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CITRONELLYL DISACCHARIDE GLYCOSIDE AS AN AROMA PRECURSOR FROM ROSE FLOWERS*

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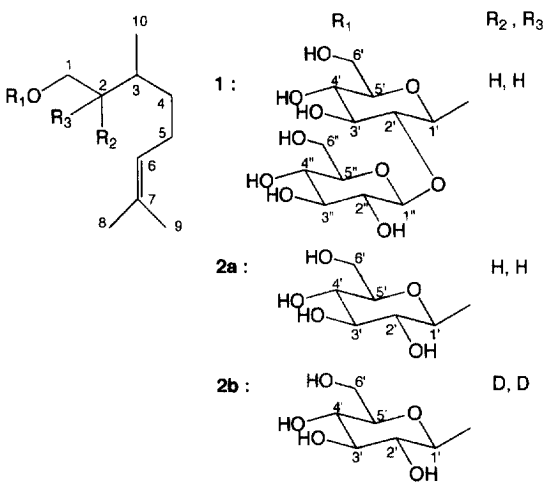
Key Word Index—*Rosa damascena* var. *bulgaria*; rose flowers; aroma precursors; flowers; aroma formation; glycoconjugates; citronellyl 2-*O*-β-D-glucopyranosyl-β-D-glucopyranoside; citronellyl β-sophoroside.

Abstract—Citronellyl 2-*O*-β-D-glucopyranosyl-β-D-glucopyranoside (citronellyl β-sophoroside) was isolated as an aroma precursor of citronellol from flowers of *Rosa damascena* var. *bulgaria*. Isolation was guided by enzymatic hydrolysis followed by GC and GC-mass spectrometric analyses. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Francis *et al.* [1] first clarified the biosynthesis of terpenols and 2-phenylethanol in flower petals of the rose 'Lady Seton'. They also reported the transformation of mevalonate into the β-glucopyranoside of terpenols in the rose petals [2, 3]. β-D-Glucopyranosides of geraniol, nellol, citronellol, and 2-phenylethanol have been also isolated from rose petals [4, 5]. Polgorel'skaya *et al.* [6] reported and hypothesized the translocation of glucosides of monoterpenols and 2-phenylethanol from leaves to flower petals. The β-glucopyranosides of alcoholic aromas have so far been regarded as aroma precursors of flowers. The possible role of β-glucosidase has also been suggested [7] to be involved in aroma formation in rose flowers. Unlike these results reported on rose flowers, we have previously reported [8] the important role of disaccharide glycosides and glycosidase involved in aroma formation in flowers of *Gardenia jasminoides* and *Jasminum sambac*. We have also isolated several disaccharide glycosides of monoterpene alcohols and aromatic alcohols as aroma precursors of the flowers [9–11]. Recently, we have reported [12], for the first time, the isolation of geranyl disaccharide glycosides, 6-*O*-β-D-apiofuranosyl-β-D-glucopyranoside and 6-*O*-α-L-arabinofuranosyl-β-D-glucopyranoside from flowers of *Rosa damascena* var. *bulgaria*. Herein we describe the isolation of a disaccharide glycoside, citronellyl 2-*O*-β-D-glucopyranosyl-β-D-glucopyranoside (citronellyl β-sophoroside) (**1**) from rose flowers.



RESULTS AND DISCUSSION

Isolation of glycoconjugates of citronellol was guided by crude enzyme assay, followed by GC and GC-mass spectrometric analyses as previously reported [6]. The aqueous residue left after steam distillation of rose flower heads was passed through a column of Amberlite XAD-2. The glycosidic fractions obtained after methanol elution were purified by ODS

* Part 5 in the series 'Studies on Aroma Formation Mechanism of Flowers'. For Part 4 see ref. [11].

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column chromatography. The fraction eluted with 30% acetonitrile was further purified by repeated recycling HPLC on an ODS column (gradient elution with 20–60% acetonitrile, 30% acetonitrile, 34% acetonitrile) to yield a disaccharide glycoside (**1**: 2.4 mg) of citronellol. Its molecular formula $C_{22}H_{40}O_{11}$ was determined by HRFAB mass spectrometry *m/z* 481.2655 $[M+H]^+$ together with the ^1H and ^{13}C NMR data. The ^{13}C NMR spectrum (125 MHz, D_2O) of **1** showed 22 carbon signals including those of a citronellyl moiety and two anomeric carbons (δ 103.9 and 105.2), indicating the presence of two hexose moieties. In the ^1H NMR, the coupling constant ($J = 8.0$ Hz) of two anomeric protons at δ 4.55 (H-1′) and 4.78 (H-1″) suggested that these hexoses are in the pyranose form. The sequential connectivities from H-1′ to 6′, and H-1″ to 6″ were confirmed by the 2D-HOHAHA (TOCSY) spectra. The large coupling constants ($J = 8$–9.2 Hz) and the splitting patterns of H-2′ to H-4′ and H-2″ to H-4″ revealed that the hexose moieties were β-D-glucopyranoses. In the ^{13}C NMR, the low-field shift of C-2′ (δ 82.9) indicated that the second glucosyl moiety was connected to this carbon. HMBC analyses showed prominent cross-peaks from H-1″ to C-2′, H-2′ to C-1″, and H-1′ to C-1. Thus, the sugar moiety of **1** was suggested to be β-sophoroside. In the ^{13}C NMR spectrum, chemical shifts of signals due to the sugar moiety coincided with those of methyl β-sophoroside [13] by direct comparison. Thus, the structure of **1** has been determined as citronellyl 2-*O*-β-D-glucopyranosyl-β-D-glucopyranoside. Although the chirality at C-3 is unclear, to the best of our knowledge this is a new compound. It is noteworthy that all the aroma precursors of monoterpene alcohols isolated from flowers of *R. damascena* are present as disaccharide glycosides not as β-glucopyranosides. Thus, we again suggest the importance of disaccharide glycosides in aroma formation of rose flowers.

Wüst *et al.* [14], recently reported the biogenesis of rose oxide in *Pelargonium graveolens* by feeding experiments using deuterium-labelled precursors. Since the plants were able to convert fed (+)- and (−)-citronellyl β-D-glucopyranosides (**2b**) into *cis*- and *trans*-isomers of rose oxide in the same ratio, it was suggested that the reaction is triggered non-enzymatically [14]. This observation strongly suggested that **2a** is one of the possible precursors of rose oxide. The transformation may involve oxidation at C-7 position of **2a** and/or citronellol produced from **2a** by enzymatic hydrolysis, followed by cyclization to generate the hydropyran ring of rose oxide, similar to well known reactions, i.e. oxygenation of citronellol by singlet oxygen, reduction, followed by the cyclization under acidic condition [15]. The disaccharide glycoside (**1**) isolated may liberate not only citronellol, but also be transformed into rose oxide in rose flowers.

EXPERIMENTAL

Plant material. Rose flowers (*Rosa damascena* var. *bulgaria*, 10 kg) were harvested at full bloom stage, June 1994, at Fukuroi, Shizuoka, Japan.

Purification and isolation. Isolation of glycoconjugates of citronellol was guided by crude enzyme assay, followed by GC and GC-MS analyses as previously reported [8–10]. The aq. residue (15 l) left after steam distillation of rose flower heads (10 kg), was passed through a column of Amberlite XAD-2 (100 × 640 mm; equilibrated with H_2O and sucessively eluted with pentane and MeOH). The MeOH fr. was purified by ODS (30 × 480 mm) CC. The fr. eluted with 30% MeCN was further purified by repeated HPLC on an ODS column (20 × 250 mm; gradient elution with 20–60% MeCN, 30% MeCN, 34% MeCN, detector: UV 210 nm) to yield the disaccharide glycoside (**1**: 2.4 mg) of citronellol.

Compound **1**. $[\alpha]_D^{22}$ −16° (H_2O; *c* 0.1). HRFABMS (positive, glycerol) *m/z* 481.2655 $[M+H]^+$ (+1.8 mu for $C_{22}H_{41}O_{11}$). ^1H NMR (500 MHz, D_2O): δ 0.89 (3H, *d*, $J = 6.4$ Hz, H-10), 1.21 (1H, *m* Ha-4), 1.36 (1H, *m*, Hb-4), 1.43 (1H, *m*, Ha-2), 1.61 (1H, *m*, Hb-2), 1.66 (1H, *m*, H-3), 1.64 (3H, *s*, H-8), 1.71 (3H, *s*, H-9), 2.02 (2H, *m*, H-5), 3.32 (1H, *dd*, $J_{2'',1''} = 8.0$ Hz, $J_{2'',3''} = 9.2$ Hz, H-2″), 3.39 (1H, *dd*, $J_{4'',3''} = 8.6$ Hz, $J_{4'',5''} = 8.9$ Hz, H-4″), 3.43 (1H, *dd*, $J_{4',5'} = 8.9$ Hz, $J_{4',3'} = 9.2$ Hz, H-4′), 3.46 (2H, *m*, overlapped, H-5′,5″), 3.51 (1H, *dd*, $J_{3'',4''} = 8.6$ Hz, $J_{3'',2''} = 9.2$ Hz, H-3″), 3.55 (1H, *dd*, $J_{2',3'} = 8.9$ Hz, $J_{2',1'} = 8.0$ Hz, H-2′), 3.69 (1H, *dd*, $J_{3',4'} = 9.2$ Hz, $J_{3',2'} = 8.9$ Hz, H-3′), 3.73 (2H, *dd*, $J_{a,b} = 12.5$ Hz, $J_{a,5' \text{ or } 5''} = 5.5$ Hz, H-6′a, 6″a), 3.74 (1H, *ddd*, $J_{1a,1b} = 11.6$ Hz, $J_{1a,2a} = 8.5$ Hz, $J_{1a,2b} = 2.0$ Hz, H-1a), 3.90 (1H, *dd*, $J_{6'b,6'a} = 12.5$ Hz, $J_{6'b,5} = 2.8$ Hz, H-6′b), 3.91 (1H, *dd*, $J_{6''b,6''a} = 12.5$ Hz, $J_{6''b,5} = 2.8$ Hz, H-6″b), 3.99 (1H, *ddd*, $J_{1b,1a} = 11.6$ Hz, $J_{1b,2b} = 7.7$ Hz, $J_{1b,2a} = 2.0$ Hz, H-1b), 4.55 (1H, *d*, $J = 8.0$ Hz, H-1′), 4.78 (1H, *d*, $J = 8.0$ Hz, H-1″), 5.23 (1H, *br t*, $J = 3.1$ Hz, H-6). ^{13}C NMR (125 MHz, D_2O): δ 19.8 (*q*, C-8), 21.6 (*q*, C-10), 27.7 (*q*, C-9), 27.8 (*t*, C-5), 31.5 (*d*, C-3), 38.6 (*t*, C-2), 39.2 (*t*, C-4), 63.6 (*t*, C-6″), 63.7 (*t*, C-6′), 72.0 (*t*, C-1), 72.4 (*d*, C-4′), 72.5 (*d*, C-4″), 76.8 (*d*, C-2″), 78.5 (*d* × 2, C-3″, -5″), 78.9 (*d* × 2, C-3′, -5′), 82.9 (*d*, C-2′), 103.9 (*d*, C-1′), 105.2 (*d*, C-1″), 128.1 (*d*, C-6), 134.0 (*s*, C-7).

Methyl β-sophoroside. ^{13}C NMR (125 MHz, D_2O): δ 59.6 (*q*, Me), 63.3 (*t*, C-6′), 63.3 (*t*, C-6″), 72.2 (*d*, C-4′), 72.2 (*d*, C-4″), 76.2 (*d*, C-2″), 78.2 (*d*, C-5′), 78.4 (*d*, C-5″), 78.6 (*d*, C-3″), 78.7 (*d*, C-3′), 82.7 (*d*, C-2′), 104.4 (*d*, C-1′), 105.2 (*d*, C-1″).

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