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Biosynthesis of anthraquinone derivatives in a *Sesamum indicum* hairy root culture

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In order to investigate the intermediacy of 2-(4-methylpent-3-en-1-yl)anthraquinone (MPAQ), a possible intermediate for the biosynthesis of anthraquinone derivatives in sesame (*Sesamum indicum*), ^{2}H-labeled MPAQ was administered to a hairy root culture of *S. indicum*. Efficient conversion of fed MPAQ to 2-[(*Z*)-4-methylpenta-1,3-dien-1-yl]anthraquinone ((*Z*)-MPDEAQ) was observed. Furthermore, administration experiment with ^{2}H-labeled 2-geranyl-1,4-naphthohydroquinone, another possible intermediate, showed that it was converted to MPAQ and (*Z*)-MPDEAQ. The results clearly demonstrated that these substrates are the actual precursors for the production of (*Z*)-MPDEAQ. In contrast, neither MPAQ nor 2-geranyl-1,4-naphthohydroquinone was converted to anthrasesamone B and 2,3-epoxyanthrasesamone B, other anthraquinone derivatives in the hairy roots, suggesting that these substrates may not be the common precursors in the biosynthesis of anthraquinone derivatives.

Key words: *Sesamum indicum*; sesame hairy root; biosynthesis; anthraquinone; naphthohydroquinone

Sesame (*Sesamum indicum* L., Pedaliaceae) is a cultivated species that is one of the most important crops throughout the world, and its seeds have been considered a valuable health food and have been utilized for millennia as an oilseed and food material.[1)] Recently, anthrasesamone F, which is a hydroxylated anthraquinone derivative possessing a potent radical scavenging activity, was identified as one of the characteristic components of black sesame seeds.[2,3)]

On the other hand, investigation of the constituents of *S. indicum* roots and hairy roots established that the roots contain several anthraquinone derivatives, including 2-(4-methylpent-3-en-1-yl)anthraquinone (MPAQ) (**1**),[4,5)] 2-[(*Z*)-4-methylpenta-1,3-dien-1-yl]anthraquinone ((*Z*)-MPDEAQ) (**2**),[6)] anthrasesamones A (**3**), B (**4**) and C[5)] and 2,3-epoxyanthrasesamone B (**5**)[7)] (Fig. 1). The carbon skeleton of MPAQ (**1**), which contains a branched C_6 side chain at C-2 in the anthraquinone ring, is identical with that of other anthraquinone derivatives (**2**–**5**). The biogenetic conversion of MPAQ (**1**) to the anthraquinone derivatives that have the dehydrogenated diene side chain (**2**) or oxygenated anthraquinone rings (**3**–**5**) was deduced from their chemical structures, but biosynthetic investigations have not been made. In addition, we previously proposed that MPAQ (**1**) would be formed from 2-geranyl-1,4-naphthohydroquinone (**7**) on the basis of the biosynthetic pathway of shikonin by *Lithospermum erythrorhizon*, a naphthoquinone derivative having the same C_6 side chain as MPAQ (**1**),[8)] as well as the formation of anthraquinone derivatives from prenylated naphthoquinone (and/or naphthohydroquinone) derivatives by plants.[9,10)] Later, 2-geranyl-1,4-naphthoquinone (**6**) corresponding to a quinone form of hydroquinone **7** has been isolated from sesame hairy roots.[11)] Moreover, the incorporation of [1-^{13}C]glucose into MPAQ (**1**) and 2-geranyl-1,4-naphthoquinone (**6**) demonstrated that these possible intermediates were biosynthesized through the same biogenetic route, the shikimate and methylerythritol phosphate (MEP) pathways.[12)] However, the biogenetic relationships among quinone derivatives in sesame have not been definitively verified. We therefore undertook administration experiments with ^{2}H-labeled MPAQ (**1a**) and 2-geranyl-1,4-naphthohydroquinone (**7a**) to elucidate the intermediacy of **1** and **7** in the biosynthesis of anthraquinone derivatives by *S. indicum* hairy roots.

Materials and methods

General experimental procedures. ^{1}H and ^{13}C NMR spectra were measured with a JEOL JNM-ECA600 FT NMR spectrometer in $CDCl_3$ at 600 MHz for ^{1}H and at 150 MHz for ^{13}C. NMR chemical shifts were referenced to the solvent (δ_H 7.24, δ_C 77.0). The deuterium content of individual hydrogen atoms was estimated by comparison of the proton signal integrals between the ^{2}H-labeled and unlabeled positions in each compound. ^{2}H NMR spectra were recorded with the same instrument in $CHCl_3$ at 92 MHz. Mass spectra were obtained with a JEOL JMS-700 mass spectrometer. [$^{2}H_8$]Naphthalene (99 atom% ^{2}H) was purchased

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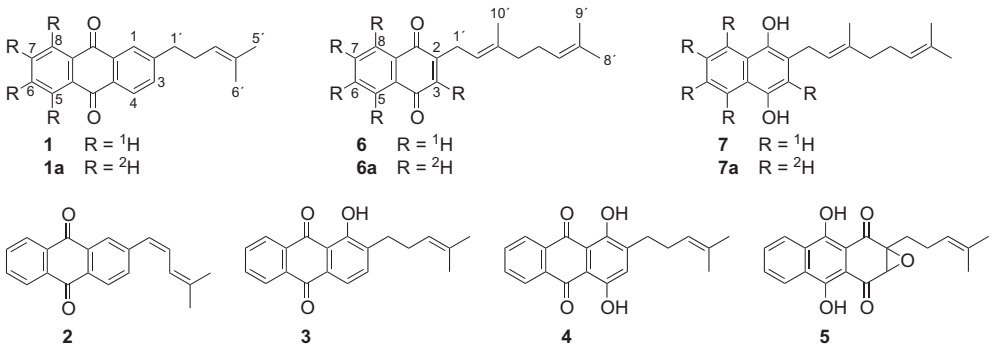

Fig. 1. Structures of MPAQ (**1**), (*Z*)-MPDEAQ (**2**), anthrasesamones A (**3**) and B (**4**), 2,3-epoxyanthrasesamone B (**5**), 2-geranyl-1,4-naphthoquinone (**6**) and 2-geranyl-1,4-naphthohydroquinone (**7**).

from Sigma-Aldrich, USA. Silica gel 60 (70–230 mesh, Nacalai Tesque, Japan) and Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Sweden) were used for column chromatography. All experimental procedures were carried out in the dark or under as low light condition as possible, because some quinone derivatives, in particular (*Z*)-MPDEAQ,[6] are unstable to light.

Synthesis of [$^2H_6$]-1,4-naphthoquinone. A solution of ammonium cerium(IV) sulfate dihydrate (7.59 g) in 2 M H_2SO_4 (100 mL) was added to [$^2H_8$]naphthalene (273 mg) in MeCN (80 mL) and 2 M H_2SO_4 (20 mL), and the solution was stirred overnight at room temperature.[13] The reaction mixture was poured into H_2O (200 mL) and treated with EtOAc (50 mL × 4). The EtOAc solution obtained was washed with saturated NaCl (100 mL × 2), dried over Na_2SO_4 and concentrated to dryness under reduced pressure to give [$^2H_6$]-1,4-naphthoquinone (315 mg, 96% yield).

*Synthesis of 2-(4-methylpent-3-en-1-yl)[5,6,7,8-$^2H_4$] anthraquinone (**1a**).* [5,6,7,8-$^2H_4$]MPAQ (**1a**) was prepared by reference to the method used for the synthesis of unlabeled MPAQ (**1**).[14] A solution of [$^2H_6$]-1,4-naphthoquinone (151 mg) and 7-methyl-3-methyleneocta-1,6-diene (β-myrcene, 277 mg, 72% purity) in dry toluene (10 mL) was allowed to stand for 5 days at 60 °C. A solution of 1 M KOH in MeOH (5 mL) was added to the reaction mixture at room temperature, and the solution was stirred vigorously for 90 min without sealing. The reaction mixture was poured into H_2O (50 mL) and treated with EtOAc (25 mL × 3). The EtOAc solution obtained was washed with saturated NaCl (40 mL × 2) and concentrated to dryness under reduced pressure. The residue was chromatographed on silica gel column by elution with EtOAc–hexane (5:95). The fractions containing **1a** were combined and further purified by recrystallization from EtOAc–hexane to afford ^{2}H-labeled **1a** (210 mg, 78% yield). ^{1}H NMR ($CDCl_3$) δ_H: 1.52 (3H, br.s, H-6′), 1.66 (3H, br.s, H-5′), 2.36 (2H, br.dt, J = 7.2, 7.7 Hz, H-2′), 2.79 (2H, t-like, J = 7.7 Hz, H-1′), 5.13 (1H, tqq, J = 7.2, 1.4, 1.4 Hz, H-3′), 7.58 (1H, dd, J = 7.9, 1.8 Hz, H-3), 8.10 (1H, d, J = 1.8 Hz, H-1), 8.20 (1H, d, J = 7.9 Hz, H-4). Two residual proton signals were observed at ^{2}H-labeled positions (>99 atom% ^{2}H, δ_H): 7.77 (H-6 and H-7), 8.29 (H-5 and H-8). ^{13}C NMR ($CDCl_3$) δ_C: 17.7 (C-6′), 25.7 (C-5′), 29.4 (C-2′), 36.3 (C-1′), 122.7 (C-3′), 127.0 (C-1), 127.4 (C-4), 131.5 (C-4a), 133.1 (C-4′), 133.5 (C-9a), 133.6 (C-8a), 133.6 (C-10a), 134.5 (C-3), 149.6 (C-2), 183.1 (C-10), 183.5 (C-9). HR-EIMS m/z (M^+): Calcd for $C_{20}{}^1H_{14}{}^2H_4O_2$, 294.1558; found, 294.1567.

*Synthesis of 2-geranyl[3,5,6,7,8-$^2H_5$]-1,4-naphthoquinone (**6a**).* 2-Geranyl[3,5,6,7,8-$^2H_5$]-1,4-naphthoquinone (**6a**) was prepared using a modification of the method described by Tsuchimoto et al.[15] (*E*)-3,7-Dimethylocta-2,6-dien-1-ol (geraniol, 463 mg, 526 μL) and scandium(III) trifluoromethanesulfonate (25 mg) were added to [$^2H_6$]-1,4-naphthoquinone (164 mg), and the solution was sonicated for 1 min and allowed to stand for 24 h at 50 °C. The reaction mixture was diluted with CH_2Cl_2 (2 mL × 5) and filtered through Celite, before the filtrate was evaporated to dryness. The residue was chromatographed on silica gel column by elution with acetone–hexane (5:95). The fractions containing **6a** were combined and further purified by preparative HPLC (column, Cosmosil Cholester, 250 × 10 mm i.d., Nacalai Tesque; detection, 254 nm) using MeOH as the mobile phase (flow rate, 1.5 mL/min) to afford ^{2}H-labeled **6a** (18 mg, 6% yield). ^{1}H NMR ($CDCl_3$) δ_H: 1.59 (3H, br.s, H-9′), 1.64 (3H, br.s, H-10′), 1.67 (3H, br.s, H-8′), 2.09 (4H, m, H-4′ and H-5′), 3.26 (2H, br.d, J = 7.3 Hz, H-1′), 5.07 (1H, tm, J = 6.7 Hz, H-6′), 5.21 (1H, tm, J = 7.3 Hz, H-2′). Four residual proton signals were observed at ^{2}H-labeled positions (approximately 99 atom% ^{2}H, δ_H): 6.74 (H-3), 7.70 (H-6 and H-7), 8.04 (H-5), 8.09 (H-8). ^{13}C NMR ($CDCl_3$) δ_C: 16.1 (C-10′), 17.7 (C-9′), 25.7 (C-8′), 26.5 (C-5′), 27.8 (C-1′), 39.7 (C-4′), 118.1 (C-2′), 123.9 (C-6′), 131.8 (C-7′), 132.1 (C-4a), 132.3 (C-8a), 140.0 (C-3′), 150.7 (C-2), 185.3 (C-4), 185.4 (C-1). HR-EIMS m/z (M^+): Calcd for $C_{20}{}^1H_{17}{}^2H_5O_2$, 299.1934; found, 299.1932.

Plant material. Hairy roots of *S. indicum* were induced by directly infecting axenic sesame seedlings with *Agrobacterium rhizogenes* ATCC 15834.[16] The established hairy root clone (SI-16) was maintained in a phytohormone-free Gamborg B5 liquid medium[17] containing 2% glucose (pH 6.1) in the dark at 25 °C on a rotary shaker at 70 rpm at intervals of 14 days.

*Administration of ^{2}H-labeled MPAQ (**1a**).* The hairy roots (about 0.3 g fresh wt.) were cultured in a 100-mL conical flask containing 50 mL of the B5 liquid medium supplemented with 2% sucrose in the dark at 25 °C at 70 rpm. Twenty flasks containing hairy roots that had been grown for 14 days were washed with sterile H_2O (25 mL × 2 per flask), before the medium was replaced with fresh B5 medium supplemented with 0.2% sucrose and 100 mM NaCl. ^{2}H-labeled **1a** (10 mg, 0.5 mg/flask) dissolved in EtOH (1.2 mL) was then fed to these flasks containing the fresh medium and washed hairy roots. The hairy roots were cultured under the same condition for four days with ^{2}H-labeled **1a**. The hairy roots and secreted metabolites were separated from the medium by gravity filtration through filter paper. The harvested hairy roots and filter paper were freeze-dried and sonicated for 30 min in MeOH (150 mL × 3). The MeOH solution obtained was concentrated to dryness under reduced pressure. The MeOH extract was partitioned between CH_2Cl_2 (100 mL × 3) and H_2O (100 mL) to give the CH_2Cl_2-soluble fraction. This fraction was subjected to silica gel column chromatography using stepwise elution with acetone–hexane containing 0.1% AcOH. The 5 and 10% acetone fractions were combined and purified by Sephadex LH-20 column chromatography eluted with MeOH–CH_2Cl_2 (1:1). The fractions containing the metabolites were further purified by reversed-phase HPLC (column, Cosmosil 5C_{18}-AR-II, 250 × 10 mm i.d., Nacalai Tesque; detection, 254 nm) using MeOH–AcOH as the mobile phase (solvent, 100:0.2; flow rate, 1.5 mL/min) to afford **1** (5 mg), **2** (3 mg), **4** (0.5 mg), **5** (3 mg) and **6** (<0.5 mg).

*Compound **2**.* ^{1}H NMR ($CDCl_3$) δ_H: 1.86 and 1.87 (each 3H, br.s, H-5′ and H-6′), 6.37 (1H, dm, J = 11.7 Hz, H-3′), 6.38 (1H, d, J = 11.7 Hz, H-1′), 6.62 (1H, dd, J = 11.7, 11.7 Hz, H-2′), 7.73 (1H, dd, J = 8.0, 1.8 Hz, H-3), 7.78 (0.48H, m, H-6 and H-7), 8.24 (1H, d, J = 1.8 Hz, H-1), 8.25 (1H, d, J = 8.0 Hz, H-4), 8.30 (0.48H, m, H-5 and H-8). EIMS *m/z* (rel. int.): 288 (M^+, 3.4), 292 (M^++4, 12.4).

*Administration of ^{2}H-labeled 2-geranyl-1,4-naphthohydroquinone (**7a**).* The administration experiment with ^{2}H-labeled **7a** was carried out by the same method as that used for ^{2}H-labeled **1a**, except for the preparation of **7a** from **6a** just before the administration of the labeled substrate. A solution of **6a** (15 mg) in Et_2O (10 mL) was shaken with 15% sodium dithionite (10 mL). The Et_2O layer obtained was washed with a mixture of saturated NaCl (10 mL) and 15% sodium dithionite (2 mL), passed through Na_2SO_4 and concentrated to dryness under reduced pressure. Without storage, the reduced product (**7a**) was dissolved in EtOH (2.4 mL) and immediately fed to the hairy root cultures. After the final purification by reversed-phase HPLC, **1** (1 mg), **2** (2 mg), **4** (0.5 mg) and **5** (1 mg) and **6** (4 mg) were isolated.

*Compound **1**.* ^{1}H NMR ($CDCl_3$) δ_H: 1.53 (3H, br.s, H-6′), 1.66 (3H, br.s, H-5′), 2.36 (2H, br.dt, J = 7.2, 7.7 Hz, H-2′), 2.80 (2H, t-like, J = 7.7 Hz, H-1′), 5.13 (1H, tqq, J = 7.2, 1.4, 1.4 Hz, H-3′), 7.59 (1H, dd, J = 7.9, 1.8 Hz, H-3), 7.77 (0.9H, m, H-6 and H-7), 8.11 (1H, d, J = 1.8 Hz, H-1), 8.21 (1H, d, J = 7.9 Hz, H-4), 8.29 (0.9H, m, H-5 and H-8). EIMS *m/z* (rel. int.): 290 (M^+, 19.5), 294 (M^++4, 22.2).

*Compound **2**.* ^{1}H NMR ($CDCl_3$) δ_H: 1.86 and 1.87 (each 3H, br.s, H-5′ and H-6′), 6.37 (1H, dm, J = 11.7 Hz, H-3′), 6.38 (1H, d, J = 11.6 Hz, H-1′), 6.62 (1H, dd, J = 11.7, 11.6 Hz, H-2′), 7.74 (1H, dd, J = 8.0, 1.8 Hz, H-3), 7.78 (0.86H, m, H-6 and H-7), 8.24 (1H, d, J = 1.8 Hz, H-1), 8.26 (1H, d, J = 8.0 Hz, H-4), 8.30 (0.86H, m, H-5 and H-8). EIMS *m/z* (rel. int.): 288 (M^+, 30.5), 292 (M^++4, 47.7).

Results and discussion

We examined whether MPAQ (**1**) and 2-geranyl-1,4-naphthohydroquinone (**7**) serve as intermediates for anthraquinone derivatives produced by *S. indicum* hairy roots. A preliminary administration experiment using a conventional B5 medium was unsuccessful because of very low or no accumulation of anthraquinone derivatives such as **3**–**5** (data not shown). In order to improve the production of anthraquinone derivatives in the hairy roots, several culture conditions were tested. The addition of sodium chloride at 100 mM in the B5 medium[7] gave a sufficient amount of 2,3-epoxyanthrasesamone B (**5**) together with a small amount of anthrasesamone B (**4**), although anthrasesamone A (**3**) unfortunately did not accumulate. Administration experiments with ^{2}H-labeled substrates were therefore carried out using this improved medium. In contrast, the conventional B5 medium was utilized as the growth medium for reducing the endogenous production of anthraquinone derivatives, prior to administration of the labeled substrates.

[5,6,7,8-$^2H_4$]MPAQ (**1a**) was synthesized by the Diels–Alder cycloaddition reaction of [$^2H_6$]-1,4-naphthoquinone and β-myrcene and subsequent aromatization of the adduct.[14] ^{2}H-labeled MPAQ (**1a**) (>99 atom % ^{2}H) was administered to a two-week-old hairy root culture of *S. indicum*. After four days of incubation with ^{2}H-labeled MPAQ (**1a**), quinone derivatives were extracted and isolated as described in the above section. The ^{1}H NMR spectrum of (*Z*)-MPDEAQ (**2**) obtained from this experiment showed that two aromatic proton signals at δ_H 7.78 (H-6 and H-7) and 8.30 (H-5 and H-8) in isolated **2** had remarkably decreased intensities (Fig. 2(A)) compared to natural abundance **2** (Fig. 2(C)) and that the content of deuterium atoms in isolated **2** was 76% at these positions, indicating that exogenously supplied **1a** was efficiently converted to **2**. Moreover, the existence of the deuterium labels in isolated **2** was confirmed by the ^{2}H NMR analysis, where two broad singlet signals having almost the same signal integral were observed at the corresponding positions. These results clearly demonstrate that MPAQ (**1**) is an actual biosynthetic precursor for the formation of (*Z*)-MPDEAQ (**2**). As expected, the hairy roots did not convert labeled MPAQ (**1a**) to 2-geranyl-1,4-naphthoquinone (**6**). Contrary to our presumption, however, the conversion of labeled MPAQ (**1a**) to anthrasesamone B

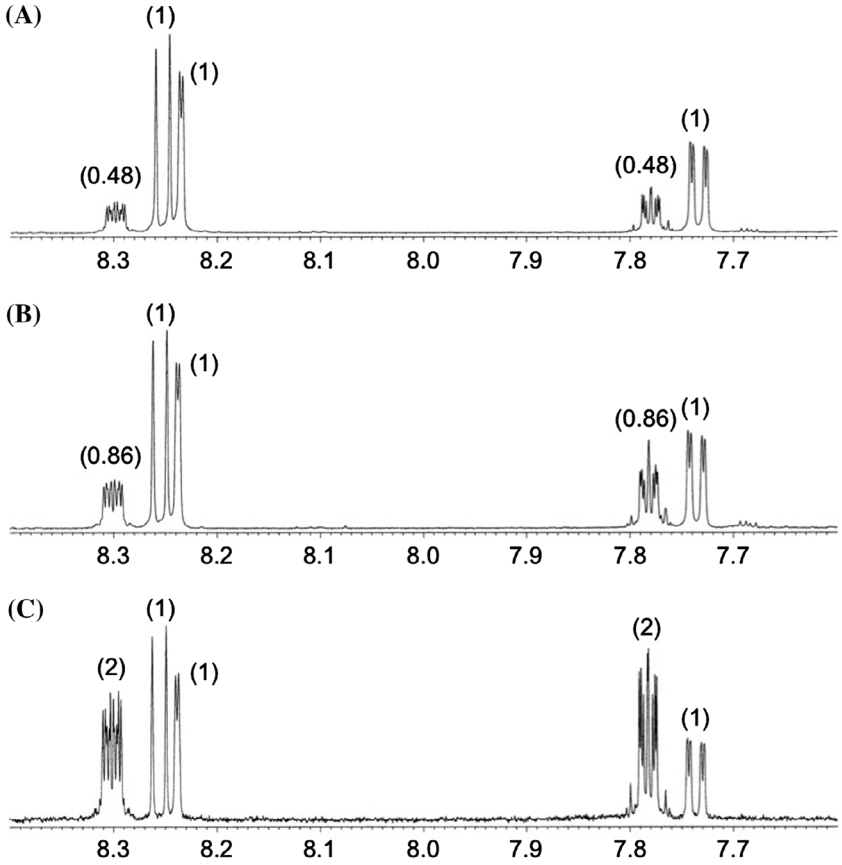

Fig. 2. Partial ^{1}H NMR spectra of (*Z*)-MPDEAQ (**2**).
Notes: (A) **2** derived from ^{2}H-labeled MPAQ (**1a**). (B) **2** derived from ^{2}H-labeled 2-geranyl-1,4-naphthohydroquinone (**7a**). (C) Natural abundance **2**. The numerals in parentheses represent the integral values.

(**4**) and 2,3-epoxyanthrasesamone B (**5**), which are respectively 1,4-dihydroxy and 2,3-epoxy-1,4-dihydroxy analogs of **1**, was not observed in the ^{1}H and ^{2}H NMR spectra of isolated **4** and **5**. Dilution of fed MPAQ (**1a**) by endogenous unlabeled **1** was excluded because recovered **1** was still fully enriched with the deuterium atoms (94%). These findings suggested that anthrasesamone B (**4**) and 2,3-epoxyanthrasesamone B (**5**) may be formed without passing through MPAQ (**1**).

It is well known that shikonin is biosynthesized through the formation of a geranylated intermediate, geranylhydroquinone, and subsequent cyclization with the geranyl side chain to construct the naphthoquinone ring.[18,19] In addition, Leistner[20] reported that 2-prenyl-1,4-naphthohydroquinone was incorporated into alizarin, an anthraquinone derivative in *Rubia tinctorum* roots, whereas 2-prenyl-1,4-naphthoquinone was not. Based on these previous findings, we selected 2-geranyl-1,4-naphthohydroquinone (**7**), but not 2-geranyl-1,4-naphthoquinone (**6**), as the most effective substrate for further administration experiment. ^{2}H-labeled **7a** was prepared by reduction of previously synthesized **6a** just before its administration, because in general, naphthohydroquinones change to the corresponding naphthoquinones by air oxidation. 2-Geranyl [3,5,6,7,8-$^{2}H_5$]-1,4-naphthoquinone (**6a**) was synthesized by the scandium triflate-catalyzed Friedel–Crafts alkylation reaction of [$^{2}H_6$]-1,4-naphthoquinone with geraniol[15] and then was reduced to 2-geranyl [3,5,6,7,8-$^{2}H_5$]-1,4-naphthohydroquinone (**7a**) with sodium dithionite. ^{2}H-labeled **7a** (about 99 atom% ^{2}H) was next administered to the hairy root culture, and quinone derivatives were isolated again as described above. Incidentally, the deuterium content of recovered **6** was 97% at the corresponding positions. In the ^{1}H NMR spectrum of MPAQ (**1**) obtained from this experiment, two aromatic proton signals at δ_H 7.77 (H-6 and H-7) and 8.29 (H-5 and H-8) had reduced intensities (Fig. 3(A)) weaker than natural abundance **1** (Fig. 3(B)), and the deuterium content was evaluated to be 55%, evidence that 2-geranyl-1,4-naphthohydroquinone (**7**) is a substantial precursor for the formation of MPAQ (**1**). Labeled **7a** was also converted to (*Z*)-MPDEAQ (**2**) (57%) (Fig. 2(B)). These data support the supposition that (*Z*)-MPDEAQ (**2**) is derived from MPAQ (**1**), which is generated from 2-geranyl-1,4-naphthohydroquinone (**7**). In contrast to MPAQ (**1**) and (*Z*)-MPDEAQ (**2**) formation, labeled **7a** was not converted to anthrasesamone B (**4**) and 2,3-epoxyanthrasesamone B (**5**), in analogy with the result using **1a**, suggesting that 2-geranyl-1,4-naphthohydroquinone (**7**), as well as **1**, does not seem to participate in the biosynthesis of **4** and **5**.

On the basis of the above findings, we propose the biosynthetic pathways of 2-geranyl-1,4-naphthohydroquinone (**7**), MPAQ (**1**) and (*Z*)-MPDEAQ (**2**) to be as shown in Fig. 4. These quinone derivatives in *S. indicum* hairy roots originate from 2-carboxy-1,4-naphthohydroquinone (1,4-dihydroxy-2-naphthoic acid) or 2-carboxy-2,3-dihydro-1,4-naphthoquinone (2-carboxy-4-oxotetralone) via the shikimate pathway and geranyl diphosphate via the MEP pathway.[12] Geranylation and

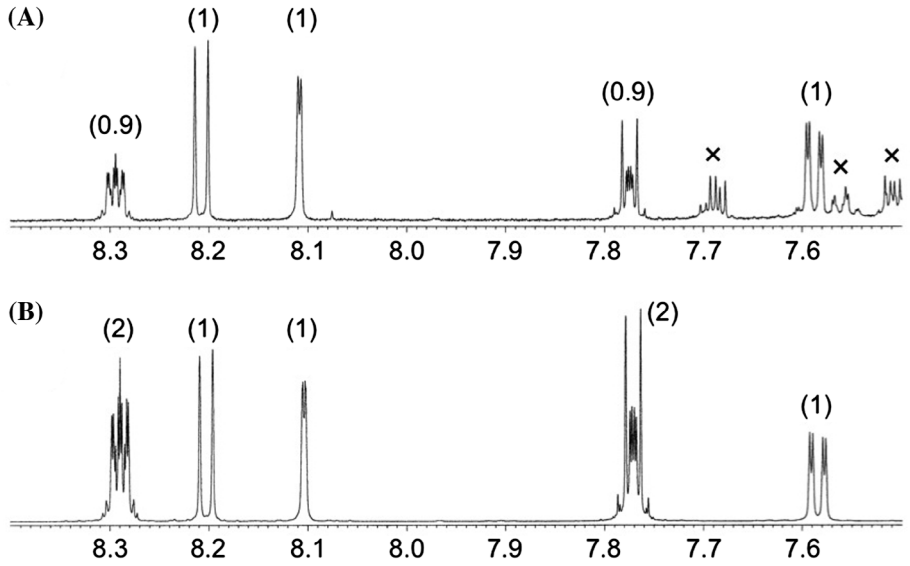

Fig. 3. Partial ^{1}H NMR spectra of MPAQ (**1**).

Notes: (A) **1** derived from ^{2}H-labeled 2-geranyl-1,4-naphthohydroquinone (**7a**). (B) Natural abundance **1**. The numerals in parentheses represent the integral values. ×, the signals of impurities.

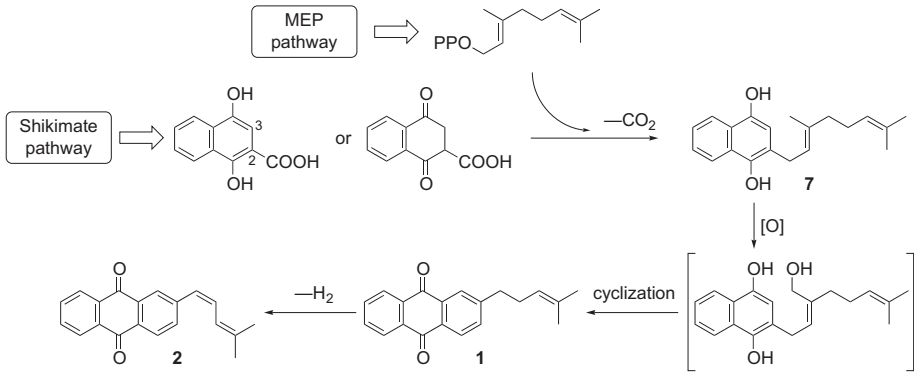

Fig. 4. Proposed biosynthetic pathway of MPAQ (**1**) and (*Z*)-MPDEAQ (**2**) from 2-geranyl-1,4-naphthohydroquinone (**7**) through the shikimate and MEP pathways in *S. indicum* hairy roots.

decarboxylation at C-2 in the aromatic intermediate produce 2-geranyl-1,4-naphthohydroquinone (**7**). In shikonin biosynthesis, 4-hydroxybenzoic acid is geranylated with geranyl diphosphate by 4-hydroxybenzoate geranyltransferase[21] to produce 3-geranyl-4-hydroxybenzoic acid, which is then converted to geranylhydroquinone. Moreover, geranylhydroquinone 3″-hydroxylase, a cytochrome P-450 monooxygenase, has been identified as a key enzyme involved in the formation of the naphthoquinone ring in shikonin.[22] This enzyme specifically hydroxylates one of the three methyl groups present in the geranyl side chain of geranylhydroquinone before the cyclization reaction to generate the naphthoquinone ring. Accordingly, oxidation of the methyl group present at C-3′ in the geranyl side chain of **7** and subsequent cyclization between the naphthohydroquinone ring and the oxidized side chain to generate the anthraquinone ring would lead to the formation of MPAQ (**1**). Dehydrogenation of the C_6 side chain in **1** would produce (*Z*)-MPDEAQ (**2**).

On the other hand, we also deduced the production of anthrasesamone B (**4**) and 2,3-epoxyanthrasesamone B (**5**) from 2-geranyl-1,4-naphthohydroquinone (**7**) and MPAQ (**1**), but our present study rules out this hypothesis. This suggests that **7** and **1** may not be the common precursors in the biosynthesis of anthraquinone derivatives by *S. indicum* and that the biogenetic route of **4** and **5** may be different from that of (*Z*)-MPDEAQ (**2**). Although several possibilities are assumed at the present state, one of the potential intermediates may be 2-carboxy-2-geranyl-2,3-dihydro-1,4-naphthoquinone, and it might be used as the substrate without conversion to **7** (Fig. S1).[23] Alternatively, 2-carboxy-3-geranyl-1,4-naphthohydroquinone may be the intermediate, which might be used without decarboxylation to **7** (Fig. S1).[10] Furthermore, cyclization of these newly presumed intermediates via certain metabolic steps might lead to the formation of hydroxylated anthraquinone derivatives such as anthrasesamones A (**3**) and B (**4**) and 2,3-epoxyanthrasesamone B (**5**) without the involvement of **1**. The difference in the biosynthetic pathways among anthraquinone derivatives produced by *S. indicum* is an interesting topic and has to be further investigated.

Author contributions

T.F. conceived and designed the experiments, discussed the results, and wrote the manuscript. T.F. and R.S. performed the experiments and analyzed the data.

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Disclosure statement

No potential conflict of interest was reported by the authors.

Supplemental data

Supplemental data for this article can be accessed at https://doi.org/10.1080/09168451.2017.1362974.

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