10,11-DHGG-OH **6b** (80 mg, 273 μmol) in 15% yield: colorless oil; ^{1}H NMR (CD_3OD, 600 MHz) δ = 5.36 (1H, tq, *J* = 7, 2 Hz, 2-H), 5.13 (1H, br-t, *J* = 7 Hz, 6-H), 5.10 (1H, br-t, *J* = 7 Hz, 14-H), 4.08 (2H, d, *J* = 7 Hz, CH_2O), 2.13 (2H, q, *J* = 7 Hz, 4-CH_2), 2.04 (2H, t, *J* = 7 Hz, 3-CH_2), 2.01–1.92 (4H, m, 7-, 12-CH_2), 1.68 (3H, s, 15-CH_3 *trans* to C14—CH_2), 1.67 (3H, s, 3-CH_3), 1.61, 1.60 (each 3H, s, 7-CH_3, 15-CH_3 *cis* to C14—CH_2), 1.47–1.25, 1.17–1.04 (5H+2H, m, 8-$CH_2CH_2CHCH_2$), 0.87 (3H, d, *J* = 7 Hz, 11-CH_3); ^{13}C NMR (CD_3OD, 151 MHz) δ = 139.44, 136.42, 131.84, 126.04, 125.10, 124.93, 59.44, 40.97, 40.75, 38.29, 37.52, 33.40, 27.40, 26.56, 26.37, 25.90, 20.08, 17.73, 16.26, 15.94; HRMS (APCI) found: *m*/*z* 291.2678, calcd for $C_{20}H_{35}O$: $[M-H]^+$, 291.2682; see also the spectral data of the (11*R*)-epimer in Ref. 27.

*4.2.3. 14,15-Dihydrogeranylgeraniol (**6a**)*

Similar to the synthesis of geranyl acetate (**2b**, see Section 4.2.2), the esterification of farnesol (**1a**, 545 mg, 2.45 mmol) with acetic anhydride (0.75 ml, 7.9 mmol) and DMAP (60 mg, 0.49 mmol) in dichloromethane (20 ml) gave farnesyl acetate (**2a**, 490 mg, 1.85 mmol) in 76% yield: colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 5.34 (1H, t, *J* = 7 Hz, 2-H), 5.11–5.06 (2H, m, 6-, 10-H), 4.58 (2H, d, *J* = 7 Hz, CH_2O), 2.14–1.95 (8H, m, 3-, 4-, 7-, 8-CH_2), 2.05 (3H, s, 1-$OCOCH_3$), 1.70 (3H, s, 3-CH_3), 1.68 (3H, s, 11-CH_3 *trans* to C10—CH_2) 1.60 (6H, s, 7-CH_3, 11-CH_3 *cis* to C10—CH_2); see also its spectral data in Ref. 15.

Similar to the synthesis of 8-hydroxy-farnesyl acetate (**3b**, see Section 4.2.2), the above acetate **2a** (490 mg, 1.85 mmol) in dichloromethane (10 ml) was oxidized by an aqueous 70% *tert*-butyl hydroperoxide solution (1.0 ml, 7.3 mmol), salicylic acid (60 mg, 0.43 mmol), and selenium oxide (13 mg, 0.12 mmol) for 1 day to give 12-hydroxy-farnesyl acetate (**3a**, 142 mg, 0.506 mmol) in 27% isolated yield: colorless oil; ^{1}H NMR ($CDCl_3$, 600 MHz) δ = 5.39 (1H, t, *J* = 7 Hz, 10-H), 5.34 (1H, t, *J* = 7 Hz, 2-H), 5.10 (1H, t, *J* = 7 Hz, 6-H), 4.59 (2H, d, *J* = 7 Hz, CH_2OAc), 3.99 (2H, s, 11-CH_2), 2.15–2.00 (8H, m, 3-, 4-, 7-, 8-CH_2), 2.05 (3H, s, 1-$OCOCH_3$), 1.71 (3H, s, 11-CH_3), 1.67 (3H, s, 3-CH_3), 1.60 (3H, s, 7-CH_3) [The 12-OH was invisible.]; see also its spectral data in Ref. 15.

Similar to the synthesis of 8-diethoxyphosphoryloxy-geranyl acetate (**4b**, see Section 4.2.2), the esterification of the above alcohol **3a** (142 mg, 506 μmol) with diethyl chlorophosphate (190 mg, 1100 μmol) and DMAP (60 mg, 491 μmol) for 3 h gave 12-diethoxyphosphoryloxy-farnesyl acetate (**4a**, 134 mg, 322 μmol) in 64% yield: colorless oil; ^{1}H NMR ($CDCl_3$, 600 MHz) δ = 5.48 (1H, t, *J* = 7 Hz, 10-H), 5.35 (1H, t, *J* = 7 Hz, 2-H), 5.11 (1H, t, *J* = 7 Hz, 6-H), 4.59 (2H, d, *J* = 7 Hz, CH_2OAc), 4.39 (2H, s, 11-CH_2O), 4.11 (4H, q, *J* = 7 Hz, $POCH_2 \times 2$), 2.15–2.00 (8H, m, 3-, 4-, 7-, 8-CH_2), 2.05 (3H, s, 1-$OCOCH_3$), 1.70 (3H, s, 11-CH_3), 1.69 (3H, s, 3-CH_3), 1.60 (3H, s, 7-CH_3), 1.33 (6H, t, *J* = 7 Hz, $POCCH_3 \times 2$).

Similar to the synthesis of 10,11-dihydrogeranylgeraniol (**6b**), isopentylmagnesium bromide prepared by magnesium (72 mg, 3.0 mmol), a piece of iodine, and 1-bromo-3-methylbutane (**5a**, isopentyl bromide, 313 μl, 375 mg, 2.48 mmol) in dry THF (10+5 ml) for 2 h, was reacted with the above phosphate **4a** (134 mg, 322 μmol) to give 14,15-DHGG-OH **6a** (26 mg, 89 μmol) in 28% yield: colorless oil; ^{1}H NMR (CD_3OD, 600 MHz) δ = 5.36 (1H, tq, *J* = 7, 2 Hz, 2-H), 5.14 (1H, tq, *J* = 7, 2 Hz, 6-H), 5.10 (1H, tq, *J* = 7, 2 Hz, 10-H), 4.08 (2H, d, *J* = 7 Hz, CH_2O), 2.13, 2.09 (each 2H, q, *J* = 8 Hz, 4-, 8-CH_2), 2.04 (2H, t, *J* = 8 Hz, 3-CH_2), 2.00 (2H, t, *J* = 8 Hz, 7-CH_2), 1.95 (2H, t, *J* = 7 Hz, 11-CH_2), 1.67 (3H, s, 3-CH_3), 1.61 (3H, s, 7-CH_3), 1.58 (3H, s, 11-CH_3), 1.53 (1H, nonet, *J* = 7 Hz, 15-H), 1.39 (2H, quintet, *J* = 7 Hz, 12-CH_2), 1.14 (2H, q, *J* = 7 Hz, 13-CH_2), 0.88 (6H, d, *J* = 7 Hz, 15-$CH_3 \times 2$) [The 1-OH was invisible.]; ^{13}C NMR (CD_3OD, 151 MHz) δ = 139.44, 136.15, 136.13, 125.29, 125.26, 124.92, 59.43, 40.96, 40.81, 40.75, 39.72, 29.07, 27.53, 27.45, 26.84, 23.04 (15-C_2), 16.26, 16.05, 15.96; HRMS (APCI) found: *m*/*z* 291.2678, calcd for $C_{20}H_{35}O$: $[M-H]^+$, 291.2682.

4.3. Preparation of tetrahydrogeranylgeraniols

4.3.1. 6,7,10,11-Tetrahydrogeranylgeraniol

Wet cells from a fully grown liquid culture of the *Rhodopseudomonas* sp. Rits strain[22] were treated with a 1:1 (v/v) mixture of

acetone and methanol (2 ml). The filtrate was stirred with a 1:1 (v/v) mixture of petroleum ether and diethyl ether (2 ml) and ice-cooled. After addition of ice-chilled distilled water (4 ml), the mixture was stirred vigorously and centrifuged. The separated ethereal phase was dried by a stream of nitrogen gas and the residue was purified with RP-HPLC (methanol:acetone:water = 16:3:1) to separate BChl-a_{THGG} from BChls-*a* esterified by the other isoprenoid alcohols.[6] The sample containing the tetrahydrogeranylgeranylated chlorophyll pigments was dissolved in methanol (2 ml) with stirring, to which was dropped a 20% methanol solution of potassium hydroxide (2 ml) at room temperature. After stirred for 20 min, the reaction mixture was extracted twice with hexane. The combined hexane phases were washed with distilled water and dried by a stream of nitrogen gas. The residue was purified with RP-HPLC (methanol) to give (7*S*,11*S*)-6,7,10,11-THGG-OH: Cosmosil 5C_{18}-AR-II 6.0 × 250 mm, 1.0 ml/min, 9.8 min. After evaporation of the eluted methanol, the residual sample was dissolved in hexane and subjected to GC analysis.

4.3.2. 6,7,14,15-Tetrahydrogeranylgeraniol

As mentioned in Section 4.3.1, wet cells of the *Halorhodospira halochloris* DSM1059 strain (DSMZ)[21] were similarly treated to give a pigment mixture. The mixture was purified with RP-HPLC (methanol:water = 19:1) to give BChl-b_{THGG} as a major component.[11,21] Caution: all the above procedures must be performed in the dark to avoid the decomposition of chlorophyll pigments. The separated BChl-*b* was methanolyzed similarly as in Section 4.3.1 to give (7*S*)-6,7,14,15-THGG-OH as the authentic sample for GC analysis.

4.3.3. 10,11,14,15-Tetrahydrogeranylgeraniol

Wet cells from a fully grown liquid culture of *Chlorobaculum tepidum* ATCC49652[19] were treated with a 1:1 (v/v) mixture of acetone and methanol (2 ml). The filtrate was stirred with hexane (2 ml), distilled water (4 ml) was added, and the separated hexane phase was washed with brine and dried by a stream of nitrogen gas. The residue was purified with RP-HPLC (methanol:acetone = 37:3) and successive NP-HPLC (hexane:acetone = 17:3) to give Chl-a_{THGG}.[12,14] The sample containing the desired Chl-*a* was methanolyzed similarly as in Section 4.3.1 to give (11*S*)-10,11,14,15-THGG-OH as the authentic sample for GC analysis.

4.4. GC analysis of di- and tetrahydrogeranylgeraniols

Regioisomeric DHGG- and THGG-OHs were analyzed by GC. Each sample was dissolved in hexane and injected into the GC system. The GC analysis was carried out with an Agilent J&W DB-WAX capillary column (122–0732, 0.25 mm × 30 m, a 0.25-μm layer of liquid phase): column temp., 230 °C; injection temp., 250 °C, detection temp., 270 °C; injection method, split (the ratio = 10.0); injection volume, 1.0 μl; carrier gas, helium (100 kPa, 22.4 cm/s); make-up gas, helium (30 ml/min); hydrogen gas, 40 ml/min; air, 400 ml/min.

4.5. HPLC analysis of chlorophyll pigments during greening

Commercially available seeds of *Hordeum vulgare* (Noguchi Seed) or *Raphanus sativus* (Tohoku Seed) were placed on wet cotton sheets and kept at 20 °C for 7 days in the dark. The etiolated leaves were illuminated with a white light (500 lx) for an appropriate time. The greening leaves (10 g) were treated with acetone and methanol (1:1, v/v) and the ground mixture was filtered. The extraction process was performed several times until the filtrate was colorless. To the combined filtrates was added the same volume of petroleum ether and diethyl ether (1:1, v/v). The stirred organic solution was mixed with the same volume of distilled water. The separated ethereal phase was washed with brine and dried by a stream of nitrogen gas. The residue was purified with RP-HPLC to give Chl-*a* species.

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A. Supplementary data

Supplementary data (^{1}H NMR spectrum of chiral esters of asymmetrically synthesized 2,3-dihydrofarnesol, ^{1}H and ^{13}C NMR spectra of DHGG-OHs, and GC profiles of C20-isoprenoid alcohols with their *t*R) associated with this article can be found, in the online version, at https://doi.org/10.1016/j.bmc.2017.10.002.

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