Highly Oxygenated Bisabolanoids in *Rosa rugosa* Leaves

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Rosa rugosa, Sesquiterpene, Bisabolanoid, Exoperoxide

Five novel bisabolane sesquiterpenes possessing a tetrahydrofuran ring or a hydroperoxy group were isolated from *Rosa rugosa* leaves and their structures elucidated by chemical and spectroscopic methods. These highly oxidized sesquiterpenes were structurally related to bisaborosaol A which is the major bisabolanoid of the plant.

Introduction

Sesquiterpenes have rarely been found as constituents of Rosaceae plants [1]. However, *Rosa rugosa* Thunb. uniquely contains bisabolanoids and carotanoids in the leaves as its major components [2–4]. Bisaborosaol A (**6**), the predominant bisabolanoid of *R. rugosa* [4], has an unmodified isoprene unit on the side chain which is possibly oxidized to yield various metabolites as Flaskamp *et al.* (1981) have reported referring to α-bisabolol whose derivatives possessed an oxygenated side chain [5, 6]. A further survey of more polar sesquiterpenes revealed the presence of two tetrahydrofurano (**1** and **2**) and three exoperoxy (**3–5**) derivatives of bisaborosaol A (**6**) (Fig. 1). In this paper, we describe the isolation and structural elucidation of these new and highly oxygenated bisabolanoids from *R. rugosa*. The biological implication of these compounds in the tissues is briefly discussed.

Results and Discussion

In a further survey of the constituents of leaf extractives, our interest was at first focused upon two substances **1** and **2** which gave a yellow pigment on thin layer plates with vanillin–H_2SO_4 test (R_f 0.38 and 0.30 in hexane–EtOAc 3:1, respectively). By column chromatography over SiO_2 gel [4], **1** and **2** were eluted with 25% EtOAc/hexane. With the guidance of the coloration with vanillin–H_2SO_4 reagent, each compound was isolated and further purified by PTLC successively in hexane–EtOAc 3:1 and $CHCl_3$–MeOH 50:2 to give a colorless syrup (**1;** 160 mg, and **2;** 120 mg from 6 kg of *R. rugosa* leaves). Both showed M^+ 282 in FI-MS and similar ^1H NMR spectra to that of **6,** suggesting that the isolates are diastereoisomers closely related to **6.** Two 3H singlets in **1** at δ_H 1.221 and 1.074 were assignable to C-13 and C-14 methyl groups, not on an olefinic but on an oxygenated aliphatic carbon. Furthermore, the detection of methine protons (δ_H 3.415 in **1** and 3.553 in **2**) attributable to the oxygenated C-12 suggested the structural modification from **6** into a cyclic ether on the side chain.

When **6** was treated with *m*-CPBA, two products identical with **1** and **2** were afforded in almost equivalent amount. The ^{13}C NMR spectra discounted the corresponding epoxy structures because of the chemical shift values of the oxygenated carbon at δ_C 86.4 (CH-12), 84.6 (C-8) and 70.1 (C-13), for example in **1,** whose values were too low to be assigned to epoxy carbons. Since a cyclization between epoxy and γ-hydroxyl groups to give a tetrahydrofuran ring is known to occur immediately on epoxidation of α-bisabolol [5], it was considered that a similar cyclization occurred on **6** in the reaction.

It is possible for **6** to undergo an oxidative conversion into two kinds of cyclic ethers as shown in Scheme 1, **1** and **2** may have two possible structures either as tetrahydrofuran or dihydropyran derivatives. On the basis of FI-MS fragments *m/z* 59 and *m/z* 223 (M^+-59) (**1,** 100 and 50%; **2,** 69 and 59%, respectively) due to a 1-hydroxy-1-methylethyl side chain, a tetrahydrofuran part structure for **1** and **2** was suggested. To prove the proposed structure, **1** was dehydrated with $POCl_3$ to yield **1a** [7], whose exomethylene group was detected by the ^1H NMR [8]. Consequently, **1** and **2**

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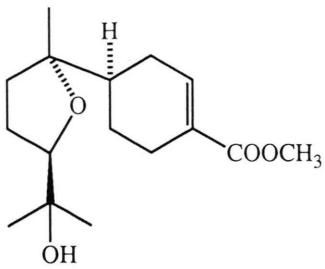
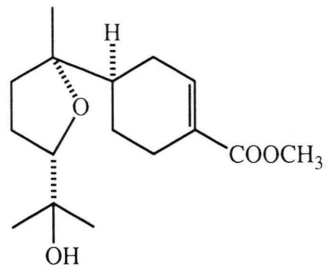


1: bisaborosarol B1



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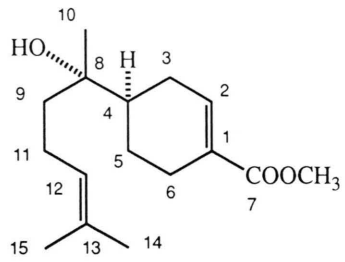
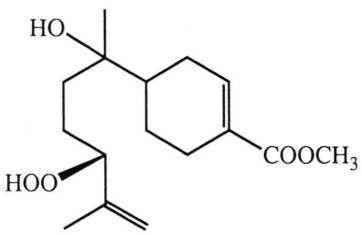
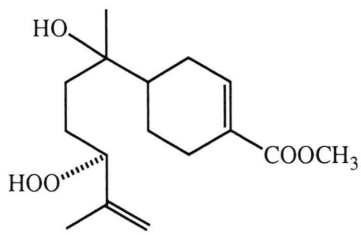


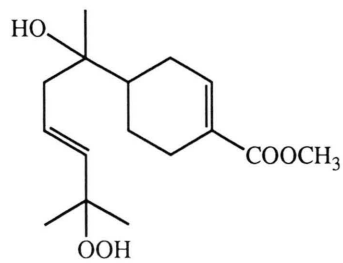
6: bisaborosaol A



3: bisaborosaol C1

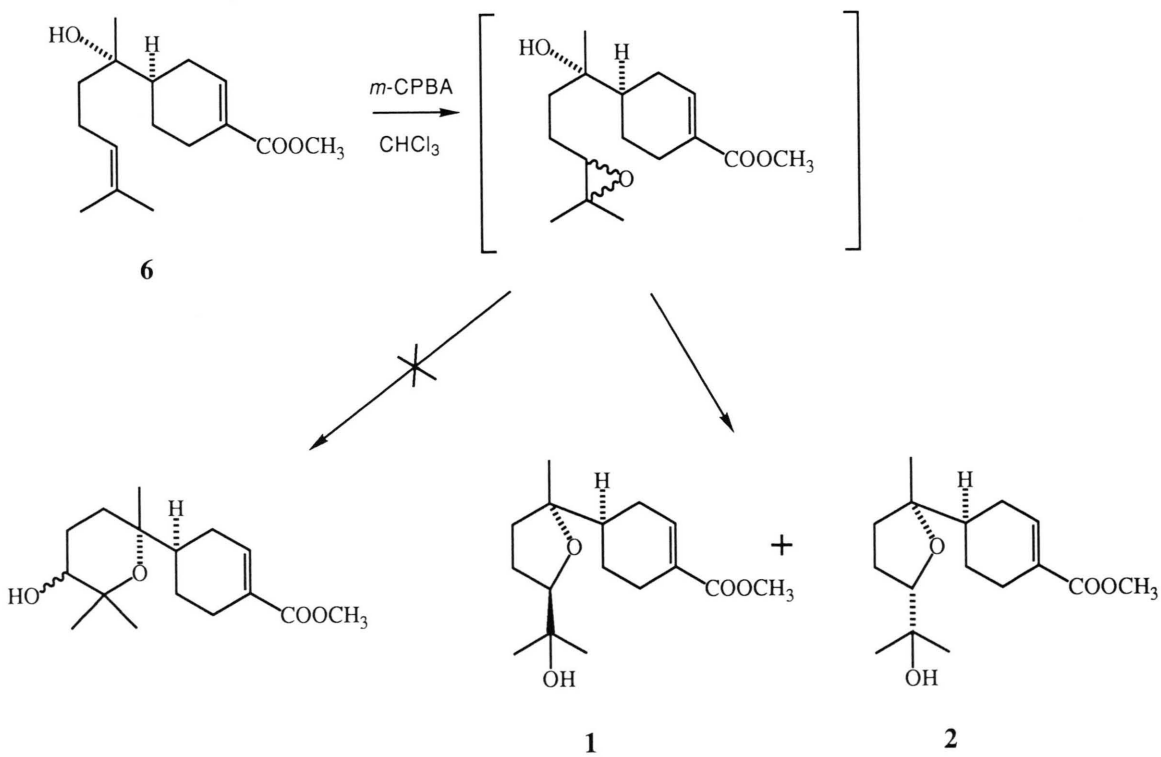


4: bisaborosaol C2



5: bisaborosaol D

Fig. 1. Highly oxygenated bisabolanoids of *R. rugosa* possibly derived from bisaborosaol A (**6**).



Scheme 1. Oxidation of bisaborosaol A (**6**) with *m*-CPBA.

were elucidated to be diastereoisomers having a tetrahydrofuran part structure.

NOE between the C-10 methyl protons and the C-12 methine proton was observed only in **2,** while **1** exhibited an NOE between C-10 methyl and C-13−OH protons. This result gave a conclusion that **1** and **2** were *trans* and *cis* isomers regarding the substitution on the tetrahydrofuran ring, respectively (Fig. 1). As the tetrahydrofuran derivatives obtained from **6** (4*R*:8*S*) were agreeable with those of the natural compounds in their optical rotation, stereostructures of **1** and **2** were solved as (4*R*:8*S*:12*R*) and (4*R*:8*S*:12*S*), respectively.

Jaensch *et al.* have reported a bisabolane derivative from *Podolepis rugata* (Compositae) having the same planar structure of **1** and **2.** Since their compound exhibited laevorotatory, its stereochemistry at the C-4 is probably inverse (4*S*), like other bisabolanoids of Compositae origin [9]. Because of the same origin, the bisabolanoid unmodified at the side chain probably possesses the *S* configuration at C-4 [4, 9].

The novel bisabolanoids thus established were named bisaborosaol B1 and B2, respectively. In fresh leaves of *R. rugosa,* **1** was found as a minor constituent together with a trace amount of **2.** It seemed likely that both compounds increase drastically when the leaves were damaged (wounding or $CuCl_2$ treatment).

On the other hand, with a guidance of the peroxide test [10], more compounds were pursued. By successive PTLC in hexane−EtOAc 1:1 and $CHCl_3$−MeOH 50:4, and multiple development PTLC in benzene−EtOAc 25:10, three compounds in the SiO_2 gel column fractions were isolated (**3, 4** and **5** with R_f 0.25, 0.23 and 0.17 in benzene−EtOAc 25:10, respectively). All isolates showed the same molecular weight (M^+ 298 in FI-MS), suggesting that these are isomers.

Compound **3** (2.1 mg) showed in FI-MS an intense fragment at *m/z* 265 (M^+−OOH, 81%) together with the parent ion (45%). Its ^1H NMR spectrum showed some signals similar to those of **6** [*e.g.* δ_H 7.012 (1H, br. s-like m) and 2.587 (1H, br. d), corresponding to C-2 and C-6 protons of **6,** respectively]. The characteristic proton signals of **3,** which include a pair of exomethylene protons (δ_H 4.967 and 4.892), an oxygenated methine proton (δ_H 4.219, t, J = 6.6 Hz), an exchangeable hydroperoxy proton (δ_H 7.550, s) [11, 12] and an allylic methyl proton (δ_H 1.646, br. s) were detected. The HH-COSY spectrum clearly revealed the presence of the 1,4-disubstituted cyclohexene part structure common to that of **6** and the side chain (■−C(OOH)H−CH_2−CH_2−■).

^{13}C NMR analyses of the compound eventually confirmed the structure of **3.** Two oxygenated sp^3 carbons at δ_C 89.5 (CH) and 73.1 (C) were assignable to the hydroperoxidated C-12 and the hydroxylated C-8, respectively. The comparatively lower chemical shift (δ_C 89.5) was reasonable for the hydroperoxidated C-12 carbon. In addition, carbons of the side chain were approximately agreeable in their chemical shift values with those of isoaminobisabolenol found in a sponge, *Theonella* sp. by Kitagawa *et al.* [13].

The second peroxide **4** (0.9 mg) showed almost the same mass fragmentation pattern as that of **3** in FI- and EI-MS. However, the proton chemical shifts assignable to C-9−H_2, C-11−H_2 and C-12−OOH were slightly but clearly different from those of **3.** As the ^{13}C NMR spectrum was almost indistinguishable from that of **3, 4** was easily characterized to be an epimer of **3** at C-12.

These novel bisabolane peroxides were named bisaborosaol C1 and bisaborosaol C2, respectively. Although the stereostructures of **3** and **4** remained unsolved, their absolute configurations at C-12 will be determined by the benzoate chirality method applicable to the compounds after reduction [14].

The third compound **5** (4.1 mg) showed a weak parent ion at *m/z* 298 in FI-MS together with the base peak at *m/z* 183. This compound indicated some signals characteristic of **6**-related bisabolanoids in the ^1H NMR and HH-COSY spectra. As the protons for the side chain, two olefinic protons were detected [δ_H 5.690 (1H, ddd, J = 14.8, 7.5 and 7.2 Hz, C-11) and 5.516 (1H, br. d, J = 14.8 Hz, C-12)] showing a clear *trans* coupling. Since the former proton was further coupled vicinally with C-9−H_2 by J = 7.5 and 7.7 Hz, partial structure ■−CH_2−CH=CH−■ became feasible. Two singlets methyl protons at δ_H 1.261 and 1.250 were reasonably assigned to C-14 and C-15 on the oxygenated C-13, respectively, and a hydroperoxy proton at δ_H 7.505 confirmed the side chain structure [■−CH_2−CH=CH−C(CH_3)$_2$−OOH] of the compound.

In the ^{13}C NMR spectrum, the carbon chemical shifts for the side chain of **5** showed a good correspondence with those of a bisabolane alkaloid, aminobisabolenol from the sponge [13]. The deshielded C-13 carbon of **5** (δ_C 81.5) reasonably indicated hydroperoxidation at that position. This novel bisabolane exoperoxide was named bisaborosaol D.

Bisaborosaol D (**5**) having a *trans* double bond is probably derived from **6,** as well as **3** and **4.** It has been proved that photoperoxidation of a 3,3-dimethylallyl compound gives three isomers, a *trans* olefinic and two exomethylene derivatives [15]. Formation of the bisabolane peroxides seems to be similar to the photoperoxidation reaction due to the fact that only *trans* form (**5**) was obtained. However, they are possibly not artifacts but metabolites of *R. rugosa* because the peroxides are found in the fresh leaves. These compounds may reflect a highly oxidative condition of some part of the leaf tissues.

Antifungal activities of **1, 2** and **6** were examined by TLC-bioautography using *Cladosporium herbarum* as a test fungus. Although those compounds showed a relatively weak antifungal activity (complete inhibition of the fungal growth at an administered ratio of 50 μg/78 mm^2 SiO_2 gel thin-layer TLC plate and slight retardation at 12.5 μg), it is hard to regard those as significant defense compounds of *R. rugosa* leaves since total antifungal activity of the bisabolanoids is *ca.* 10 times lower than that of rugosal A found as the major sesquiterpene of the plant [2]. Compounds **2** and **3,** exhibiting some increase in their concentration in the wounded tissues, are probably meaningless as far as the defensive mechanism concerned; however, it should be noted that the wounded tissues are under more oxidative conditions than the intact ones.

Experimental

General

^1H and ^{13}C NMR were recorded by JEOL JX-270 and a Bruker AM-500, and coupling constants were given in Hz. UV spectra and optical rotations were measured by Hitachi Model ESP-3T and JASCO Model 285, respectively, for MeOH solutions. Merck silica gel 60 F_{254nm} precoated on glass or aluminum sheet was used for analytical or preparative TLC (PTLC). R_f values referred to spots quenching under UV_{254} light or giving coloration with vanillin–H_2SO_4 spray reagent. *N,N*-Dimethyl-*p*-phenylenediamine sulfate reagent was used for peroxide detection [9].

Materials and isolation of the constituents

The leaf extracts were prepared and column-chromatographed as previously described [4]. Compounds **1** and **2** contained in the fractions FFr-3 and FFr-4 were isolated by PTLC. On the other hand, fresh leaves (uninjured, 6.0 kg collected in early July) were directly extracted with 95% MeOH (*ca.* 50 l). The methanol extract was once defatted with *n*-hexane and then the solvent was removed to obtain *ca.* 1 l of a water suspension. The suspension saturated with NaCl was mixed with 1.5 l of acetone, and kept stirring overnight. The partitioned acetone layer was concentrated and the resulting extracts were diluted into 1.5 l of EtOAc. The EtOAc solution was once washed with an equivalent volume of saturated NaCl solution, and then the solvent removed. The residue was again dissolved in 800 ml of benzene. The solution successively washed with 5% $NaHCO_3$ (700 ml) was dried over Na_2SO_4 and concentrated. The concentrate (*ca.* 48 g of dark oil) was applied to silica gel column chromatography (Wako-gel C-200, 750 ml vol.) and eluted with 2 l of 20% EtOAc/hexane. The eluates (12.8 g) were re-chromatographed on 250 ml of silica gel column to wash with 30% Et_2O/hexane (900 ml), and finally eluted with 50% Et_2O/hexane to obtain Fr2-1, Fr2-2 and Fr2-3 (50% EtOAc/hexane). Fr2-2 and Fr2-3 were subjected to PTLC to isolate **3, 4** and **5.**

Physicochemical properties of isolated compounds

^1H and ^{13}C NMR data for isolated compounds (**1**–**5**) are shown in Tables I and II, respectively.

*Bisaborosaol B1 (**1**).* A colorless syrup. Vanillin–H_2SO_4 color: yellow. $[\alpha]_D$ +63° (*c* = 0.19, MeOH). UV λ_{max}(MeOH): 218 nm (ε 14,300). FI-MS (rel. int.): *m/z* 283 (M^++1, 81%), 282 (M^+, 42), 265 (21), 223 (50), 143 (82), 139 (34), 133 (22), 112 (18), 59 (100). EI-MS (rel. int.): *m/z* 267 (1.2%),

Table I. ^{1}H NMR* chemical shift values of the isolated compounds.

Compound / Proton	**1**	**2**	**3**	**4**	**5**
2-H	7.057 br. m	7.010 br. m	7.012 br. s	7.019 br. s	7.135 br. s
3-Ha	2.075 br. d (19.3)	2.106 br. d (19.8)	1.965 br. d (18.9)	1.949 br. d (19.0)	2.082 br. d (19.4)
3-Hb	1.682 m	1.753 m	1.711 m	1.717 m	1.800 m
4-H	*ca.* 1.43 m	*ca.* 1.40 m	1.221 m	1.222 m	1.396 m
5-Ha	1.559 m	1.553 m	*ca.* 1.54 m	1.907 m	1.639 br. d
5-Hb	0.930 dddd (12.5, 12.4 / 12.4, 5.1)	0.945 dddd (12.4, 12.4 / 12.3, 5.1)	0.900 dddd (12.6, 12.4 / 12.4, 5.2)	0.906 dddd (12.5, 12.5 / 12.4, 5.2)	0.958 dddd (12.4, 12.4 / 12.4, 5.1)
6-Ha	2.655 br. d (15.0)	2.588 br. d (17.8)	2.587 br. d (18.0)	2.590 br. d (17.7)	2.630 br. d (15.3)
6-Hb	2.192 m	2.138 m	2.112 m	2.123 m	2.182 m
9-Ha	1.403 dd (9.8, 8.6)	1.408 ddd (12.2, 9.7, 5.4)	1.390 ddd (13.8, 10.8, 6.4)	1.421 m	2.019 dd (13.7, 7.5)
9-Hb	1.249 dd (9.8, 9.0)	1.237 ddd (12.2, 8.6, 7.2)	1.278 ddd (13.8, 10.8, 5.0)	1.225 dd (12.5, 5.0)	1.937 dd (13.7, 7.2)
10-H_3	0.834 s	0.863 s	0.752 s	0.752 s	0.860 s
11-Ha	1.719 m	1.620 m	*ca.* 1.55 (2H) m	*ca.* 1.65 m	5.690 (1H) ddd (14.8, 7.5, 7.2)
11-Hb	*ca.* 1.39 m	1.488 dddd (17.7, 8.7 / 6.8, 5.4)		*ca.* 1.46 m	
12-H	3.405 dd (10.3, 5.2)	3.552 dd (8.0, 6.0)	4.219 t (6.6)	4.183 dd (6.9, 5.6)	5.516 dd (14.8, 0.8)
14-H_x	1.221 (3H) s	1.216 (3H) s	4.967 (1H) d (0.9) 4.892 (1H) dd (1.0, 0.9)	4.958 (1H) br. s 4.894 (1H) dd (1.5, 1.5)	1.261 (3H) s
15-H_3	1.074 s	1.070 s	1.646 s	1.648 s	1.250 s
7′-OCH_3	3.481 s	3.479 s	3.473 s	3.473 s	3.472 s
13-OH	1.975 br. s	1.788 s	–	–	–
12-OOH	–	–	7.550 s	7.432 s	–
13-OOH	–	–	–	–	7.505 s

* ^{1}H NMR spectra (NON and COSY) were determined at 500 MHz in C_6D_6 (TMS reference). *J* are in Hz.

Table II. ^{13}C NMR* assignments of the isolated compounds.

Compound / Carbon	**1**	**2**	**3**	**4**	**5**
C-1	130.4	130.4	130.4	130.4	130.4
C-2	139.5	139.5	139.5	139.4	139.6
C-3	26.4	26.2	26.8	26.8	26.9
C-4	43.3	43.5	42.9	42.7	42.9
C-5	24.1	24.5	23.4	23.5	23.5
C-6	25.7	25.5	25.7	25.7	25.7
C-7	167.3	167.3	167.3	167.3	167.4
C-8	84.6	84.5	73.1	73.1	73.3
C-9	35.8	35.8	35.3	35.4	42.8
C-10	27.9	27.5	23.6	23.6	23.8
C-11	27.7	27.7	25.2	25.1	126.2
C-12	86.4	84.7	89.5	89.5	138.4
C-13	70.1	70.9	144.3	144.4	81.5
C-14	24.8	25.1	113.8	113.8	24.7
C-15	23.4	21.7	17.6	17.6	24.4
C-7′	51.1	51.1	51.1	51.1	51.1

* ^{13}C NMR spectra (COM and DEPT) were determined at 125 MHz in C_6D_6 (TMS reference).

264 (M^+-H_2O, 1.5), 251 (0.9), 235 (5.4), 224 (3.7), 223 ($M^+-COOCH_3$, 4.5), 205 ($M^+-H_2O-COOCH_3$, 14), 192 (14), 191 (61), 181 (11), 173 (7.2), 163 (10), 145 (19), 143 (89), 137 (13), 125 (50), 119 (12), 107 (23), 105 (20), 93 (15), 91 (16), 85 (43), 83 (15), 81 (21), 79 (25), 77 (11), 71 (43), 69 (18), 67 (18), 59 (48), 57 (11), 55 (25), 53 (18), 43 (100), 41 (37).

*Bisaborosaol B2 (**2**).* A colorless syrup. Vanillin–H_2SO_4 color: yellow. $[\alpha]_D$ +68° (*c* = 0.2, MeOH). UV λ_{max}(MeOH): 218 nm (ε 12,200). FI-MS (rel. int.): *m/z* 283 (M^++1, 66%), 282 (M^+, 23), 265 (11), 223 (59), 143 (100), 139 (56), 59 (69). EI-MS (rel. int.): *m/z* 267 (1.8%), 264 (M^+-H_2O, 2.1), 251 (1.4), 235 (9.4), 233 (3.9), 224 (8.0), 223

($M^+-COOCH_3$, 8.9), 205 ($M^+-H_2O-COOCH_3$, 23), 192 (28), 191 (95), 181 (21), 147 (20), 145 (24), 143 (100), 125 (39), 107 (19), 105 (20), 93 (16), 91 (15), 85 (42), 81 (18), 79 (23), 71 (28), 59 (45), 55 (18), 53 (16), 43 (58), 41 (27).

*Bisaborosaol C1 (**3**).* A colorless syrup. Vanillin–H_2SO_4 color: pinkish red. FD-MS (rel. int.): *m/z* 299 (M^++1, 57%), 298 (M^+, 45), 281 (29), 265 (81), 183 (40), 159 (100), 141 (41), 139 (76). EI-MS (rel. int.): *m/z* 262 (4.2%), 246 (3.1), 230 (6.4), 202 (5.7), 192 (12), 178 (20), 177 (21), 176 (13), 145 (19), 139 (20), 137 (45), 125 (28), 119 (23), 107 (32), 105 (34), 93 (27), 91 (43), 79 (63), 77 (40), 70 (35), 69 (39), 55 (23), 43 (100), 41 (85).

*Bisaborosaol C2 (**4**).* A colorless syrup. Vanillin–H_2SO_4 color: pinkish red. FD-MS (rel. int.): *m/z* 299 (M^++1, 86%), 298 (M^+, 43), 281 (36), 265 (100), 183 (34), 159 (92), 141 (57), 139 (73). EI-MS (rel. int.): *m/z* 262 (6.1%), 260 (3.6), 246 (3.1), 230 (8.5), 202 (7.1), 192 (8.0), 178 (26), 177 (31), 176 (21), 163 (11), 148 (21), 145 (29), 137 (47), 125 (29), 119 (32), 107 (32), 105 (42), 93 (31), 91 (51), 79 (56), 77 (38), 70 (36), 69 (47), 55 (27), 43 (100), 41 (99).

*Bisaborosaol D (**5**).* A colorless syrup. Vanillin–H_2SO_4 color: pinkish red. FD-MS (rel. int.): *m/z* 299 (M^++1, 57%), 298 (M^+, 45), 281 (29), 265 (81), 183 (40), 159 (100), 141 (41), 139 (76). EI-MS (rel. int.): *m/z* 262 (4.2%), 246 (3.1), 230 (6.4), 202 (5.7), 192 (12), 178 (20), 177 (21), 176 (13), 145 (19), 139 (20), 137 (45), 125 (28), 119 (23), 107 (32), 105 (34), 93 (27), 91 (43), 79 (63), 77 (40), 70 (35), 69 (39), 55 (23), 43 (100), 41 (85).

Chemical conversion of isolated compounds

*Oxidation of **6** with m-CPBA*

Compound **6** (11.5 mg) [4] and *m*-CPBA (10.0 mg) were dissolved in ice-cold $CHCl_3$ (2 ml), and the mixture was stirred for 1 h. At which point, EtOAc (25 ml) was added to the reaction mixture, and then the mixture washed with 5% Na_2CO_3 solution (25 ml × 2). From the organic layer, two major products agreeable with **1** and **2** on TLC (R_f values and response to vanillin–H_2SO_4 reagent) were isolated by PTLC. The spectroscopic properties (EI-MS, ^1H and ^{13}C NMR and $[\alpha]_D$) of oxidation products **1** (5.2 mg, 43% yield, $[\alpha]_D$ +69°) and **2** (4.5 mg, 37% yield, $[\alpha]_D$ +65°) were completely identical with those isolated from *R. rugosa* leaves.

*Dehydration of **1***

To prove the 1-hydroxy-1-methylethyl-substituted part structure in **1** and **2,** compound **1** was dehydrated [7]. In pyridine (1 ml), **1** (12.7 mg) was dissolved, and then $POCl_3$ (60 µl) was added to the solution at 0 °C. The mixture was kept at −20 °C for 24 h and left at room temperature for 1 h. The mixture was then poured an ice water (5 ml) and extracted with Et_2O (3 ml). The product less polar than the starting material was isolated by PTLC in hexane–EtOAc 10:1 to give a colorless syrup (3.2 mg, 26%).

*The dehydration product **1a**.* R_f: 0.56 (hexane–EtOAc 10:1). EI-MS (rel. int.): *m/z* 264 (M^+, 1.2%), 249 (0.2), 233 (M^+-OCH_3, 2.2), 217 (0.2), 207 (0.6), 205 (0.7), 204 (0.7), 178 (3.1), 161 (2.8), 137 (7.7), 126 (10), 125 (100), 107 (28), 91 (11), 81 (11), 79 (23), 77 (13), 67 (15), 55 (10), 53 (13), 43 (90), 41 (22). ^1H NMR δ(C_6D_6, 500 MHz): 7.061 (1H, br. s-like m, C-2–H), 2.190 (1H, multiple-divided d, *J* = 19.8 Hz, C-3–Ha), 1.749 (1H, t-like m, C-3–Hb), 1.499 (1H, m, C-4–H), 1.614 (1H, m, C-5–Ha), 0.976 (1H, dddd, *J* = 12.3, 12.2, 12.1 and 5.3 Hz, C-5–Hb), 2.660 (1H, br. d, *J* = 17.0 Hz, C-6–Ha), 2.218 (1H, t-like m, C-6–Hb), 1.422 (1H, m, C-9–Ha), 1.294 (1H, ddd, *J* = 12.0, 7.8 and 2.6 Hz, C-9–Hb), 0.964 (3H, s, C-10–H_3), *ca.* 1.64 (1H, m, C-11–Ha), 1.538 (1H, m, C-11–Hb), 4.130 (1H, dd, *J* = 9.6 and 5.9 Hz, C-12–H), 5.169 (1H, br. s, C-14–Ha), 4.836 (1H, br. s, C-14–Hb), 1.668 (3H, s, C-15–H_3), 3.471 (3H, s, C-7′–H_3). ^{13}C NMR δ(C_6D_6, 125 MHz): 130.4 (C, C-1), 139.7 (CH, C-2), 27.9 (CH_2, C-3), 43.4 (CH, C-4), 24.2 (CH_2, C-5), 25.7 (CH_2, C-6), 167.4 (C, C-7), 84.8 (C, C-8), 35.8 (CH_2, C-9), 23.7 (CH_3, C-10), 31.6 (CH_2, C-11), 82.8 (CH, C-12), 146.6 (C, C-13), 109.9 (CH_2, C-14), 18.2 (CH_3, C-15), 51.5 (CH_3, C-7′).

TLC bioautography

On TLC plates Kieselgel 60 F_{254}, 0.25 mm thickness), 15 µl of bisaborosaol A (**6**) dissolved in acetone in several concentrations was charged to a test zone (78 mm^2). The plate dried in a dessicator *in vacuo* was sprayed with a spore suspension of *Cla-*

dosporium herbarum, and left under a moist condition at 25 °C for 3 days. As a reference, rugosal A was also tested, and this antifungal sesquiterpene showed a clear inhibition at 2 μg/78 mm^2. Bisaborosaols B 1 and B 2 also showed about the same activity as **6.**

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[1] J. S. Glasby, Encyclopedia of the Terpenoids, John Wiley & Sons, Chichester 1982.
[2] Y. Hashidoko, S. Tahara, and J. Mizutani, Phytochemistry **28,** 425 (1989).
[3] Y. Hashidoko, S. Tahara, and J. Mizutani, Phytochemistry **29,** 867 (1990).
[4] Y. Hashidoko, S. Tahara, and J. Mizutani, Z. Naturforsch. **46c,** 349 (1991).
[5] E. Flaskamp, G. Nonnenmacher, G. Zimmerman, and O. Isaac, Z. Naturforsch. **36b,** 1023 (1981).
[6] C. Zdero, F. Bohlmann, J. C. Solomon, R. M. King, and H. Robinson, Phytochemistry **28,** 531 (1989).
[7] J. Kawabata, Y. Fukushi, S. Tahara, and J. Mizutani, Agric. Biol. Chem. **48,** 713 (1984).
[8] S. Tahara, J. L. Ingham, S. Nakahara, J. Mizutani, and J. B. Harborne, Phytochemistry **23,** 1889 (1984).
[9] M. Jaensch, J. Jakupovic, R. M. King, and H. Robinson, Phytochemistry **28,** 3497 (1989).
[10] E. Knappe and D. Peteri, Z. Anal. Chem. **190,** 386 (1962).
[11] K. Koseki, F. Saito, N. Kawashima, and M. Noma, Agric. Biol. Chem. **50,** 1917 (1986).
[12] J. Jakupovic, T. V. Chau-Thi, U. Warning, F. Bohlmann, and H. Greger, Phytochemistry **25,** 1663 (1986).
[13] I. Kitagawa, N. Yoshioka, C. Kamba, M. Yoshikawa, and Y. Hamamoto, Chem. Pharm. Bull. **35,** 928 (1987).
[14] T. Kato, Y. Yamaguchi, T. Hirano, T. Yokoyama, T. Uyehara, T. Namai, S. Yamanaka, and N. Harada, Chemistry Lett. **1984,** 409.
[15] L. M. Stephenson, Tetrahedron Lett. **21,** 1005 (1980).