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Identification of (3S, 9R)- and (3S, 9S)-Megastigma-6,7-dien-3,5,9-triol 9-O-β-D-glucopyranosides as Damascenone Progenitors in the…

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Note

Identification of (3*S*, 9*R*)- and (3*S*, 9*S*)-Megastigma-6,7-dien-3,5,9-triol 9-*O*-*β*-D-glucopyranosides as Damascenone Progenitors in the Flowers of *Rosa damascena* Mill.

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The progenitors of damascenone (1), the most intensive C_{13}-norisoprenoid volatile aroma constituent of rose essential oil, were surveyed in the flowers of *Rosa damascena* Mill. Besides 9-*O*-*β*-D-glucopyranosyl-3-hydroxy-7,8-didehydro-*β*-ionol (4b), a stable progenitor already isolated from the residual water after steam distillation of flowers of *R. damascena* Mill., two labile progenitors were identified to be (3*S*, 9*R*)- and (3*S*, 9*S*)-megastigma-6,7-dien-3,5,9-triol 9-*O*-*β*-D-glucopyranosides (2b) based on their synthesis and HPLC-MS analytical data. Compound 2b gave damascenone (1), 3-hydroxy-*β*-damascone (3) and 4b upon heating under acidic conditions.

Key words: rose flower; damascenone; C_{13}-norisoprenoid; progenitor; megastigma-6,7-dien-3,5,9-triol 9-*O*-*β*-D-glucopyranoside

The flowers of *Rosa damascena* Mill. are utilized for the production of rose essential oil, in which several volatile C_{13}-norisoprenoids have been identified. Among them, damascenone [**1**, 1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-2-buten-1-one] was the first constituent to be identified[1)] in the essential oil of *R. damascena* Mill., and showed an extremely low-threshold sensory value (2 pg/g of water).[2)] This compound has been also identified in various types of plant tissue and in plant-derived beverages.[3–5)] Compound **1** was neither produced nor released from the flowers of *R. damascena* Mill., whereas the essential oil contained **1**. Thus, it has been suggested that **1** was produced from the progenitors during the steam distillation of the rose flowers. Many other C_{13}-norisoprenoid volatile compounds have been suggested to also be produced from their progenitors, such as glycosidic or polyhydroxylated compounds, by heat treatment or acidic hydrolysis.[6,7)] Isoe *et al.*[8)] and Ohloff *et al.*[9)] have originally postulated that the progenitors of **1** were produced by the enzymatic cleavage of carotenoids, such as neoxanthin, as shown in Fig. 1. The primary degradation product was grasshopper ketone, which was expected to yield the key intermediate, megastigma-6,7-dien-3,5,9-triol (**2a**). Compound **2a** was transformed to **1** and 3-hydroxy-*β*-damascone (**3**) under the acidic conditions.[10,11)] Several progenitors of **1** have been isolated from various plant tissues and beverages. Two important polyols, **2a** and 3-hydroxy-7,8-didehydro-*β*-ionol (**4a**), have been identified in wine as progenitors of **1**.[10,12)] The C-9- and C-3-*O*-*β*-D-glucopyranosides (**4b**, **4c**) of the latter polyol, and megastigma-6,7-dien-3,5,9-triol 9-*O*-*β*-D-glucopyranoside (**2b**) have been isolated and characterized from Riesling wine[13)] and *Lycium halimifolium*,[14)] respectively. Apples have been reported to contain several disaccharide glycosides of **4a**.[15)] Quite recently, Kumazawa *et al.*[16)] have reported the presence of the glucoside of **4a** as a progenitor of **1** in a black tea extract. Compound **4b** has also been isolated as a progenitor of **1** from the residual water after steam distillation of the flowers of *R. damascena* Mill.[17)] However, **4b** would have been derived from an unstable compound such as **2b** during steam distillaiton of the flowers. Therefore, the genuine progenitor of **1** in rose flowers is still elusive. This paper describes the identification and characterization of progenitors of **1** in the flowers of *R. damascena* Mill.

Identification of damascenone progenitors from the flowers of R. damascena Mill. Frozen flowers

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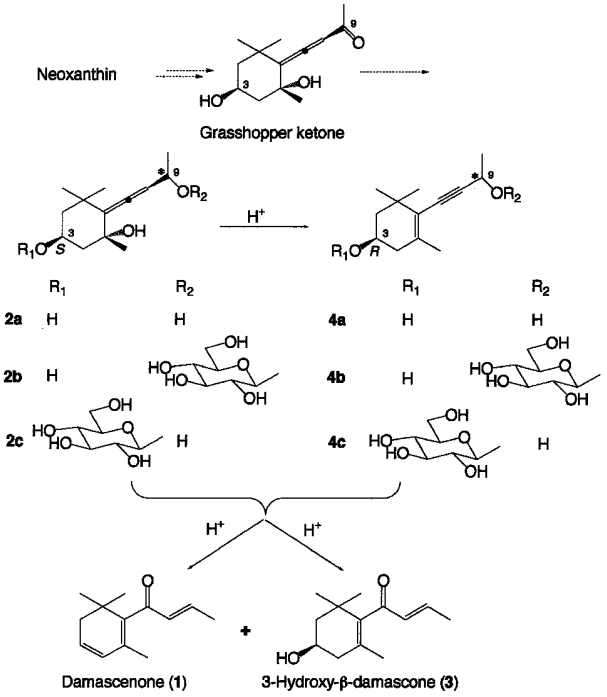

Fig. 1. Hypothetical Biogenetic Pathway for Damascenone Progenitors **2a**–**2c** and **4a**–**4c**, and Their Transformation to Damascenone (**1**) and 3-Hydroxy-β-damascone (**3**).

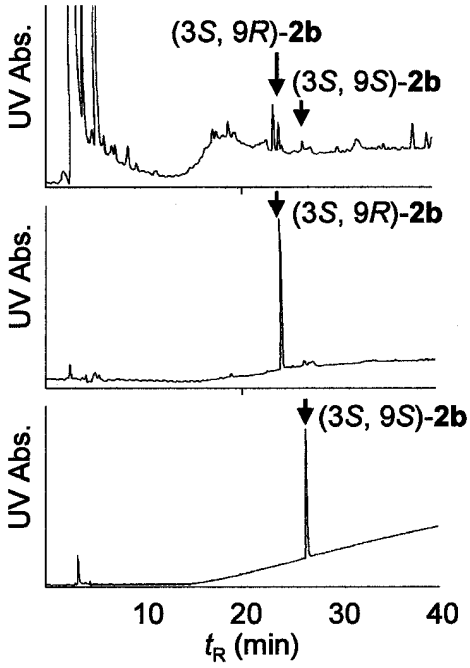

Fig. 2. HPLC Analyses of a Sample (A) Purified from the Flowers of *Rosa damascena* Mill., and of Authentic (3S, 9R)-**2b** (B) and (3S, 9S)-**2b** (C).

(10 kg fr. wt) at the full bloom stage were extracted with 70% MeOH, and the resulting extract was subjected to chromatography on an Amberlite XAD-2 column (water, MeOH). The MeOH eluate was acetylated and purified by chromatography on silica gel cartridge column with hexane-EtOAc as the solvent. The progenitor fractions of **1** yielded 3-hydroxy-β-damascone (**3**) together with **1** after an acid treatment according to the method of Skouroumounis *et al.*[11,12] Each fraction was further purified, being guided by detection of the ion peaks for *m/z* 190 (M^+) and *m/z* 175 (M^+-CH_3) at t_R = 29.0 min for **1**, and *m/z* 208 (M^+) at t_R = 52.2 min for **3** by GC-MS chromatography. Successive flash chromatography on an LP-75 silica gel cartridge (hexane-*t*-butyl methyl ether) and HPLC on an ODS-AM column (MeCN-water) yielded four fractions containing the progenitors.

The most lipophilic fraction was subjected to repeated HPLC (ODS-AQ; MeCN-water) to give **4b** as its pentaacetate. The ^{13}C-NMR spectral data coincided with those of authentic 3,2′,3′,4′,6′-penta-*O*-acetyl-(3*R*, 9*R*)-**4b**,[18] whose chemical shift (δ 67.5) of C-9 showed a characteristic difference in the chemical shift (δ 64.3) at C-9 of 3,2′,3′,4′,6′-penta-*O*-acetyl-(3*R*, 9*S*)-**4b**. Thus, **4b** that had been isolated from the frozen flowers was identified as (3*R*, 9*R*)-**4b**. We have already reported[17] the isolation and characterization of **4b** from the residual water after steam distillation of the flowers of *R. damascena* Mill. In addition, **4b** has been reported as a damascenone progenitor in wine by Sefton *et al.*[11] and Skouroumounis *et al.*[13] Although at least three additional fractions were obtained as the progenitors besides **4b**, we could not isolate any others due to their instability. This fact strongly suggests the presence of other labile progenitors such as allenic triol (**2a**), its 9-*O*-glucopyranoside (**2b**), and 3-*O*-glucopyranoside (**2c**) in these fractions. As already suggested by Juglisi *et al.*,[19] **2a** was quite labile to yield **4a**, **1**, and **3** at room temperature and at pH 3.0 in an aqueous environment. They have also postulated that the β-D-glucosyl moiety at C-3 and/or C-9 stabilized **2a**, although it may lower the rate of dehydration at these positions.[20] The progenitor fractions can thus be expected to contain 9-*O*-β-D-glucopyranoside (**2b**) or 3-*O*-β-D-glucopyranoside (**2c**) as a progenitor of **1**. As **4b** had already been isolated from the residual water after steam distillation of the flowers, **4b** is thought to be one of the products transformed from **2b** during steam distillation of the flowers and/or evaporation of aqueous MeOH used as an extraction solvent. We therefore surveyed **2b** in the flowers of *R. damascena*.

To confirm the chemical structures of the progenitors, we first tried to synthesize **2b**. Two diastereomers of **2b** were obtained from grasshopper ketone[8,21] in four steps. The absolute stereo-structures were determined by a direct comparison with (3*S*, 9*R*)- and (3*S*, 9*S*)-**2b**, respectively, which had been enantioselectively synthesized. This enantio-selective synthesis will be reported elsewhere.

The stability of **2b** and its 3,2′,3′,4′,6′-penta-*O*-

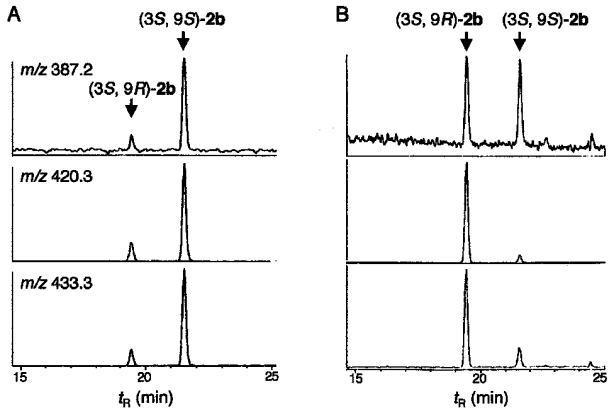

Fig. 3. Selected Ion Traces from the LC-APCI/MS Analysis at *m/z* 387.2, *m/z* 420.3, and *m/z* 433.3 of Authentic (3*S*, 9*R*)-**2b** and (3*S*, 9*S*)-**2b** (A) and of a Sample (B) Purified from the Flowers of *Rosa damascena* Mill.

acetate was investigated by evaporating *in vacuo* the samples with MeOH or MeCN in water. Compound **2b** was revealed to be unstable, whereas its acetate was relatively stable. Thus, the majority of the progenitors found in the MeOH extract already mentioned must have been gradually transformed to other compound(s) during the course of the purification.

Based on the empirical results just mentioned, the flowers (50 g fr. wt) were extracted with an HCOOH–NH_4OH buffer to give a glycosidic progenitor fraction. The extract was lyophilized, and the materials were acetylated and then purified by successive chromatography on a silica gel cartridge column (hexane-*t*-butyl methyl ether) and by preparative TLC (hexane-diethyl ether) guided by the chromatographic behavior of the pentaacetate of **2b**. After treating with MeONa, the sample was further purified by preparative HPLC (ODS-AM, MeCN-water) to give the desired fraction. This fraction was analyzed by HPLC apparatus equipped with a photodiode array detector. As shown in Fig. 2, peaks at t_R 24.04 and 26.51 min were detected at the same retention times for synthetic (3*S*, 9*R*)-**2b** and (3*S*, 9*S*)-**2b**, respectively. Finally, the presence of (3*S*, 9*R*)-**2b** and (3*S*, 9*S*)-**2b** was confirmed by an LC-APCI/MS analysis. As shown in Fig. 3, the progenitor fraction gave two prominent peaks with selected ion monitoring: SIM traces at *m/z* 387.2 $(M-H)^-$, 420.3 $(M+O_2)^-$ and 433.3 $(M-H+HCOOH)^-$ at t_R 19.26 and 21.34 min, respectively. The ion traces of the authentic compounds, (3*S*, 9*R*)-**2b** and (3*S*, 9*S*)-**2b**, showed the peaks at the same retention times as those for the desired fraction, indicating the presence of (3*S*, 9*R*)-**2b** and (3*S*, 9*S*)-**2b**. The ratio of (3*S*, 9*R*)-**2b** and (3*S*, 9*S*)-**2b** could be roughly calculated to be ca. 10–4 to 1 based on the ion intensities at *m/z* 420.3 and 433.3, although the ratio of the ion intensity at *m/z* 387.2 was 1 to 1. This latter ratio is not reliable due to the low (1/5–1/10th) intensity being compared with those for *m/z* 420.3 and *m/z* 433.3.

Although Kotseridis *et al.*[22] have reported a quantitative determination of free and hydrolytically liberated **1** in wine by a stable isotope dilution assay, there is almost no quantitative data on the generation of **1** from **2b**. To confirm the transformation of **2b** to **1**, we surveyed the reaction conditions for hydrolysis. Compound (3*S*, 9*S*)-**2b** was treated under the conditions shown in the Experimental section. Compound **1** was produced only when **2b** was treated in an aqueous solution at pH 2.0 at 90°C. Within half an hr, (3*S*, 9*S*)-**2b** was transformed or degraded to give **1** (4.8 mol%) and **3** (8.2 mol%) as volatile compounds, and (3*R*, 9*S*)-**4b** (13.0 mol%), as well as several unidentified compounds which were found in the non-volatile fraction. A longer reaction period (1.0 hr) did not significantly increase the amount of **1** (5.1 mol%). The reaction at pH 4.0 gave neither **1** nor **3** (data not shown). As a conclusion, labile progenitors (3*S*, 9*R*)- and (3*S*, 9*S*)-**2b** for **1** were identified for the first time in the flowers of *R. damascena* Mill. The instability of **2b** was confirmed to explain the reason for the disappearance of progenitors during purification of the MeOH extract from *R. damascena* Mill. A portion of (3*R*, 9*R*)-**4b** found in the residual water after steam distillation was strongly suggested to be one of the artifacts from (3*S*, 9*R*)-**2b**. We are now surveying all the possible progenitors of **1** in the flowers of *R. damascena* Mill. by LC-MS.

Experimental

Instruments. Optical rotation data were measured with a DIP-370 (Jasco) polarimeter at ambient temperature. ^{1}H- and ^{13}C-NMR and two-dimensional spectra were recorded by either a Jeol JNM-LA500 or a Jeol JNM-EX270 spectrometer. Mass spectra (MS) were acquired with a Jeol JMS-DX303-HF spectrometer. GC-MS data were measured with a Jeol JMS-DX302 instrument with a Jeol JMA-DA5000 MS data system equipped with GC apparatus (Yokogawa Hewlett Packard 5890) was used under the following conditions: column, PEG-20M (0.25 mm × 50 m); oven temp., a linear temperature gradient from 60°C to 220°C (3°C/min); injector temp., 150°C; carrier gas, He (1 ml/min). To detect **1** and **3** after the acid treatment, ions were monitored at *m/z* 208 $[M]^+$ for **3**, 190 $[M]^+$ for **1** and/or $[M-H_2O]^+$ for **3**, and 175 $[190\text{-}CH_3]^+$. The t_Rs for **1** and **3** were 29.0 and 52.2 min, respectively.

Isolation and characterization of damascenone progenitors from the flowers of R. damascena Mill. The flowers of *Rosa damascena* Mill. were harvested at the full-bloom stage in Fukuroi (Shizuoka, Japan) in 1998, then stored in a freezer (−30°C). The same cultivar was grown at the University Farm of

Shizuoka University (Japan) in 2000. The frozen flowers (10 kg) were extracted by 70% MeOH under ice-cooling, and the extract was loaded into a column of Amberlite XAD-2 (120×660 mm, Vt=7500 ml) that has been equilibrated with water and developed with MeOH to give a progenitor fraction. After concentration, the MeOH extract was acetylated (pyridine-acetic anhydride) and purified by flash chromatography on an LP-75 silica gel cartridge (75×300 mm, Vt=1325 ml; Wako, Japan; hexane-EtOAc=8:2–3:7 v/v) to yield eight fractions.

To detect the progenitors, a portion (10–20 g fr. wt. eq.) of each fraction was deacetylated by treating with MeONa and then heated at 90°C for 2 h at pH 2 in a sealed tube.[6,7] The volatile compounds formed were extracted by an azeotropic mixture of pentane-CH_2Cl_2 (2:1) under neutral conditions and analyzed by GC-MS. Two desired fractions were separately further purified by silica gel flash chromatography on an LP-40 cartridge (40×150 mm, Vt=188 ml; hexane-*t*-butyl methyl ether=9:1–3:7 v/v) and then by HPLC (column: ODS-AM 20×250 mm, YMC, Japan; solvent: MeCN-water 50:50–75:25 v/v) to give four progenitor fractions. Compound **4b** (1.2 mg) was isolated from one of the less-polar fractions as its pentaacetate.

*3,2′,3′,4′,6′-penta-O-acetyl-(3R, 9R)-3-hydroxy 7,8-didehydro-β-ionol 9-O-β-D-glucopyranoside (3,2′,3′,4′,6′-penta-O-acetyl-(3R, 9R)-**4b**).* ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 22.3 (CH_3-C5), 23.1 (CH_3-C9), 28.5 (CH_3-C1), 30.1 (CH_3-C1), 35.9 (C1), 37.3 (C4), 42.1 (C2), 62.0 (C6′), 67.5 (C9), 67.8 (C3), 68.4 (C4′), 71.8 (C2′), 71.9 (C5′), 73.0 (C3′), 84.0 (C7), 91.9 (C8), 98.9 (C1′), 123.2 (C6), 137.9 (C5), 20.6–21.4 (CH_3×5 of acetyl group), 169.3–170.7 (C=O×5 of acetyl group). All the signals were assigned based on the ^{1}H–^{1}H-COSY, HMQC, and HMBC spectra.[12,17]

*Synthesis of (3S, 9S)- and (3S, 9R)-megastigma-6,7-diene-3,5,9-triol 9-O-β-D-glucopyranosides ((3S, 9S)- and (3S, 9R)-**2b**).* To a solution of 3-*O*-acetyl-grasshopper ketone, which had been prepared according to the literature method,[18] (96 mg, 0.36 mmole) in 3 ml of MeOH was added $NaBH_4$ (38 mg, 1.0 mmole), and the mixture stirred for 30 min to give 3-*O*-acetyl-megastigma-6,7-diene-3,5,9-triol (3-*O*-acetyl-**2a**; 81 mg, 0.30 mmole, 83% yield). 3-*O*-Acetyl-**2a** (76 mg, 0.28 mmole) was stirred with 2,3,4,6-tetra-*O*-pivaloyl-D-glucopyranosyl bromide (489 mg, 0.84 mmole) and tetramethylurea (252 mg, 2.2 mmole) in dry CH_2Cl_2 at 0°C for 1 h in the presence of molecular sieves 4A (2 g). To this solution, silver trifluoroacetate (292 mg, 1.0 mmole) was added, and the solution further stirred for 5.5 hours at 0°C. The diastereomeric mixture of tetra-*O*-pivaloyl-**2b** obtained was partially purified by preparative silica gel TLC (CH_2Cl_2:hexane:EtOAc=6:2.5:1.5) to give the (3*S*, 9*S*)- and (3*S*, 9*R*)-diastereomers. These were treated with $LiOH \cdot H_2O$ in the usual manner and further purified by HPLC (MeCN-water, YMC pack ODS-AQ, 20×250 mm) to give (3*S*, 9*S*)-**2b** (0.8 mg, 0.75% yield) and (3*S*, 9*R*)-**2b** (3.2 mg, 2.9% yield).

Spectral data:

*3-O-Acetyl-megastigma-6,7-diene-3,5,9-triol (3-O-acetyl-**2a**).* ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 1.08, 1.10 (each 3/2H, s, H-11), 1.30 (3H, d, J=6.6 Hz, H-10), 1.36 (3H, s, H-13), 1.37 (1H, overlapped with H-13, H-2ax), 1.38, 1.39 (each 3/2H, s, H-12), 1.47 (1H, dd, J=12.9, 8.8 Hz, H-4ax), 1.96 (1H, ddd, J=2.3, 4.2, 12.2 Hz, H-2eq), 2.03 (3H, s, acetate), 2.25 (1H, ddd, J=2.3, 4.2, 12.9 Hz, H-4eq), 4.33 (1H, m, H-9), 5.33 (1H, m, H-3), 5.41 (1H, d, J=5.7 Hz, H-8); ^{13}C-NMR (67.5 MHz, $CDCl_3$) δ: 23.3 (C10), 29.2 (C12), 31.2 (C13), 32.2 (C11), 35.1 (C1), 45.1, (C4), 45.3 (C2), 66.3 (C3), 67.9 (C9), 72.9 (C5), 100.1 (C8), 117.5 (C6), 197.6 (C7), 21.4 and 170.5 (acetate).

*(3S, 9S)-megastigma-6,7-diene-3,5,9-triol 9-O-β-D-glucopyranoside ((3S, 9S)-**2b**).* ^{1}H-NMR (500 MHz, D_2O) δ: 1.07 (3H, s, H-11), 1.26 (3H, s, H-12), 1.32 (1H, dd, J=11.9, 8.9 Hz, H-2ax), 1.36 (3H, d, J=6.4 Hz, H-10), 1.41 (1H, dd, J=12.0, 8.8 Hz, H-4ax), 1.41 (3H, s, H-13), 1.89 (1H, ddd, J=2.0, 4.5, 11.9 Hz, H-2eq), 2.16 (1H, ddd, J=2.0, 4.5, 12.0 Hz, H-4eq), 3.27 (1H, t, J=8.5 Hz, H-2′), 3.33 (1H, ddd, J=1.2, 5.8, 8.8 Hz, H-5′), 3.37 (1H, dd, J=8.5, 8.8 Hz, H-4′), 3.45 (1H, dd, J=8.9, 8.5 Hz, H-3′), 3.70 (1H, dd, J=5.8, 12.5 Hz, H-6′a), 3.89 (1H, dd, J=1.5, 12.5 Hz, H-6′b), 4.21 (1H, m, H-3), 4.54 (1H, m, H-9), 4.64 (1H, d, J=8.3 Hz, H-1′), 5.29 (1H, d, J=8.0 Hz, H-8); ^{13}C-NMR (125 MHz, D_2O) δ: 23.7 (C10), 30.9 (C12), 33.1 (C13), 34.6 (C11), 37.3 (C1), 50.1 (C4), 50.4 (C2), 63.6 (C6′), 66.7 (C3), 71.3 (C4′), 74.7 (C5), 75.8 (C2′), 76.8 (C9), 78.7 (C3′), 78.9 (C5′), 98.5 (C8), 101.9 (C1′), 117.9 (C6), 203.2 (C7); FABMS (negative ion, glycerol) m/z 387 $(M-H)^-$; $[\alpha]_D^{27}$ −29.1° (c 1.00, MeOH).

*(3S, 9R)-megastigma-6,7-diene-3,5,9-triol 9-O-β-D-glucopyranoside ((3S, 9R)-**2b**).* ^{1}H-NMR (500 MHz, D_2O) δ: 1.10 (3H, s, H-11), 1.24 (3H, s, H-12), 1.29 (1H, dd, J=11.5, 9.2 Hz, H-2ax), 1.32 (3H, d, J=6.1 Hz, H-10), 1.36 (3H, s, H-13), 1.42 (1H, dd, J=11.9, 8.8 Hz, H-4ax), 1.89 (1H, dd, J=2.3, 4.2, 11.5 Hz, H-2eq), 2.15 (1H, dd, J=2.3, 4.2, 13.2 Hz, H-4eq), 3.23 (1H, t, J=8.2 Hz, H-2′), 3.33 (1H, ddd, J=1.2, 4.6, 7.9 Hz, H-5′), 3.37 (1H, dd, J=7.5, 7.9 Hz, H-4′), 3.39 (1H, t, J=7.5 Hz, H-3′), 3.71 (1H, dd, J=4.6, 12.2 Hz, H-6′a), 3.88 (1H, dd, J=1.2, 12.2 Hz, H-6′b), 4.20 (1H, m, H-3),

4.48 (1H, m, H-9), 4.56 (1H, d, $J=7.9$ Hz, H-1′), 5.45 (1H, d, $J=6.7$ Hz, H-8); ^{13}C-NMR (125 MHz, D_2O) δ: 22.5 (C10), 31.0 (C12), 33.0 (C13), 34.3 (C11), 37.3 (C1), 50.1 (C4), 50.5 (C2), 63.5 (C6′), 66.8 (C3), 72.3 (C4′), 74.9 (C5), 76.0 (C2′), 78.6 (C9), 78.8 (C3′), 79.0 (C5′), 99.6 (C8), 103.7 (C1′), 118.2 (C6), 202.4 (C7); FABMS (negative ion, glycerol) m/z 387 $(M-H)^-$; $[\alpha]_D^{23}$ 0° (c 0.77, MeOH).

Identification of allenic triol 9-O-β-D-glucopyranoside (2b) in flowers of R. damascena Mill. The frozen flowers (50 g fr. wt., harvested in 2000) at the full-bloom stage were homogenized with 100 ml of a 10 mM HCOOH–NH_4OH buffer (pH 7.0) under ice-cooling. The homogenate was extracted by stirring in 400 ml of the same buffer at room temperature. After the centrifugation (3800 g, 0°C, 15 min), the supernatant was lyophilized. The resulting powdery material (3.2 g) was acetylated (pyridine-acetic anhydride) and purified by silica gel column chromatography (hexane-*t*-butyl methyl ether, diethyl ether, stepwise) to yield ten fractions. The diethyl ether fraction was further purified by preparative TLC (Merck 1.13792) in hexane-*t*-butyl methyl ether = 1:6 as the developing solvent. The silica gel in the R_f region, corresponding to those for both the diastereomers of 3,2′,3′,4′,6′-penta-*O*-acetyl-**2b**, was scrapped off and extracted with dichloromethane. The resulting extract was deacetylated by treating with NaOMe, and then further purified by HPLC under the following conditions: column, YMC-ODS-AM5, 4.6 × 250 mm (YMC Co., Kyoto, Japan); solvent, 5% MeCN (0–10 min), the concentration of MeCN then being increased to 30% in 30 min at a flow rate of 1.0 ml/min at 40°C; detector, photodiode array (Jasco MD-1510). The purified fraction eluted between 23.6 and 27.0 min (Fig. 2), wheen both diastereomers of **2b** were eluted, was subjected to an LC-APCI/MS analysis. LC-APCI/MS was carried out under the following conditions: Agilent-1100 LC system; column, Mightysil ODS, 4.6 × 150 mm (Kanto Reagent, Chemicals & Biologicals, Tokyo, Japan); solvent, 5% MeOH (0–10 min), the concentration of MeCN then being increased to 30% in 30 min at a flow rate of 0.2 ml/min at 40°C; MS JMS-LC Mate (Jeol, Tokyo, Japan) equipped with an APCI interface and ionization in the APCI negative mode. The retention times were 19.26 and 21.34 min for (3*S*, 9*R*)-**2b** (2 ng) and (3*S*, 9*S*)-**2b** (10 ng), respectively (Fig. 3).

Screening of conditions for the conversion of (3S, 9S)-2b to damascenone (1). GC analyses were carried out to prepare a standard curve for the estimation of **1** and **3** in the concentration ranges of 0.01–0.15 μg and 0.1–1.5 μg, respectively. Ethyl decanoate (0.1 μg) was added to each standard solution as an internal standard. In each experiment, 25 μg of (3*S*, 9*S*)-**2b** was dissolved in 100 μl of acidic water. The conditions for the acid treatment were as follows: pH was set at 2.0 or 4.0; temperature was set to 40°C, 60°C or 90°C; incubation period was 0.5, 1.0 or 4.0 hr. After cooling to below −20°C, the reaction mixture was then neutralized with 0.1 M NaOH. After adding 1 μg of ethyl decanoate as an internal standard, each solution was extracted three times with 1 ml each of a mixture composed of pentane:CH_2Cl_2 = 2:1. The organic layers were combined and dried over $MgSO_4$, and then concentrated to 10–20 μl. One μl of the concentrate was injected for the GC-MS analysis as already mentioned. To analyze the non-volatile compounds, 10 μl of the aqueous layer was analyzed by HPLC without further pre-treatment. The HPLC conditions were the same as those described for the synthesis and identification of the allenic triol (**2a**) and its 9-*O*-glucopyranoside (**2b**). Under these conditions, (3*S*, 9*S*)-**2b** and (3*R*, 9*S*)-**4b** were detected at 26.3 and 29.3 mins, respectively.

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