Biosci. Biotechnol. Biochem., **64** (4), 907–910, 2000

Note

(+)-4-*epi*-α-Bisabolol as a Major Sesquiterepene Constituent in the Leaves of Two *Rosa rugosa* Hybrids, Martin Frobisher and Vanguard

Yasuyuki HASHIDOKO,[1,2] Keiko ENDOH,[1] Toshihiro KUDO,[3] and Satoshi TAHARA[1,2]

[1]*Division of Applied Bioscience, Graduate School of Agriculture, Hokkaido University, Kita-ku, Sapporo 060-8589, Japan*
[2]*CREST, Japan Science and Technology Corporation, Honmachi 4-1-8, Kawaguchi 332-0012, Japan*
[3]*Yurigahara Park, Sapporo Parks Green Development Association, 210 Shinorocho-Taihei, Kita-ku, Sapporo 002-8051, Japan*

Received October 29, 1999; Accepted December 21, 1999

During an investigation of the sesquiterpene phases and contents in leaves of several *Rosa rugosa* hybrids (hybrid rugosas), Martin Frobisher and Vanguard were found to accumulate a large amount of (+)-4-*epi*-α-bisabolol (1) as a single constituent. Although glandular trichomes of Martin Frobisher on the leaves are dense, this *R. rugosa* hybrid produces none of the carota-1,4-dienaldehyde (2) or bisaborosaol A (3) that are both found as representative sesquiterpenes of the carotane and bisabolane classes, respectively, in a glandular trichome exudate of wild-type *R. rugosa*. Compound 1 was also apparent as a nearly single constituent detectable by GC in the leaf constituents of Vanguard possesses sparse glandular trichomes on the leaf. Martin Frobisher and Vanguard had likely lost their capability to form carotane-type sesquiterpenes and had also lost their activity to oxygenate the C-7 allyl methyl carbon of compound 1 to convert 3. The presence of (+)-4-*epi*-α-bisabolol-accumulating *R. rugosa* hybrids is significant when considering the sesquiterpene biogenesis of *Rosa rugosa*.

Key words: Martin Frobisher; Vanguard; *Rosa rugosa* hybrid; glandular trichome; (+)-4-*epi*-α-bisabolol

Rosa rugosa is a particular Rosaceae plant which can produce large amounts of sesquiterpenoids. These sesquiterpenes are mainly exuded from mushroom-shaped glandular trichomes located along the running leaf veins on the undersurface of the leaflet.[1)] Most of the species of the Rosaceae family, known as the tanniferous family, are polyphenol-accumulating plants and commonly have poor ability to biosynthesize mono- and sesqui-terpenes.[2)] Thus, the capability of *R. rugosa* to produce carotane and bisabolane sesquiterpenoids is unique in the Rosaceae family as its phytochemical feature.[3)]

Among *Rosa* genus plants, those so far known to produce sesquiterpenoids in the glandular trichomes so far are also limited to *R. rugosa*, its natural sports, varieties and some of their hybrids (so-called *R. rugosa* hybrids or hybrid rugosas). The major skeletal classes of the sesquiterpenes of *R. rugosa* are carotane and bisabolane, and carota-1,4-dienaldehyde (**2**) and bisaborosaol A (**3**) are representative sesquiterpenes of these respective classes in an analysis by a gas-chromatography (GC). The sesquiterpene components in several *R. rugosa* hybrids were qualitatively and quantitatively analyzed by GC with an OV-1 glass capillary column (TC-1, GL-Science, 30 m × 0.32 mm i.d.).

We have analyzed over 40 of the *R. rugosa* hybrids, and both Martin Frobisher ((*R. rugosa* × *R. bracteata*) × unknown, Canadian Explorer series, 1968), which possessed relatively dense glandular trichomes to produce colorless mucilage,[4)] and Vanguard ((*R. wichuraiana* × *R. rugosa alba*) × a hybrid tea rose, Eldorado), which also had glandular trichomes in young leaves, showed a totally different sesquiterpene composition from that of wild *R. rugosa*.[4)] These *R. rugosa* hybrids contained none of compounds **2** and **3**, even as trace amounts. Instead, both showed a large single peak at *t*R 13.2 min in GC (*cf.* 14.0 and 20.7 min for **2** and **3**, respectively, and 18.5 min for methyl palmitate used as the internal standard) (Fig. 1). This characteristic constituent showed *m*/*z* 204 (M^+-H_2O) by GC-MS, and initially fractionated extracts of Martin Frobisher with small-scale preparative TLC monitoring by GC revealed that the compound possessed a certain polarity nearly corresponding to that of sesquiterpene mono-alcohols.

Accordingly, 17.3 g of the leaves of Martin Frobisher were extracted with EtOAc, and the soluble substance was chromatographed in a silica gel column that was eluted with 10% EtOAc/*n*-hexane. The major constituent thus isolated (2.3 mg) was

To whom correspondence should be addressed. Fax: +81-11-706-4182; Tel: +81-11-706-3839; E-mail: yasu-h@abs.agr.hokudai.ac.jp

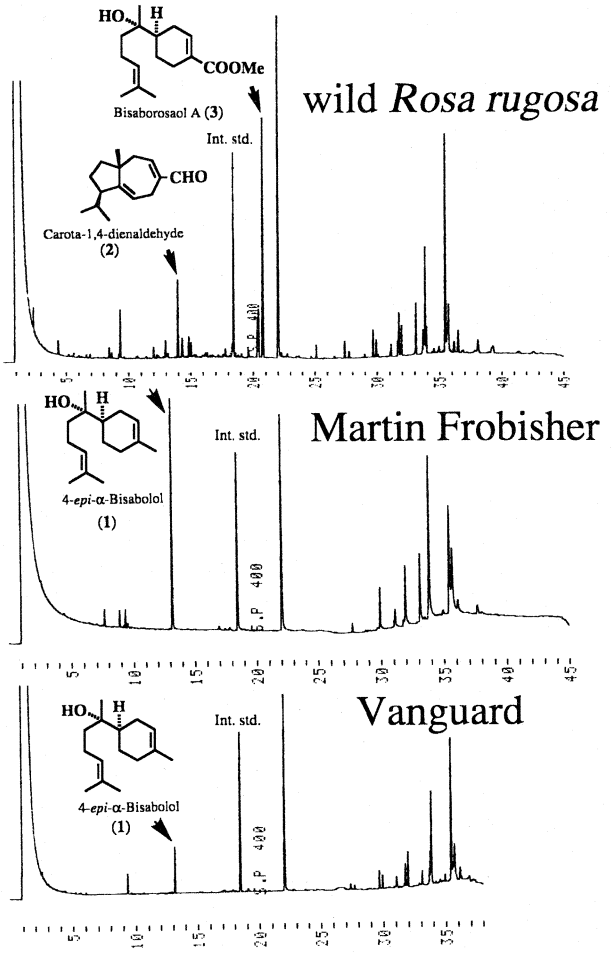
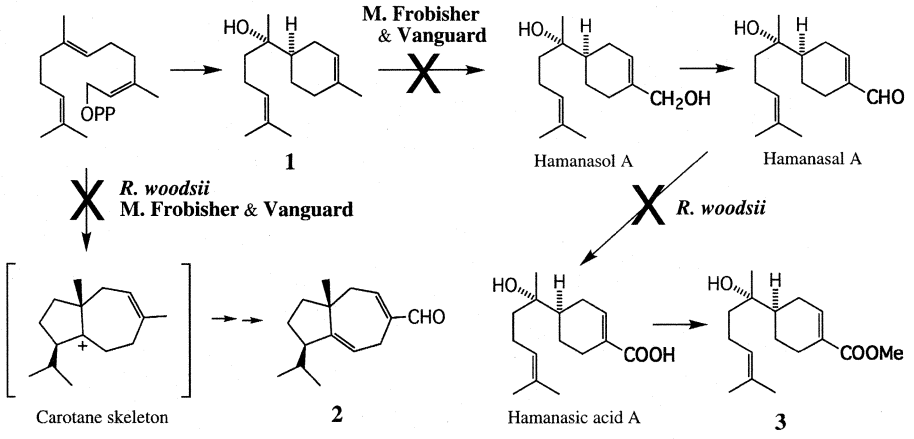


Fig. 1. Gas Chromatograms of Leaf Extracts of Wild-type *R. rugosa* (Top) and the Two Hybrids, Martin Frobisher (Middle) and Vanguard (Bottom).

identical to (+)-4-*epi*-α-bisabolol (**1**) in its ^{1}H- and ^{13}C-NMR spectra and optical rotation.[5-7] Compound **1** has been found as a naturally occurring rare enantiomer of α-bisabolol in the essential oils of *Salvia stenophylla*[5] and *Santalum spicatum*,[6] and we have also isolated **1** from the ethanol rinsates of *R. rugosa* leaves, although the amount chromatographically obtained from 11.4 kg of the leaves (146 g of the rinsate) was only 10 mg (*ca.* 1 μg/g of fresh leaves).[7] Considering the amount of **1** that was practically obtained from the leaves of Martin Frobisher, ability of the hybrid to produce **1** (*ca.* 130 μg/g of fresh leaves) is over 100 fold more than that of wild-type *R. rugosa*. By using a standard curve for compound **2**,[8] the contents of compound **1** in Martin Frobisher and Vanguard were calculated to be 466 and 69 μg/g of fresh leaves, respectively. The rinsate obtained by washing the leaves of Martin Frobisher with 95% EtOH for several seconds contained compound **1** as a nearly single constituent. Similarly, young and mature leaves of Vanguard contained compound **1** in the ethanolic rinsate as the predominant sesquiterpene constituent. Since the leaves of Vanguard lose their glandular trichomes when the leaves mature, the hybrid rugosa is likely to accumulate compound **1** on the surface of the mature leaves.

We have found that *R. woodsii*, a wild rose possessing no glandular trichomes on its leaves, contained a bisabolol-type sesquiterpene, whose major constituent was one oxidized at the C-7 allyl methyl carbon into a formyl group, but not being oxidized into a carboxyl group.[9] We therefore speculate that *R. woodsii* lacks an enzyme that catalyzes oxidation of the C-7 formyl group into a carboxyl group. Like *R. woodsii*, these **1**-accumulating *R. rugosa* hybrids are likely to have lost the enzyme activity to introduce an oxygen atom to the C-7 carbon of the bisabolane skeleton, because compound **1** is a sesquiterpene of the bisabolol type without any oxygenation at the C-7 allyl methyl group. These hybrids are consequently unable to yield compound **3** (Scheme 1). Moreover, a key enzyme that catalyzes the cyclization of *Z,E*-farnesyl diphosphate to produce a 5,7-bicyclic (carotane) sesquiterpene is likely to have been lost in the hybrids, so that the



Scheme 1. Sesquiterpene Biogenesis of Wild-type *Rosa rugosa*, Its Hybrids Martin Frobisher and Vanguard, and *R. woodsii*. →available biosynthetic pathways; -X→blocked pathway. Roses with blocked or lacking pathways are noted.

sesquiterpene biosynthetic pathway of the hybrids would only yield and accumulate compound **1** in the glandular trichomes.

A molecular biological investigation of the mechanism by which the hybrids suppress particular metabolic steps in the sesquiterpenoid pathway of *R. rugosa* seems significant, because genetic aspects will give us a better understanding of the origin of the sesquiterpene-producing capability of *R. rugosa*, in comparison of the gene regulation among wild-type *R. rugosa, R. woodsii* and the **1**-accumulating hybrids. The phytochemical properties of the hybrids have thus encouraged a biochemical approach to *R. rugosa* sesquiterpene biosynthesis.[10,11] The chemical composition and evaluation of other *R. rugosa* hybrids will be reported elsewhere.

General. Gas chromatography was conducted with a Hitachi G 5000 instrument equipped with a TC-1 glass-capillary column (GL Science, 30 m × 0.32 mm i.d., corresponding to OV-1), and ^{1}H- and ^{13}C-NMR spectra were measured by a Jeol EX 270 instrument at 270 and 68 MHz, respectively, using TMS as an internal standard. GC-MS analyses were performed by a ThermoQuest GC-Q mass spectrometer combined with ThermoQuest Trace 2000 GC apparatus equipped with a TC-1 glass-capillary column.

Plant materials. The leaves (0.5–2.5 g) of *R. rugosa* and other sports, varieties and hybrids were sampled from Yurigahara Park in Sapporo in late May 1998. All of the hybrids had originally been purchased from Spring Valley Roses (Spring Valley, USA), Pickering Nurseries (Pickering, Canada), Hortico (Waterdown, Canada) and Peter Beales Roses (Norfolk, England). The parentage of each hybrid was based on Verrier's "*Rosa rugosa*"[4] and Beales' "Roses."[12]

Extraction, cleaning and GC analysis. The leaves of Martin Frobisher (1.04 g) and Vanguard (1.53 g) were individually soaked in 10 ml of EtOAc and kept at −20°C in the dark. The resulting EtOAc extract of each was adjusted to 25 ml in a volumetric flask, and precisely 10 ml of the solution was concentrated and re-dissolved in 1 ml of 20% EtOAc/*n*-hexane to apply to a Sep Pak silica cartridge (Waters, 3 ml in volume) that had previously been conditioned with the same solvent. The sample-charged cartridge was eluted with 10 ml of 20% EtOAc/*n*-hexane, and the resulting eluate was evaporated nearly to dryness, before being mixed with 1 ml of an internal standard solution containing 1.67 mg of methyl palmitate. A 0.5-μl aliquot of the sample solution thus prepared was applied to GC. The oven temperature being initially set to 100°C for 1 min, then programmed from 100 to 200°C at a rate of 5°C/min and finally from 200 to 300°C at a rate of 7.5°C/min. The injector and detector temps. were both set at 220°C.

Isolation and identification of 4-epi-α-bisabolol from the leaves of Martin Frobisher. 17.3 g of fresh leaves of Martin Frobisher that had been collected at Yurigahara Park in mid-July 1999 were soaked in EtOAc for one week. The EtOAc-soluble substances were partitioned between aqueous ethanol and *n*-pentane. The target compound, which was transferred mainly in the aqueous ethanol phase (*ca.* 1 g), was evaporated to dryness, and the resulting residue was chromatographed in a silica gel column (30 g), eluting with 10% EtOAc/*n*-hexane, to give 50-ml fractions. The target compound was eluted in the 4th faction to give a single spot at R_f 0.4 on silica gel thin-layer plates developed in *n*-hexane:EtOAc = 9:1, and the purified compound was analyzed by its ^{1}H- and ^{13}C-NMR spectra and optical rotation. (+)-4-*epi*-α-Bisabolol was obtained as colorless oil; EI-MS (*m/z*, %): 204 (45). 189 (10), 161 (26), 119 (100), 109 (29), 93 (28), and 67 (27); $[\alpha]_D^{23}$ + 63° (methanol, *c* = 0.29, lit. + 67.4°[13]). The ^{1}H- and ^{13}C-NMR spectra of the isolated compound were identical with those of the reference compound that had been obtained from *R. rugosa*.[7] ^{1}H-NMR (270 MHz, in C_6D_6): 5.44 (1H, br. d, *J* = 3.6 Hz), 5.21 (1H, br. t, *J* = 7.0 Hz), 1.68 (3H, br. s), 1.64 (3H, br. s), 1.58 (3H, br. s), and 0.98 (3H, s). ^{13}C-NMR (68 MHz, in C_6D_6): 133.4, 131.1, 125.5, 121.5, 73.5, 43.7, 39.9, 31.4, 26.5, 25.8, 24.3, 24.1, 23.5, 22.7, and 17.6.

References

1) Hashidoko, Y., and Urashima, M., Efficient preparation of browning-free glandular trichome tissues from the surface of leaves of *Rosa rugosa* Thunb. *Plant Cell Physiol.*, **36**, 127–132 (1995).
2) Kokubun, T., and Harborne, J. B., A survey of phytoalexin induction in leaves of the Rosaceae by copper ions. *Z. Naturforsch.*, **49c**, 628–634 (1994).
3) Hashidoko, Y., The phytochemistry of *Rosa rugosa*. *Phytochemistry*, **43**, 535–549 (1996).
4) Verrier, S., "Rosa rugosa," Capability's Books, Deer Park, USA, (1991).
5) Brunke, E. J., and Hammerschmidt, F. J., Constituents of the essential oil of *Salvia stenophylla*-first identification of (+)-epi-α-bisabolol in nature. In Baerheim, S. A., and Scheffer, J. J. C., *Essent. Oils Aromat. Plants, Proc. Int. Symp.*, Martinus Nijhoff/Dr W. Junk, Dordrecht, **15**, 145–150 (1985).
6) Brophy, J. J., Fookes, C. J. R., and Lassak, E. V., Constituents of *Santalum spicatum* (R.Br.) A. DC. wood oil. *J. Essent. Oil. Res.*, **3**, 381–385 (1991).
7) Hashidoko, Y., Tahara, S., and Mizutani, J., Six sesquiterpenoids from glandular trichome exudates of *Rosa rugosa*. *Phytochemistry*, **35**, 325–329 (1994).
8) Hashidoko, Y., Iwaya, N., Tahara, S., and Mizutani, J., Changes in carotane sesquiterpenoids in *Rosa rugosa* leaves (Jpn). *Nippon Nougeikagaku Kaishi* (in Japanese), **65**, 1483–1488 (1991).

9) Hashidoko, Y., Tahara, S., and Mizutani, J., Bisabolane sesquiterpenes and a 2-phenoxychromone from *Rosa woodsii* leaves. *Phytochemistry*, **31**, 2148–2149 (1992).
10) Yencho, G. C., Bonierbale, M. W., Tingey, W. M., Plasted, R. L., and Tanksley, S. D., Molecular markers locate genes for resistance to the Colorado potato beetle, *Leptinotarsa decemlineata*, in hybrid *Solanum tuberosum*×*S. berthaultii* potato progenies. *Entomologia Experimentalis Applicata*, **81**, 141–154 (1996).
11) Kowalski, S. P., Plaisted, R. L., and Steffens, J. C., Immunodetection of polyphenol oxidase in glandular trichomes of *Solanum berthaultii, Solanum tuberosum* and their hybrids. *Am. Potato J.*, **70**, 185–199 (1993).
12) Beales, P., "Roses, An Illustrated Encyclopaedia and Grower's Handbook of Species Roses, Old Roses and Modern Roses, Shrub Roses and Climbers," Harvill/Harper Collins Publishers, London (1992).
13) Gunther, K., Carle, R., Fleischhauer, I., and Merget, S., Semi-preparative liquid-chromatographic separation of all four stereoisomers of α-bisabolol on tribenzoylcellulose. *Fresenius J. Anal. Chem.*, **345**, 787–790 (1993).