Novel Bisabolanoids in *Rosa rugosa* Leaves

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Z. Naturforsch. **46c,** 349–356 (1991); received June 5/October 10, 1990

Rosa rugosa, Sesquiterpene, Bisabolanoid

Bisaborosaol A (**1**), a novel sesquiterpene belonging to the bisabolane class, was isolated from *Rosa rugosa* leaves. The absolute configuration of **1** at C-4 was shown to be inverted in comparison with bisabolanoids from Compositae. Bisaborosaol A (**1**) and its corresponding carboxylic acid (**2**) may be biogenetically related to the carotanoids which constitute the major sesquiterpenes of *R. rugosa* leaves.

Introduction

Rosaceae which include *Rosa rugosa* is the third family found as the source of carotanoids among higher plants [1]. Rugosa rose *(R. rugosa)* leaves have been shown to contain various kinds of carotane sesquiterpenoids, which were regiospecifically oxygenated at the C-14 carbon yielding an α,β-unsaturated aldehyde or an α,β-unsaturated carboxylic acid (or its methyl ester) [1–3]. In a survey of the constituents of *R. rugosa* leaves, a new sesquiterpene alcohol (**1**) having a bisabolane skeleton and the corresponding acid (**2**) were isolated. The structures were elucidated by chemical and spectroscopic methods. The compound **1,** (4*R*:1′*S*)-1-methoxycarbonyl-4-(1′-hydroxy-1′,5′-dimethyl-4-hexenyl)-1-cyclohexene (= 7-methoxycarbonyl-4-hydro-8-hydroxy-α-bisabolene) was called bisaborosaol A. The oxygenation of **1** at C-7 indicated the biogenetic relationship between the bisabolanoids and carotanoids [4] previously reported as leaf constituents of *R. rugosa*. Naturally occurring bisabolanoids having a C-8–OH and/or oxygenated C-7 have been found in some Compositae [5–9]. The bisabolanoids of *R. rugosa,* however, were found epimeric at C-4 to those of Compositae origin. Isolation and structure elucidation including stereochemistry of these bisabolanoids are described. Correlation of these bisabolanoids with the carotanoids of *R. rugosa* origin is briefly discussed.

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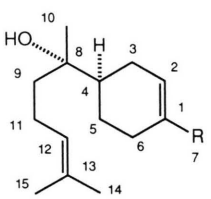
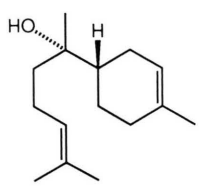
0939–5075/91/0500–0349 $ 01.30/0

Results and Discussion

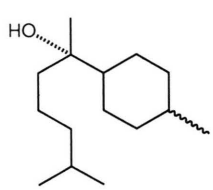
The rugosa rose *(R. rugosa)* leaves were initially soaked in 3 mM $CuCl_2$ solution to survey their stress compounds [10]. Although this attempt failed, a unique quenching spot showing a reddish purple by vanillin-H_2SO_4 test was detectable both in the $CuCl_2$-treated and control (soaked in tap water) extracts. To obtain this compound, the diffusates from 6.0 kg of fresh rugosa rose leaves into 3 mM $CuCl_2$ solution were extracted with EtOAc, and the extracts were chromatographed on silica gel column.



1:R=$COOCH_3$ bisaborosaol A
1a:R=CH_2OH
1b:R=CH2OAc
1c:R=CHO
2:R=COOH hamanasic acid A



3: (-)-α-bisabolol



1d

Fig. 1. Chemical structures of isolated and related compounds.

Compound **1** obtained as a colorless syrup (*ca.* 200 mg) showed the parent ion at *m/z* 266 (100%) and its molecular formula $C_{16}H_{26}O_3$ (found 266.184, calcd. 266.188) in FI- and EI-HR-MS, respectively. The EI-MS fragment at *m/z* 248 (M^+-H_2O, 23%) indicated the presence of a hydroxyl group. UV spectrum of **1** showed λ_{max} at 218 nm due to an α,β-unsaturated carboxyl group. The infra-red spectrum also supported the presence of a hydroxyl (KBr; 3500 cm^{-1}, br.) and an α,β-unsaturated carbonyl (1720 cm^{-1}, s) groups.

In ^1H and ^{13}C NMR spectra (in $CDCl_3$), two olefinic protons [δ_H 7.005 (1H) and 5.135 (1H)] and four sp^2 carbons [δ_C 139.7 (C), 132.0 (CH), 130.0 (CH) and 124.3 (C)] attributable to two >C=CH moieties were detected. The latter olefinic proton (C-12−H, δ_H 5.135, 1H, multiple-divided triplet, J = 7.3 and 1.0 Hz) and two allylic methyl groups (C-15 and C-14−H_3, δ_H 1.626, br. s and 1.692, br. d, J = 1.0 Hz, respectively, allylic coupled with C-12−H) were indicative of a 3,3-dimethylallyl group. In the HH-COSY, C-11−H_2 (2H, multiplet, centered at δ_H 2.0) showed a cross peak with C-12−H, and a further coupling with equivalent C-9 methylene protons (δ_H 1.536, 2H, J = 8.3 Hz). The proton-proton coupling sequence was ended there. The above data led to the elucidation of substructure **A** as in Fig. 2 [$(CH_3)_2C=CHCH_2CH_2-$■]. Vicinal couplings in the substructure were also confirmed by two point-homodecoupling experiments irradiated at 5.135 ppm and 1.536 ppm. The C-11−H_2 became feasible as non-equivalent methylene protons (δ_H 2.090 and 2.029 geminally coupled by J = 14.6 Hz).

Another substructure involving the conjugation system was also revealed by the NMR analyses. The methoxycarbonyl protons [δ_H 3.729 (3H, s, C-7′−H_3)] and methoxycarbonyl carbons (δ_C 51.0, 7′-CH_3 and 167.0, 7-C) were observed. Since two sp^2 carbons out of four have already been assigned to the substructure **A**, the remaining two should be attributed to the α,β-unsaturated methoxycarbonyl moiety. Its geometry on the conjugated C,C-double bond was deduced to be (*E*) (*cis* as regard to −COOMe and C-2−H) since the chemical shift value for the olefinic proton (δ_H 7.005) is comparatively low by the deshielding effect of the carbonyl group [11]. The C-2 proton showed a vicinal coupling with C-3 methylene protons at δ_H 2.337 and 2.087. In the HH-COSY, the C-3 protons showed further cross peaks with C-4 methine proton at δ_H 1.610 (1H, overlapped with C-15 methyl protons) that exhibited a correlation with carbon signal of a methine at δ_C 42.5 in the CH-COSY spectrum. On the other hand, C-2 proton showed allylic couplings with C-6−H_2 at δ_H 2.525 (br. d, J = 17.6 Hz) and 2.168 (m). These allylic methylene protons, showing downfield shift probably due to the β-carbonyl group, was further coupled with C-5−H_2 at δ_H 1.907 (1H, br. d, J = 12.7 Hz) and 1.249 (1H, dddd, J = 12.7, 12.2, 12.2 and 5.4 Hz). The latter proton also exhibited a cross peak with the C-4 proton. Consequently, substructure **B** was elucidated to be a 1,4-disubstituted 1-cyclohexene ring (Fig. 2). An isolated methyl signal at δ_H 1.160 was reasonably assigned to 10-CH_3 situated on a hydroxylated tertiary carbon (δ_C 69.8) as shown in the third substructure (**C**). The combination of the structures **A** and **B** through the hydroxylated tertiary carbon of substructure **C** suggested a bisabolane skeleton for the isolate in which all the 16 carbon atoms have been characterized.

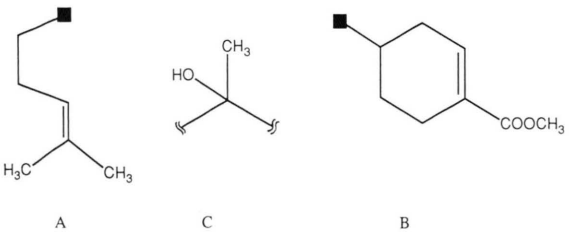


Fig. 2. The spectroscopically elucidated substructures for **1**.

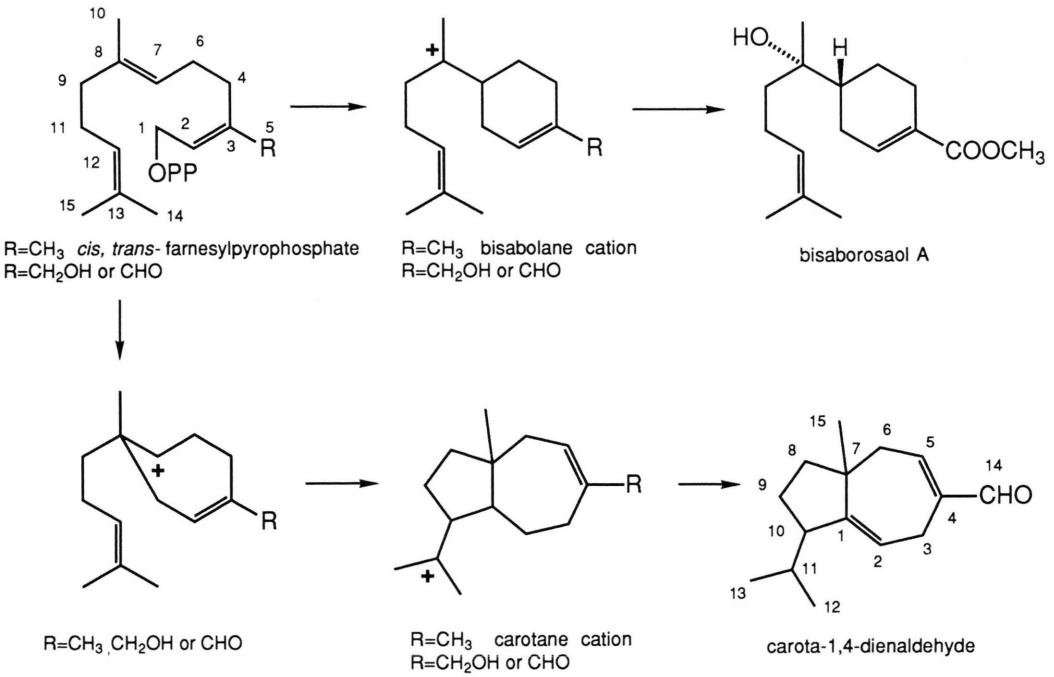
Bisabolane alcohols having the same substitution as that of **1** have been synthesized as an epimeric product by an acid-catalyzed prenylation reaction [12], in which the synthetic mixture (4*S*:8*RS*) showed $[\alpha]_D$ −68.0°. While about the same $[\alpha]_D$ values in 4*S*:8*R*- and 4*S*:8*S*-7-keto bisabolols (−71.3° and −68.2°, respectively) were observed [13], this suggested that C-4 chiral carbon contributes mostly to the optical rotation in this type of bisabolane alcohols, and both 4*S*:8*R*- and 4*R*:8*S*-7-methoxycarbonyl derivative may give about −70° in $[\alpha]_D$. Because compound **1** gave $[\alpha]_D$ +78° (dextrorotatory), the configuration of **1** at C-4 was expected to be inverse to that of the synthetic diastereo mixtures, and as described later, the configuration of **1** at C-4 was shown to be *R*. All the

8-hydroxybisabolanoids of Compositae origin whose configuration has been established so far possess the *S* configuration at C-4, and that with *R* at C-4 is quite unique. Although the naturally occurring 7-methoxycarbonyl-4-hydro-8-hydroxy-α-bisabolene was recently isolated from a Compositae plant without optical reference [9], the properties of its related compounds suggest that C-4 configuration of the bisabolanoid is *S* [9, 13]. Among naturally occurring bisabolanoids, C-7 oxygenated derivatives are rather rare [6–9, 14–16], and the isolation of bisabolanoid is the first example from Rosaceae.

Stereostructure of **1** was unambiguously confirmed by chemical correlation. By $LiAlH_4$ reduction, **1** was converted into an alcohol derivative **1a** in a good yield. The monoacetate of **1a** (**1b**) showed $[\alpha]_D$ +33° whose positive sign was opposite to that of the corresponding derivative from 7-hydroxy-α-bisabolol of Compositae origin [6]. In addition, **1c** obtained by MnO_2 oxidation of **1a** also showed dextrorotatory, proving C-4-*R* in **1** [13]. The stereochemistry at C-8 was elucidated by chemical conversion of **1** into a stereochemically known derivative. Compound **1b** was hydrogenated to **1d** (*cis* and *trans* mixture) to compare with the hydrogenation product from (−)-α-bisabolol (**3**, 4*S*:8*S*) [17]. Each mixture from **1b** and **3** exhibiting about the same *cis*/*trans* ratio (*ca.* 1:2) showed a good accordance in their spectroscopic properties. Both product mixtures were dextrorotatory in a short-wave range (250–350 nm in ORD). Thus, the C-8 absolute configuration of **1** was elucidated to be *S*.

In addition to **1**, the corresponding free acid, hamanasic acid A (**2**) was also found in *R. rugosa* leaf extractives as a major constituent. This acid was identical with the hydrolysis product of **1** in the EI-MS and ^1H NMR spectra. In addition, the optical rotation of methylation product of **2** was agreeable with that of **1.**

These bisabolanoids suggested a biogenetic correlation with carotanoids of *R. rugosa* origin which were the major antifungal sesquiterpenes of *R. rugosa* leaves [1, 2]. Because of the same *trans*, *cis*-farnesylpyrophosphate pathway for bisabola-



Scheme 1. Hypothetical biosynthetic relation between bisabolanoids and carotanoids of *R. rugosa*. The oxygenated methyl groups of the bisabolanoid and the carotanoid probably originated from the C-5 methyl group of their common precursor.

Table I. ^{1}H NMR* assignments of compounds **1** and **2.**

Compound Proton	**1**	**2**
2-H	7.005 br. m	7.145 br. m
3-Ha	2.337 m-divided d** (18.6)	2.377 m-divided d (18.8)
3-Hb	2.087 m-divided d (18.6)	*ca.* 2.12 m
4-H	1.610 m	*ca.* 1.62 m
5-Ha	1.907 m	1.916 m-divided d (11.3)
5-Hb	1.249 dddd (12.7, 12.2, 12.2, 5.4)	1.251 dddd (12.4, 12.3, 12.3, 5.4)
6-Ha	2.525 m-divided d (17.6)	2.520 br. d (17.5)
6-Hb	2.168 t-like m*** (17.6)	2.165 t-like m
9-H_2	1.536 t (8.3)	1.542 t (8.2)
10-H_3	1.160 s	1.167 s
11-Ha	2.090 ddd (14.6, 8.3, 7.3)	2.063 (2H) m
11-Hb	2.029 ddd (14.6, 8.3, 7.3)	
12-H	5.135 m-divided td (7.3, 1.0)	5.133 br. t (7.0)
13-H_3	1.692 d (1.0)	1.692 br. s
14-H_3	1.626 br. s	1.626 br. s
7′-OCH_3	3.729 s	

* ^{1}H NMR spectra were determined at 500 MHz in $CDCl_3$ (TMS reference); *J* are in Hz.
** Multiple-divided doublet.
*** Triplet like multiplet.

noids and carotanoids [4], the presence of **1** and **2** in *R. rugosa* leaves may be reasonable. It is more likely that the characteristic oxygenation at C-7 in the bisabolanoids (**1, 2**) indicates their biogenetic correlation, because their oxygenated carbons (C-7 of the bisabolanoids and C-14 of the carotanoids, respectively) both arise from the same allylic methyl carbon of their common precursor (Scheme 1). However, it is still a matter of conjecture whether the biosynthetic precursor undergoes oxygenation at C-7 position prior to cyclizations.

Experimental

General

^{1}H and ^{13}C NMR were recorded by JEOL JX-270, JEOL JX-500 and Bruker AM-500 instruments with TMS as internal standard. IR and UV spectra were taken in KBr disc and MeOH solution, respectively. Optical rotations were measured by Hitachi Model 047-2 for solutions in acetone, MeOH or EtOH at 23 °C. Merck silica gel 60 F_{254} precoated on a glass plate or an aluminum sheet was used for analytical or preparative TLC. R_f values referred to spots quenching under $UV_{254\,nm}$ light or giving coloration with vanillin–H_2SO_4 spray reagent.

Table II. ^{13}C NMR* assignments of compounds **1** and **2.**

Compound Carbon	**1**	**2**
1-C	132.0	129.5
2-CH	139.8	142.3
3-CH_2	26.8	27.0
4-CH	42.5	42.3
5-CH_2	23.3	23.2
6-CH_2	25.7	24.8
7-C	167.9	172.4
8-C	74.1	74.2
9-CH_2	39.5	39.4
10-CH_3	23.8	23.8
1-CH_2	22.3	22.2
12-CH	124.3	124.2
13-C	129.9	132.1
14-CH_3	25.2	25.7
15-CH_3	17.7	17.7
7′-OCH_3	51.5	–

* ^{13}C NMR spectra (COM, DEPT and CH-COSY) were determined at 125 MHz in $CDCl_3$ (TMS reference).

Materials and isolation of the constituents

The fresh leaves of *R. rugosa* were collected at Ishikari near Sapporo, during June–July in 1986–1987. After 2.0 kg of the leaves were soaked in 3 mM of $CuCl_2$ solution (25 l) for 19 h and successively in the same volume of tap water for another 24 h, the water layers were extracted with EtOAc

(1000 ml water/1200 ml), and the organic layer was dried over Na_2SO_4. As the concentrated extractives (2.1 g and 1.5 g, respectively) showed almost indistinguishable patterns on TL chromatograms, both concentrates were subjected to column chromatography on Wako gel settled in hexane (gel volume, 190 ml). The column was eluted with Et_2O/hexane mixtures as follows: Fr-1, Fr-2, and Fr-3; eluted with 10% Et_2O/hexane (100, 200 and 200 ml, respectively), Fr-4; 20% Et_2O/hexane (200 ml), Fr-5 and Fr-6; 35% EtOAc/hexane (150 ml each), Fr-7 and Fr-8; 50% Et_2O/hexane (150 ml each). The fraction Fr-8 contained bisaborosaol A (**1**). Other 4.0 kg of leaves were tightly soaked in 30 l of 3 mM $CuCl_2$ solution for 19 h and successively in 30 l of tap water for 24 h. Total 60 l of water layers were also extracted with EtOAc to obtain 9.5 g of a dark syrup, and the extracts were fractionated over silica gel column (volume 500 ml) with the same procedure.

The fraction containing **1** from the second extracts was combined with the former one (total 1.4 g) to rechromatograph on SiO_2 gel column (volume 150 ml) and successively eluted as follows: FFr-1; eluted with 25% EtOAc/hexane (100 ml), FFr-2; 25% EtOAc/hexane (50 ml), FFr-3; 25% EtOAc/hexane (50 ml). Compound **1** was contained in FFr-2, appearing as a single spot on TLC. Compound **1** (*ca.* 50 mg of a colorless syrup) was finally obtained by PTLC in hexane–acetone 4:1 (R_f 0.28). FFr-3 also contained marked amount of **1** together with more polar compounds. Consequently, *ca.* 200 mg of **1** was totally obtained.

Hamanasic acid A (**2**) was isolated from the water diffusates of damaged leaves (11 kg). The acidic constituents soluble in EtOAc (21.6 g) were obtained in the usual way. The acidic extracts were successively fractionated on SiO_2 gel column (gel volume 1500 ml) as follows: Fr-A-1; eluted with 40% EtOAc/hexane (1500 ml), Fr-A-2, Fr-A-3 and Fr-A-4; 60% EtOAc/hexane (500 ml each), Fr-A-5; EtOAc alone (500 ml). Fr-A-2 and Fr-A-3 mainly contained **2.** From a part of Fr-A-3 (*ca.* 1/3), the focused acid showing coloration into a reddish pink with vanillin–H_2SO_4 reagent was isolated by PTLC developed in hexane–EtOAc–HCOOH 25:25:1 (R_f 0.44) to give *ca.* 50 mg of a colorless syrup. This acid was also present in non-damaged *R. rugosa* leaves.

Physicochemical properties of the isolated compounds

*Bisaborosaol A (**1**).* Vanillin–H_2SO_4 color: reddish purple. $[\alpha]_D$ +78° (*c* = 0.06, acetone). UV λ_{max} (MeOH): 218 nm (ε 17,800). FI-MS (rel. int.): *m/z* 266 (100%). EI-HR-MS: $C_{16}H_{26}O_3$ (found 266.184, calcd. 266.188). EI-MS (rel. int.): *m/z* 266 (M^+, 1.1%), 248 (M^+-H_2O, 23), 233 ($M^+-H_2O-CH_3$, 4.4), 217 ($M^+-H_2O-CH_3O$, 5.2), 216 ($M^+-H_2O-CH_3OH$, 6.3), 205 (17), 192 (6.9), 189 ($M^+-H_2O-COOCH_3$, 16), 188 (8.0), 178 (6.2), 164 (15), 163 (48), 151 (25), 140 (57), 139 (11), 137 (22), 110 (11), 109 (100), 108 (12), 107 (12), 105 (17), 93 (18), 91 (12), 83 (10), 82 (29), 81 (19), 80 (13), 79 (29), 77 (13), 71 (17), 69 (87), 67 (26), 59 (10), 57 (10), 55 (27), 53 (13), 45 (28), 43 (97), 41 (72). IR KBr (cm^{-1}): 3450, 2920, 1710, 1440, 1260, 1090. ^1H and ^{13}C NMR data are shown in Tables I and II, respectively.

*Hamanasic acid A (**2**).* A colorless syrup. Vanillin–H_2SO_4 test; reddish pink. FI-MS (rel. int.): *m/z* 252 (100%). EI-MS (rel. int.): *m/z* 234 (M^+, 4.5%), 219 (1.3), 191 (5.6), 189 (2.7), 178 (2.0), 177 (2.6), 164 (2.6), 151 (6.4), 150 (5.3), 149 (11), 126 (7.8), 123 (9.0), 110 (10), 109 (100), 105 (8.5), 93 (7.9), 82 (22), 81 (12), 79 (17), 71 (13), 69 (80), 67 (19), 55 (14), 45 (20), 43 (77), 41 (51). ^1H and ^{13}C NMR data are shown in Tables I and II, respectively.

Chemical conversions of the isolated compounds

*Hydrolysis of **1***

To 19.8 mg of **1** dissolved in EtOH (2 ml) was added 1 M NaOH solution (2 ml), and the mixture was left overnight at room temperature. The reaction mixture was diluted with saturated NaCl solution (20 ml) and then extracted with EtOAc (15 ml). The main product detected at R_f 0.35 in hexane–EtOAc–HCOOH 30:10:0.5 was isolated by PTLC to give 14.5 mg of a colorless syrup (77%). The hydrolysis product showed a good accordance with **2** in comparisons of R_f values, EI-MS and ^1H NMR spectra.

*Reduction of **1** with $LiAlH_4$*

To **1** (24.4 mg) dissolved in $CHCl_3$ (1.5 ml) was added excess amounts of $LiAlH_4$ (*ca.* 200 mg), and the reaction mixture was stirred overnight at room

temperature. EtOAc (1 ml) was added to the reaction mixture and cooled in an ice bath for 3 h. The resulting mixture was diluted with EtOAc (15 ml) and washed directly with saturated NaCl solution containing HCl (0.2 M). The organic layer was dried over Na_2SO_4 and successively chromatographed on TLC plates developed in *n*-hexane–EtOAc 1:1. A non-quenching product (**1a**) at R_f 0.32 and showing pinkish blue with vanillin–H_2SO_4 reagent was isolated as a colorless syrup (13.1 mg, 60%).

The reduction product ***1a.*** UV λ_{max} (MeOH): featureless above 210 nm. FI-MS (rel. int): 238 (M^+, 100%). EI-MS (rel. int.): *m/z* 220 (M^+-H_2O, 4.1%), 202 (M^+-2H_2O, 3.2), 189 (3.4), 187 (2.2), 177 (2.6), 159 (4.0), 135 (24), 133 (10), 119 (8.1), 109 (66), 107 (14), 105 (16), 95 (13), 94 (24), 93 (40), 91 (18), 82 (20), 81 (12), 79 (39), 71 (18), 69 (84), 67 (21), 55 (21), 44 (79), 43 (100), 41 (55). ^{1}H NMR δ ($CDCl_3$, 500 MHz): *ca.* 5.71 (1H, br., C-2–H), 5.137 (1H, br. t, *J* = 7.1 Hz, C-11–H), 4.026 (1H, d, *J* = 11.1 Hz, C-7–Ha), 3.994 (1H, d, *J* = 11.1 Hz, C-7–Hb), *ca.* 2.15 (2H, m × 2, C-3–Ha and C-6–Ha), *ca.* 2.07 (1H, overlapped, C-3–Hb), 2.062 (2H, m, C-10–H_2), 1.937 (1H, m, C-6–Hb), 1.881 (1H, m, C-3–Hb), 1.693 (3H, br. d, *J* = 0.7 Hz, C-14–H_3), 1.629 (3H, br. s, C-13–H_3), 1.612 (1H, m, C-4–H), 1.527 (2H, t, *J* = 8.1 Hz, C-9–H_2), 1.283 (1H, dddd, *J* = 12.2, 12.2, 12.1 and 5.5 Hz, C-5–Hb), 1.154 (3H, s, C-15–H_3). ^{13}C NMR δ($CDCl_3$, 125 MHz): 137.5 (C, C-1), 132.1 (C, C-13), 124.6 (CH, C-12), 122.9 (CH, C-2), 74.5 (C, C-8), 67.4 (CH_2, C-7), 43.6 (CH, C-4), 39.6 (CH_2, C-9), 26.8 (CH_2, C-3), 26.0 (CH_3, C-10), 25.9 (CH_2, C-6), 24.2 (CH_3, C-14), 23.8 (CH_2, C-5), 22.5 (CH_2, C-11), 17.9 (CH_3, C-15).

Acetylation of ***1a***

The diol **1a** (8.2 mg) was treated with acetic anhydride/pyridine (1 ml, 1:1, v/v) at 80 °C for 1.5 h. To the reaction mixture was added toluene to remove the solvent under reduced pressure. The main product **1b** was obtained as a colorless syrup by PTLC (hexane–EtOAc 3:1, R_f = 0.43, 7.0 mg, 73%).

Monoacetate ***1b.*** Vanillin–H_2SO_4 color: pinkish blue. $[\alpha]_D$ +33° (*c* = 0.1, EtOH). UV λ_{max} (MeOH): featureless above 210 nm. EI-MS (rel. int.): *m/z* 280 (M^+, 1.1%), 248 (M^+-H_2O, 23), 233 ($M^+-H_2O-CH_3$, 4.4), 217 ($M^+-H_2O-CH_3O$, 5.2), 216 ($M^+-H_2O-CH_3OH$, 6.3), 189 ($M^+-H_2O-COOCH_3$, 16), 164 (15), 163 (48). ^{1}H NMR δ($CDCl_3$, 500 MHz): *ca.* 7.27 (1H, br., C-2–H), 5.132 (1H, br. t, *J* = 6.9 Hz, C-11–H), 4.456 (2H, s, C-7–H_2), 2.158 (1H, br. d, *J* = 19.1 Hz, C-6–Ha), 2.073 (3H, s, C-7′ –OCOC$\underline{H}_3$), *ca.* 2.08 (1H, overlapped, C-3–Ha), *ca.* 2.06 (1H × 2, C-2–Hb and C-6–Hb), 1.945 (1H, m, C-5–Ha), 1.868 (1H, br. d, *J* = 12.4 Hz, C-4–H), 1.691 (3H, br. s, C-13–H_3), 1.627 (3H, br. s, C-14–H_3), 1.519 (2H, t, *J* = 8.3 Hz, C-9–H_2), 1.294 (3H, s, C-15–H_3), 1.148 (1H, dddd, *J* = 12.5, 12.4, 12.4 and 5.7 Hz, C-5–Hb). ^{13}C NMR δ($CDCl_3$, 125 MHz, DEPT): 171.0 (–O$\underline{C}$OCH$_3$, C-7′), 132.7 (=C$\swarrow$, C-1), 131.9 (=C$\swarrow$, C-12), 126.2 (=CH, C-2), 124.4 (=$\dot{C}$H, C-11), 74.2 (–$\dot{C}$–O, C-8), 68.4 (–CH_2–O, C-7), 43.1 (CH, C-4), 39.4 (CH_2, C-9), 26.9 (CH_2, C-10), 25.9 (CH_2, C-6), 25.7 (CH_3, C-13), 24.0 (CH_3, C-15), 23.5 (CH_2, C-5), 22.3 (CH_2, C-3), 21.0 (–OCO$\underline{C}$H$_3$, C-7′), 17.7 (CH_3, C-14).

Oxidation of ***1a***

To compound **1a** (4.5 mg) dissolved in 2 ml $CHCl_3$ was added *ca.* 50 mg of active MnO_2, and the mixture was stirred overnight at room temperature. The reaction mixture was then diluted with EtOAc (20 ml) and washed with water (15 ml). The dried and concentrated mixture was subjected to PTLC (R_f 0.17 in $CHCl_3$–MeOH 50:1) to yield 0.8 mg of a colorless syrup (**1c,** 18% yield) and unchanged **1a.**

The oxidation product **1c.** Vanillin–H_2SO_4 color: pinkish blue. $[\alpha]_D$ +77° (*c* = 0.1, MeOH). EI-MS (rel. int.): *m/z* 218 (M^+-H_2O, 3.4%), 203 ($M^+-H_2O-CH_3$, 2.0), 190 (M^+-CO, 3.0), 175 (5.3), 162 (4.5), 148 (5.0), 135 (7.8), 133 (7.6), 110 (28), 109 (100), 93 (32), 82 (24), 79 (21), 69 (93), 67 (23), 55 (21), 43 (80), 41 (66). ^{1}H NMR δ($CDCl_3$, 500 MHz): 9.442 (1H, s, C-7–H), 6.848 (1H, br. m, C-2–H), 5.143 (1H, br. t, *J* = 7.0 Hz, C-12–H), *ca.* 2.53 (1H, overlapped, C-6–Ha), 2.520 (1H, m-divided d, *J* = 20.0 Hz, C-3–Ha), 2.237 (1H, t-like m, C-6–Hb), 2.075 (2H, m, C-11–H_2), *ca.* 2.04 (1H, m, C-3–Hb), 1.938 (1H, d-like m, C-5–Ha), 1.698 (3H, s, C-14–H_3), *ca.* 1.68 (1H, overlapped, C-4–H), 1.633 (3H, s, C-15–H_3),

1.564 (2H, overlapped with H_2O, C-9−H_2), 1.218 (1H, dddd, J = 12.4, 12.4, 12.1 and 5.4 Hz, C-5−Hb), 1.182 (3H, s, C-10−H_3). ^{13}C NMR δ($CDCl_3$, 125 MHz): 193.9 (C-7), 151.3 (C-2), 141.3 (C-1), 134.5 (C-13), 124.2 (C-12), 74.1 (C-8), 43.1 (C-4), 39.6 (C-9), 27.5 (C-3), 25.7 (C-6), 23.8 (C-10), 22.7 (C-14), 22.3 (C-5), 22.2 (C-11), 17.7 (C-15).

Hydrogenation of ***1b***

The starting material **1b** (19.5 mg, in 2 ml of MeOH) was mixed with 6.3 mg of platinum black, and the mixture was bubbled with H_2 gas for 5 h at room temperature, at which point further platinum black (6 mg) was added and the reaction was continued for another 5 h. The concentrate was chromatographed (PTLC in hexane−EtOAc 6:1) to yield 8.9 mg of a major product (R_f 0.59, 57% yield) and 3.1 mg of a minor product (R_f 0.26, 16% yield).

Hydrogenation products. The minor product was identified to be a mixture of *cis*- and *trans*-1-acetoxymethyl-4-(1′-hydroxy-1′-methyl-5′-methylhexyl)cyclohexanes (1:2) (in NMR and FD-MS). A colorless syrup. Vanillin−H_2SO_4 color: pinkish blue. FI-MS (rel. int.): m/z 285 (M^++1, 11%), 284 (M^+, 15), 267 (25), 199 (71), 155 (15), 129 (100). ^1H NMR δ($CDCl_3$, 500 MHz): 4.093 (d, J = 7.8 Hz), 3.885 (d, J = 7.8 Hz), 2.062 (s), 2.054 (s), 1.160 (d, J = 7.3 Hz), 1.100 (s), 1.091 (s). On the other hand, the major product was confirmed to be a mixture of the desired derivatives (**1d**). A colorless syrup. Vanillin−H_2SO_4 color: pinkish blue. FI-MS (rel. int.): m/z 226 (M^+, 7.0%), 209 (4.6), 208 (M^+-H_2O, 3.6), 141 (27), 130 (14), 129 (100), 97 (11). ^1H NMR δ($CDCl_3$, 500 MHz): 1.097 and 0.950 (each d, J = 6.3 Hz and 6.1 Hz, respectively, C-7−H_3), 0.887, 0.885, 0.880, 0.873, 0.866. ^{13}C NMR δ($CDCl_3$, 125 MHz): 74.6, 74.5, 47.6, 46.9, 40.2, 40.1, 39.7 (2C), 35.5, 35.4, 32.8 (2C), 32.12, 32.07, 28.0, 27.4, 26.9, 26.7, 24.10, 24.09, 22.67, 22.65, 22.6 (2C), 21.4, 21.1 (2C), 20.7, 17.4 (2C). These geometric isomers were distinguishable from the chemical shift values for C-7−H_3.

Comparison of ***1d*** *with hydrogenation products from* ***3***

As with **1b,** 82.5 mg of (−)-α-bisabolol (**3,** purchased from Extrasynthese) was also hydrogenated. After 5 h stirring, the starting material disappeared and a major product was detected on TLC. By PTLC (hexane−EtOAc 6:1), 59.6 mg of the product was obtained in 71% yield. R_f and spectroscopic (FI-MS, ^1H and ^{13}C NMR) data of the product were completely identical to the hydrogenation product from **1b.** Since the relative ratio of *cis*/*trans* isomers from **3** showed a good accordance to that of **1d** (calculated from the height of C-7 methyl signals as the index), ORD spectrum of each mixture was measured. The two compounds showed dextrorotatory in the range of 250−350 nm [**1d:** +0.11° (c = 0.4) and the hydrogenation product from **3:** +0.11° (c = 2.0) at 300 nm], which indicated that the configuration at C-8 in **1** is *S* same as that of **3.**

Methylation of ***2***

To 4.6 mg of **2** dissolved in EtOAc was added excess CH_2N_2 in CH_2Cl_2 and the mixture was left overnight. The reaction mixture after removal of the solvent was chromatographed (PTLC in hexane−EtOAc 3:1). The main product (3.0 mg) corresponding to **1** in TLC was isolated as a colorless syrup. The methylation product was indistinguishable from **1** by EI-MS, and ^1H and ^{13}C NMR (in $CDCl_3$, 500 and 125 MHz, respectively) spectrometries. Its optical rotation $[\alpha]_D$ +68° in acetone (c = 0.1) revealed that **1** and **2** had the same configuration.

Acknowledgements

The authors are grateful to Mr. K. Watanabe, Mrs. Y. Misu and Mr. M. Ikura for FI-MS and NMR measurements, Mrs. Y. Sugiyama and Miss E. Matsumiya for EI-MS analyses and Dr. L. Lajide for reading the manuscript.

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