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ANTIMICROBIAL SESQUITERPENE FROM DAMAGED *ROSA RUGOSA* LEAVES

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Key Word Index—*Rosa rugosa*; Rosaceae; rugosa rose; antimicrobial sesquiterpene; carotanoid; endoperoxide.

Abstract—An antimicrobial sesquiterpene has been isolated from diffusates of damaged leaves of *Rosa rugosa*. Its structure, including relative stereochemistry, was deduced by chemical and spectroscopic (UV, IR, NMR and mass spectrometry) methods to be a novel carotane with an α,β-unsaturated aldehyde group, an *endo*-peroxide bridge and an allyl alcohol partial structure. The corresponding carboxylic acid was found in intact leaves of *R. rugosa*, but the compound is non-fungitoxic.

INTRODUCTION

The rugosa rose (*Rosa rugosa* Thunb.) is a wild rose species commonly distributed along the coastal area of north east Asia. It has been used in the breeding of some commercial varieties. In the course of an investigation of antimicrobial agents in higher plants, we found that an aqueous extract of damaged leaves of *R. rugosa* inhibited growth of microorganisms, and that an antimicrobial principle was readily extracted from the diffusates of damaged leaves. The isolated principle appeared to be a novel sesquiterpene which we named rugosal A (**1**). Chemical and spectroscopic determinations proved it to be an α,β-unsaturated aldehyde belonging to the carotane class of sesquiterpenes, which contains an allyl alcoholic hydroxyl group and an endoperoxide bridge. The compound exhibits antimicrobial properties and might function as a natural and biochemical defence system against infection. Interestingly, from the uninjured leaves we were able to isolate the corresponding carboxylic acid, rugosic acid A (**2**), which possesses no antifungal activity. In the present paper we describe the isolation, purification and chemical properties of these two sesquiterpenes and some of their derivatives.

RESULTS AND DISCUSSION

Initially, we observed that water washings from wounded leaves of rugosa rose prevented the growth of microorganisms, contrary to water in which non-wounded ones had been soaked. This observation suggested that injured leaf tissues release some antimicrobial substances. Accordingly 1.3 kg of *R. rugosa* fresh leaves were mechanically injured and steeped in tap water for 24 hr, after which time the water layer was collected and extracted with ethyl acetate to yield *ca* 3 g of diffusates. Antifungal substances in the diffusates were monitored by TLC bioautography using *Cladosporium herbarum* as test fungus [1]. The diffusate extract gave a distinct spot on silica gel TLC at R_f 0.43 after development in hexane–ethyl acetate (3:1) which showed a clear zone of growth inhibition. Neither the spot nor the inhibition zone was, however, detected in extracts of the diffusates from uninjured leaves. The antifungal principle isolated by silica gel column chromatography was successively purified by recrystallization (see Experimental) to give 119 mg of colourless needles, and was named rugosal A.

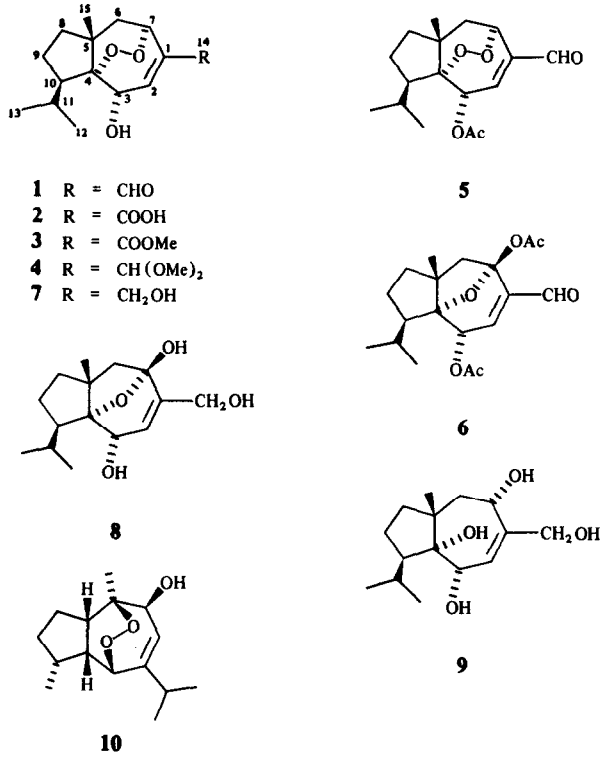

Fig. 1. Structures of sesquiterpene endoperoxides and related compounds.

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Table 1. NMR data for rugosal A (**1**), rugosic

	Rugosal A (**1**) in C_6D_6			Dimethyl acetal (**4**)		Monoacetate (**5**)	
	^{1}H NMR	J = Hz	^{13}C NMR	^{1}H NMR in C_6D_6	J = Hz	^{1}H NMR in C_6D_6	J = Hz
C-1			146.5 *s*				
C-2-H	6.031 *dd*	6.35 1.10	149.4 *d*	5.967 *dd*	6.23 1.10	6.491 *dd*	5.86 0.73
C-3-H	4.198 *dd*	11.72 6.35	69.1 *d*	4.319 *dd*	11.72 6.23	5.685 *d*	5.86
C-4			94.8 *s*				
C-5			39.6 s				
C-6-H_a	1.384 *dd*	13.92 2.57	42.0 *t*	1.775 *dd*	13.92 2.20	1.389 *dd*	14.29 2.57
C-6-H_b	1.942 *dd*	13.92 5.13		2.121 *dd*	13.92 5.13	2.010 *dd*	14.29 5.50
C-7-H	5.277 *ddd*	5.13 2.57 1.10	70.1 *d*	4.897 *ddd*	5.13 2.20 1.10	5.323 *ddd*	5.50 2.57 0.73
C-8-H_a	1.275 *m*		38.5 *t*	1.355 *m*		1.248 *m*	
C-8-H_b	1.630 *m*			1.816 *m*		1.659 *m*	
C-9-H_a	1.105 *m*		20.2 t	1.220 *m*		1.097 *m*	
C-9-H_b	1.295 *m*			1.412 *m*		1.271 *m*	
C-10-H	1.912 *ddd*	10.62 9.71 2.57	54.6 *d*	2.036 *ddd*	10.63 9.16 2.57	2.011 *m*	
C-11-H	2.739 *dsept*	6.84 2.57	24.8 *d*	2.890 *dsept*	6.80 2.57	2.023 *dsept*	6.59 2.57
C-12-H_3	0.732 *d*	6.84	18.4 *q*	0.787 *d*	6.59	0.795 *d*	6.59
C-13-H_3	0.845 *d*	6.84	22.8 *q*	0.883 *d*	6.96	0.814 *d*	6.59
C-14	9.046 *s*[a]		190.7 *d*	4.308 *s*[b]		9.003 *s*[a]	
C-15-H_3	0.393 *s*		25.9 *q*	0.762 *s*		0.454 *s*	
C-3-OH	2.758 *d*	11.72		2.836 *d*	11.72		
Others				3.042 *s*, OMe × 2	3.092 *s*	1.722 *s* C-3-OAc	

NMR δ values at 270 MHz for ^{1}H NMR and 68 MHz for ^{13}C NMR.
[a]aldehyde proton, [b]acetal proton, [c]carbinol methylene, [d]carboxyl proton.

Rugosal A (**1**) showed a $[M]^+$ at m/z 266.156 (EI-HR-mass spectrometry) indicative of the molecular formula $C_{15}H_{22}O_4$ (requires 266.152). The IR spectrum of **1** exhibited absorption bands assignable to an intramolecularly hydrogen-bonded hydroxyl group (ν_{max} 3450 cm^{-1}, KBr disc and ν_{max} 3563 cm^{-1}, 0.31 mM in CCl_4), and an α,β-unsaturated aldehyde group (ν_{max} KBr disc: 2820 and 2730 cm^{-1}; CHO and 1690 cm^{-1}; C=O). The UV spectrum λ_{max}^{MeOH} at 228 nm (ε = 7400) also supported the presence of α,β-unsaturated carbonyl partial structure. The EI-mass spectral fragments at m/z 248 $[M-H_2O]^+$ (3.1%), 237 $[M-CHO]^+$ (4.5%) and 219 $[M-H_2O-CHO]^+$ (2.8%) are presumed to have arisen by the loss of the aldehyde group and/or the hydroxyl group. Together with the molecular formula, the presence of other mass fragments at m/z 109, 97, 69 (base peak), 55 and 41 is suggestive of a sesquiterpene nature for **1**.

Analyses of ^{1}H–, ^{13}C–, ^{1}H–^{1}H correlated 2D (two dimensional)- and ^{1}H–^{13}C correlated 2D-NMR spectra of **1** in C_6D_6 (Table 1) defined three partial structures (substructures **A**, **B** and **C** in Fig. 2). The largest substructure **A** involves an α,β-unsaturated aldehyde moiety; the aldehyde proton was detected at δ9.046 (C-14-H) as a singlet. Therefore, C-1 (α carbon to C-14) was deduced to be a non-hydrogen-bearing olefinic carbon. The three sp^2 carbons detected at 190.7 ppm (CHO), 149.4 (CH) and 146.5 (C) were attributed to the α,β-unsaturated aldehyde partial structure (C-14, C-2 and C-1, respectively). The proton signal δ6.031 (1H, *dd*, J = 6.35, 1.10 Hz) was assigned to the olefinic C-2-H from the ^{1}H–^{13}C correlated 2D-NMR spectrum. Two allylic methine protons at C-3 (δ4.198 *dd*, J = 11.72, 6.35 Hz) and at C-7 (δ5.277 *ddd*, J = 5.13, 2.57, 1.10 Hz), both coupling with the C-2 proton (J = 6.35, 1.10 Hz), resonated at lower field, suggest in turn that they are oxygenated. On addition of D_2O, the proton resonating at δ2.758 (1H, *d*, J = 11.72) disappeared and the signal of the C-3 methine proton became a doublet (J = 6.35 Hz). This result indicates that the signal due to an exchangeable proton can be assigned to the hydroxyl on C-3 and that the coupling sequence on this side of the molecule is closed. The other allylic methine proton showing an allyl coupling with C-2-H (J = 1.10 Hz) further coupled with vicinal methylene protons (C-6-H_2, two *dd*) to yield double-double-doublets; thus, the sequence was also closed here. *cis*-Geometry at the carbon–carbon double bond was eventually deduced from NOE measurements (Fig. 4).

The ^{1}H–^{1}H correlated 2D-NMR spectrum showed

acid (**2**) and related compounds

Alcohol (**7**) ^{1}H NMR in C_6D_6		Rugosic acid (**2**) in $CDCl_3$		Methyl ester (**3**)		
^{1}H NMR	J = Hz	^{1}H NMR	J = Hz	^{1}H NMR	J = Hz	^{13}C NMR
						137.1 s
5.697 *m*; + D_2O *dd*	6.23 1.10	7.204 *dd*	6.59 0.90	7.075 *dd*	5.86 1.10	140.9 *d*
4.256 *dd* 11.72 6.23; + D_2O *d*	6.23	4.429 *d*	6.59	4.449 *dd*	9.52 5.86	68.5 *d*
						94.6 *s*
						39.3 s
1.504 *dd* 13.92 2.20		1.892 *dd*	14.28 2.57	1.882 *dd*	13.92 2.19	38.3 *t*
2.018 *dd* 13.92 5.13		2.234 *dd*	14.28 5.12	2.218 *dd*	13.92 4.76	
	5.13		5.12		4.76	
4.392 *m*; + D_2O *ddd*	2.20 1.10	5.219 *ddd*	2.57 0.90	5.253 *ddd*	2.19 1.10	72.4 *d*
1.387 *m*		1.602 *m*		1.616 *m*		41.6 *t*
1.789 *m*		*ca.* 1.75 *m*		1.742 *m*		
1.210 *m*		1.450 *m*		1.431 *m*		19.8 t
1.416 *m*		*ca.* 1.73 *m*		1.505 *m*		
2.060 *m*		1.862 *m*		1.874 *m*		53.9 *d*
2.886 *dsept* 6.80 2.20		2.613 *dsept*	6.80 2.20	2.608 *dsept*	6.80 2.20	25.4 *d*
0.896 *d* 6.96		0.928 *d*	6.60	0.945 *d*	6.60	18.0 *q*
0.793 *d* 6.60		0.972 *d*	6.97	0.968 *d*	6.96	24.5 *q*
3.569 *m* (2H); + D_2O s[c]		undetected[a]				165.4 *s*
0.678 *s*		0.909 *s*		0.902 *s*		22.8 *q*
2.836 *d* 11.72		undetected		2.698 *d*	9.52	
0.63 *br s*; + D_2O disappear				3.781 s		51.9 *q*
C-14-OH				OMe		OMe

another coupling sequence [–CH_2–CH_2–CH–CH(Me)$_2$] for the substructure **B** containing an isopropyl group. The observed $^1J_{C-H}$ 129.1 and 130.1 Hz for C-8 and C-9, respectively, suggest that substructure **B** does not possess a four-membered ring ($^1J_{C-H}$ for cyclobutane 136 ±1 Hz) but a five-membered ring ($^1J_{C-H}$ for cyclopentane 131±2 Hz) [2]. Including a bridge-head methyl group [substructure **C**: ^{13}C NMR, 25.9 ppm *q*; ^{1}H NMR δ0.393 in C_6D_6 or 0.883 in $CDCl_3$ (3H, *s*)], 13 carbons out of 15 in **1** are thus assigned. Accordingly, partial structures **A-C** must be connected to each other by the two non-hydrogen-bearing carbons detected at 94.8 ppm (**I**) and 39.6 ppm (**II**). The former ought to be linked to an oxygen because of its resonance at lower field, and the latter can be reasonably attributed to the quarternary carbon on which the bridge-head methyl group is located.

Two oxygen atoms out of the total of four in **1** have already been assigned to aldehyde and hydroxyl groups in substructure **A**. The remaining two, one linked to C-7 and the other to the non-hydrogen-bearing carbon (**I**) were presumed to form a peroxide bridge between these two carbons, since no other oxygenated carbon remains. The presence of an endoperoxide group was confirmed by the potassium iodide–starch test [3] and by EI-HR-mass spectrometric detection of a $[M-33]^+$ fragment [1.6%, $C_{15}H_{21}O_2$ (233.151 = **1** – HO_2)] due to fission of the endoperoxide linkage. The $[M-33]^+$ fragment is also detected in mass spectrum of hanalpinol (**10**) [4], a guaianoid containing an endoperoxide bridge, present in rhizomes of *Alpinia japonica* (Zingiberaceae).

After consideration of the above requirements, possible structures for rugosal A (**1**) were finally constructed to give **D-G** depicted in Fig. 3. The structures **D** and **E** were discounted for two reasons; firstly, the C-10 methine carbon resonating at a very low field (δ54.6) is presumably located adjacent to the oxygenated carbon (δ94.8, **II**), and secondly, both structures do not comply with the isoprene rule. A NOESY experiment exhibited the interactions as shown in Fig. 4 and the one between C-12-H_3 and C-3-H can only be obtained from structure **G**. Thus structure **1** including its relative configuration was proposed. An intramolecular hydrogen-bond between the alcoholic OH and one of the endoperoxide oxygen occurs in hanalpinol (**10**) [4] resembling **1** in the relative position and stereochemistry of the endoperoxide bridge and the allyl alcohol partial structure.

The structure **1** for rugosal A was confirmed by spectroscopic and chemical analyses of the corresponding

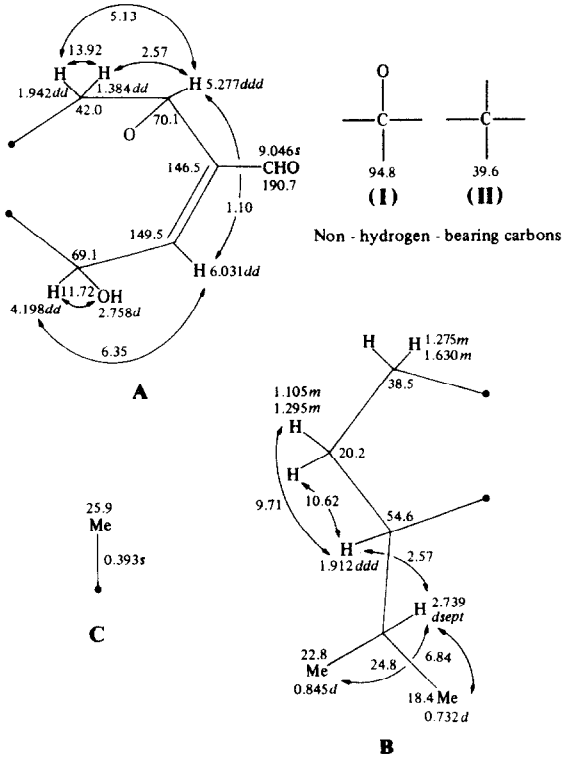

Fig. 2. Part structures for rugosal A inferred from ^{1}H-, ^{1}H-^{1}H-2D- and ^{1}H-^{13}C-2D-NMR analyses. Spectral data were recorded in C_6D_6 at 270 and 68 MHz for ^{1}H and ^{13}C, respectively, and coupling constants are shown in Hz.

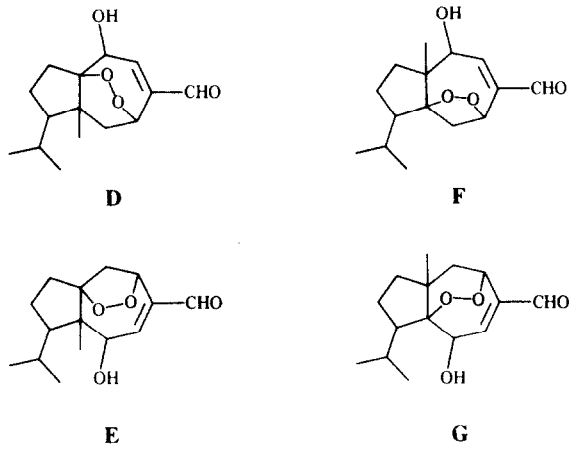

Fig. 3. Possible structures (D–G) for rugosal A.

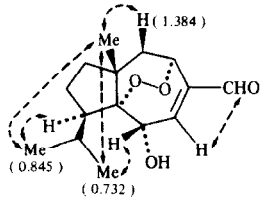

Fig. 4. Nuclear Overhauser effects observed by NOESY.

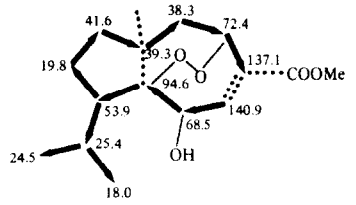

Fig. 5. Carbon sequence of rugosic acid A methyl ester confirmed by ^{13}C-^{13}C-2D-NMR (INADEQUATE). ➡: observed correlations and chemical shift values, -----: incomplete results, ——: carbon–oxygen or oxygen–oxygen bond.

carboxylic acid. The sesquiterpene acid **2** isolated from both intact and damaged leaves of *R. rugosa* (*ca* 100 mg/kg of fresh material) showed the molecular formula $C_{15}H_{22}O_5$ (EI-HR-mass spectrum). Together with the IR absorption at ν_{max}^{KBr} disc, 3560 and 3400–3000 cm^{-1} (*br*), the EIMS fragments at *m/z* 264 $[M-H_2O]^+$ (4.8%) and 220 $[M-H_2O-CO_2]^+$ (12%) are indicative of the presence of a hydroxyl and a carboxyl group. Although the carboxylic proton was undetectable in the ^{1}H NMR spectrum of **2**, the chemical shifts and coupling patterns for the remainder of the signals closely resembled those of **1** except for the formyl proton (Table 1). The acid **2** named rugosic acid A was converted into the methyl ester (**3**) and the ^{1}H and ^{13}C NMR signals were unambiguously assigned by ^{1}H–^{13}C correlated 2D-NMR analysis (Table 1). The carbon skeleton of **3** was proved by ^{13}C–^{13}C correlated 2D-NMR spectrometry, and the C–C sequence depicted in Fig. 5 became feasible. Thus, **3** was shown to be a carotane bridged by an endoperoxide group between C-4 and C-7.

The chemical relationship between **1** and **3** was successfully established. According to Corey's method [5], **1** was converted into the methyl carboxylate which was chromatographically (TLC) and spectroscopically (IR and $[\alpha]_D$) indistinguishable from **3**. Therefore, the two sesquiterpenes (the aldehyde **1** and the carboxylic acid **2**) of *R. rugosa* have the same carbon skeleton and absolute configuration. Consequently, rugosal A (**1**) and rugosic acid A (**2**) were established to be new naturally occurring carotane sequiterpenoids containing an endoperoxide group.

The structure of **1** was also supported by the additional chemical reactions described below. Treatment of **1** with a small amount of 0.5 M HCl in MeOH gave the dimethyl acetal (**4**) which is UV transparent above 210 nm and exhibits signals for protons in the dimethyl acetal part at δ3.042 and 3.092 (2 × OMe) and δ4.308 (–O–CH–O–), in addition to the $[M]^+$ at *m/z* 312 and an intense mass fragment at *m/z* 75 $[(MeO)_2CH]^+$. Acetylation of **1** with acetic anhydride/dry pyridine gave the monoacetate (**5**, C-3-OAc; δ1.722 in C_6D_6; $[M]^+$ at *m/z* 308) as the major product. The minor product was tentatively deduced to be **6**, in which the endoperoxide bridge was converted into an acetylated hemiacetal at C-7 (97.1 ppm, ^{13}C NMR). Reduction of **1** with sodium borohydride yielded the corresponding primary alcohol as the major product (**7**: $[M]^+$ *m/z* 268 and δ3.569 –CH_2OH) together with a minor one presumed to be **8**. Furthermore, treatment of **1** with lithium aluminium hydride gave a tetraol identified as **9** ($[M]^+$ 270); this compound was labile in potassium periodate solution.

Antifungal activities of **1** and related compounds were examined by silica gel TLC bioautography [6]. Rugosal A (**1**) caused a prominent growth inhibition of *C. herbarum* at concentrations of more than 3.75 μg/78 mm^2 and a clear growth retardation even at 0.9 μg/78 mm^2. The methyl ester of rugosic acid A showed activity comparable to that of **1**, whilst the free acid (**2**) showed only a slight inhibition at 100 μg/78 mm^2. Results of our preliminary examination suggested that the amount of **1** released from crushed leaves of *R. rugosa* is very high at flowering. However, at budding or at fruiting stages little **1** was released from damaged leaves. Treatment of intact leaves with a copper chloride solution [7] or water did not cause the diffusion of **1** even though the material was collected at the flowering stage. We suggest that this sesquiterpene has a specific and significant role as a protective agent against microbial invasion if the tissue is wounded.

Plant species containing carotane sesquiterpenoids reported so far are restricted to those of Umbelliferae [8–17] with one exception from the Compositae [18]. We have now found that *R. rugosa* (Rosaceae) is a new and abundant source of carotane sesquiterpenoids.

EXPERIMENTAL

General. Mps uncorr. ^{1}H NMR were recorded at 270 and 500 MHz, ^{13}C NMR at 67.5 MHz using TMS as int. std. Coupling constants are given in Hz. UV and IR spectra were recorded for solns in MeOH or for KBr discs. Optical rotations were determined for solns in Me_2CO or MeOH.

Merck silica gel 60 F_{254} precoated on glass or aluminium was used for analytical or prep. TLC; R_f values refer to spots which quench under $UV_{254\,nm}$ light or give colour reactions with vanillin–H_2SO_4 or phosphomolybdic acid spray reagents.

Extraction and isolation of constituents. Young, fresh leaves of *R. rugosa* Thumb. (1.3 kg collected in late June in 1986 at Ishikari near Sapporo) were bruised by gently hitting them with a wooden hammer. They were then covered with H_2O (7 l), allowed to stand for 24 hr at 25° and the H_2O decanted off and filtered by suction. The filtrate was extd once with EtOAc (2 l) and the extract reduced *in vacuo* to 300 ml followed by washing once with 5% $NaHCO_3$ soln (500 ml). The neutral constituents (*ca* 0.8 g) in the EtOAc layer and the acidic constituents (*ca* 2.2 g) recovered from the aq. $NaHCO_3$ layer by EtOAc extn were further fractionated by silica gel CC after being acidified to pH 3.5.

The neutral fraction (0.8 g) was loaded onto a Wako-gel C-200 (200 ml) column packed in hexane and subsequently eluted with EtOAc–hexane (1:1) to give Fr-1 (100 ml), and Fr-2–5 (40 ml each). Fr-4 was evapd *in vacuo* to give the major constituent rugosal A (**1**, R_f 0.49 in hexane–EtOAc, 3:1) as crude crystals. Recrystallization from EtOAc–hexane yielded 74 mg of pure **1** as colourless needles, and a further amount of **1** (45 mg) was recovered from Fr-3 and Fr-4 by prep. TLC in hexane–EtOAc (3:1).

The acidic fraction (7.3 g from 4.4 kg of crushed leaves) as fractionated over 800 ml of Wako-gel C-200 as described for the fractionation of neutral constituents, using hexane–EtOAc (3:2) as eluting solvent. The eluate (250 ml) collected from 750 to 1000 ml was concd to yield 650 mg of crude rugosic acid A (**2**, R_f 0.28 in hexane–EtOAc–HCO_2H, 200:200:1) which was washed with hexane–EtOAc (6:1) and recrystallized from $CHCl_3$–MeOH (10:1) as colourless needles.

Rugosal A (**1**). Colourless needles from EtOAc–hexane, mp 145–147°. Vanillin–H_2SO_4 test; greyish brown. $[\alpha]_D^{23}$ +183° (MeOH; *c* 0.1). UV λ_{max}^{MeOH} 228 nm (ε 7400). FIMS: *m/z* 266 $[M]^+$ (100%). EI-HR-MS: $[M]^+$ 266.156 ($C_{15}H_{22}O_4$ requires 266.152). EIMS *m/z* (rel. int.): 266 $[M]^+$ (1.9), 248 $[M-H_2O]^+$ (3.1), 237 $[M-CHO]^+$ (4.5), 233 $[M-HO_2]^+$ (1.6), 219 $[M-H_2O-CHO]^+$ (2.8), 205 $[M-H_2O-HO_2]^+$ (4.0), 109 (28), 97 (25), 69 (100), 55 (62), 41 (73). IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 2950 (CH), 2820 and 2730 (CHO), 1690 (C=O), 1450, 1380, 1260 and 1160 (C-O), 1080, 1050, 1020, 990, 940, IR ν_{max} (0.31 mM in CCl_4): 3563 (intramolecular hydrogen-bonded OH).

Derivatives of rugosal A. (a) Dimethyl acetal (**4**). To a MeOH soln of **1** (3.5 mg/ml) was added one drop of 0.5 N HCl. The mixt. was allowed to stand for 30 min at room temp. and the product extracted with EtOAc after diln with satd NaCl soln. The EtOAc extract contained unreacted **1** and the reaction product (R_f 0.76 hexane–EtOAc, 3:1), isolated by prep. TLC in hexane–Me_2CO (4:1) as colourless needles (2.3 mg). The compound, mp 119–120.5°, could not be detected by TLC on silica gel F_{254} but could be visualized by spraying with vanillin–H_2SO_4 reagent (reddish yellow spot) or with phosphomolybdic acid reagent (dark blue spot). UV λ_{max}^{MeOH}: featureless above 210 nm. FIMS: $[M]^+$ 312 (**1** + 46 mu, 100%). EIMS *m/z* (rel. int.): 312 $[M]^+$ (0.6), 294 $[M-H_2O]^+$ (0.2), 280 (2.4), 263 (1.4), 248 (4.4), 237 (7.1), 205 (3.3), 163 (11), 139 (14), 125 (28), 111 (17), 99 (19), 97 (19), 83 (19), 75 (100), 69 (97), 55 (45), 41 (68). ^{1}H NMR data see Table 1.

(b) Acetylation products. Acetylation of **1** (12 mg) with Ac_2O–pyridine (3 hr at 80°) gave two products, AAC-1; 7.5 mg in 54% yield, R_f 0.57 in hexane–EtOAc (3:1) and AAC-2; 2.4 mg in 15% yield, R_f 0.23 in same solvent. The former was confirmed to be a monoacetate ($[M]^+$ 308 = **1** + 42 mu). *AAC*-1 (**5**). Colourless small plates, mp 108–109°. UV λ_{max}^{MeOH} 226 nm. MS *m/z* (rel. int.): 308 $[M]^+$ (1.1), 266 $[M-42]^+$ (3.1), 249 (1.4), 248 (2.2), 234 $[M-42-32]^+$ (1.5), 233 (1.6), 220 (2.1), 216 (2.1), 205 (2.4), 203 (2.0), 193 (1.8), 192 (1.5), 191 (4.1), 187 (1.8), 179 (1.7), 177 (3.4), 175 (1.5), 173 (3.0), 166 (1.6), 165 (3.5), 164 (2.0), 163 (3.7), 161 (2.6), 160 (1.7), 109 (12), 83 (14), 81 (11), 70 (11), 69 (62), 55 (31), 43 (100), 41 (43). ^{1}H NMR data see Table 1. *AAC*-2 (**6**). A colourless oil. MS *m/z* (rel. int.): 350 $[M]^+$ (2.7), 233 (2.5), 230 (2.9), 220 (8.3), 205 (7.3), 202 (8.6), 163 (7.3), 151 (7.2), 139 (20), 138 (11), 128 (8.5), 109 (7.2), 95 (7.2), 81 (7.6), 69 (15), 55 (13), 43 (100), 41 (19). ^{1}H NMR C_6D_6 (270 MHz): δ 9.138 (*s*), 6.535 (*d*, *J* = 5.13), 5.243 (*d*, *J* = 5.13), 2.443 (*dd*), 2.215 (*d*, *J* = 12.09), 2.073 (*d*, *sept*), 1.967 (*d*, *J* = 12.09), 1.792 (*m*), 1.714 (3H, *s*), 1.627 (3H, *s*), 1.488 (2H, *m*), 1.351 (*m*), 0.846 (3H, *d*, *J* = 6.59), 0.698 (3H, *s*), 0.679 (3H, *d*). ^{13}C NMR C_6D_6 δ: 189.9 (C-14), 170.3 and 168.5 (2 × $\underline{C}$OMe), 147.7 (C-1), 139.0 (C-2), 101.1 (C-4), 97.1 (C-7), 67.0 (C-3), 54.2 (C-6), 53.0 (C-10), 48.1 (C-5), 44.1 (C-8), 25.7 (C-15), 25.4 and 24.4 (2 × CO$\underline{C}$H$_3$), 24.2 (C-11), 20.8 (C-9), 20.4 (C-12)*, 20.1 (C-13)*; * signals may be interchanged.

(c) $NaBH_4$ reduction products. To a soln of **1** (7.5 mg) in Et_2O (1.6 ml) and MeOH (0.4 ml) was added 8 mg of $NaBH_4$. The mixt. was stirred overnight at room temp. and then dil. with EtOAc followed by washing with satd NaCl soln. The EtOAc layer was concd and subjected to prep. TLC in hexane–EtOAc (1:1). Two reaction products were isolated: A-RB-1, R_f 0.53, 4.4 mg, colourless needles, and A-RB-2, R_f 0.07, 2 mg, colourless semi-solid. *A-RB*-1 (**7**): mp 117–118°. UV λ_{max}^{MeOH}: featureless above 210 nm. FIMS: $[M]^+$ 268 (100%). EIMS *m/z* (rel. int.): 268 $[M]^+$ (0.13), 250 (1.4), 205 (1.7), 193 (12), 153 (7.5), 140 (11), 139 (23), 123 (9.3), 121 (9.4), 111 (19), 97 (40), 95 (25), 83 (24), 69 (100), 55 (48), 43 (54), 41 (72). The MS ($[M]^+$ *m/z* 268) and ^{1}H NMR (with or without D_2O) spectra (Table 1) presented enough evidence to conclude that the product was the primary alcohol (**7**) corresponding to **1**. *A-RB*-2 (**8**): FIMS: $[M]^+$ 268 (68%), *m/z* 250 $[M-H_2O]^+$ (100%). EIMS *m/z* (rel. int.): 268 (0.07), 250 (6.8). The compound was tentatively identified as the triol (**8**) containing a tetrahydrofuran structure in place of the endoperoxide ring.

(d) $LiAlH_4$ reduction products. To a THF soln of **1** (7 mg) was added an excess of $LiAlH_4$ (5 mg) and the mixt. stirred for 1 hr at room temp. A satd NaCl soln was then added to the reaction mixt. and the resulting soln extracted with 3 × 15 ml EtOAc after acidification (pH 4). The combined exts were dried over Na_2SO_4. The major reaction product (R_f 0.09, hexane–EtOAc, 1:5) was isolated from the concd ext. by prep. TLC in hexane–EtOAc (1:6). The compound isolated (*ca* 1 mg) showed a prominent peak at *m/z* 271 $[M+1]^+$ (100%) on FDMS ($[M]^+ = 1+4H$), probably caused by reductive cleavage of the endoperoxide bridge to give 2 × OH and reduction of –CHO to $-CH_2OH$. Although the ^1H NMR measured in MeOH-d_4 was somewhat ambiguous mainly because of large solvent absorptions, all methyl, methylene and methine protons including an olefinic proton corresponding to those of **1** were observed. The tetraol thus prepared (*ca* 200 μg in 100 μl MeOH) was mixed with a soln of KIO_4 (400 μg) and K_2CO_3 (200 μg) in 100 μl of H_2O, and stirred for 2 hr at room temp. By the end of the reaction period, the tetraol had disappeared and two products were detected on TLC (R_f 0.83 and 0.51 in hexane–EtOAc, 1:5). Both products isolated by prep. TLC (hexane–EtOAc, 1:4) were subjected to FIMS. The higher R_f product exhibited a base peak at *m/z* 250 but no ion at *m/z* 268, the lower R_f product having a base peak at *m/z* 250 and a small peak at *m/z* 268 (15%). If the estimated structure for the starting tetraol (**9**) is correct, oxidative cleavage would be expected to produce a keto aldehyde M_r 268. The present results, however, were insufficient to confirm such a reaction process. The tetraol presumably is easily decomposed by KIO_4.

(e) *Methoxycarbonyl derivatives.* Rugosal A (**1**) was converted into the methoxycarbonyl derivative according to the method of ref. [5]. To a MeOH soln of **1** (6.7 mg in 2.5 ml) were added 7.5 mg of KCN, 3.6 mg of HOAc and 60 mg of MnO_2. The reaction mixt. was stirred for 2 hr at room temp. and then filtered. The filtrate dil. with H_2O was extd with Et_2O. The concd Et_2O ext. was subjected to prep. TLC in hexane–EtOAc (3:1) to give 2.4 mg of reaction product (R_f 0.67) and 1.6 mg of **1** (R_f 0.48). The former was indistinguishable from the Me ester of rugosic acid A by silica gel TLC, IR and MS. The optical rotation of the product, $[\alpha]_D^{23}+128°$ (Me_2CO; *c* 0.02), showed that both Me esters were also identical in absolute configuration.

Rugosic acid A (**2**). Colourless needles from $CHCl_3$, mp 142–144°. Vanillin–H_2SO_4 test: greyish blue. *N,N*-Dimethyl-*p*-phenylenediamine test: reddish purple [19]. $[\alpha]_D^{23}$ +159° (MeOH; *c* 0.08). UV λ_{max}^{MeOH}: 216 nm. FIMS: *m/z* 283 $[M+1]^+$ (100%), 282 $[M]^+$ (91%). EI-HR-MS: $[M]^+$ 282.146 ($C_{15}H_{22}O_5$ requires 282.147). EIMS *m/z* (rel. int.): 282 $[M]^+$ (0.6), 264 $[M-H_2O]^+$ (4.8), 246 (7.6), 220 (12), 203 (11), 181 (15), 152 (16), 140 (32), 139 (92), 125 (20), 121 (21), 109 (29), 97 (56), 83 (31), 81 (36), 70 (33), 69 (94), 55 (83), 43 (60), 41 (100). IR - ν_{max}^{KBr} cm^{-1}: 3360 (OH), 3400–3100 (COOH), 2960, 1710 (C=O), 1675, 1410, 1180, 1060, 810, 720. ^1H NMR data shown in Table 1.

Rugosic acid A methyl ester (**3**). Rugosic acid A (350 mg) was treated with excess CH_2N_2 in CH_2Cl_2 to yield 220 mg of the corresponding Me ester (EIMS: $[M]^+$ 296, 0.8%) and a diazomethane adduct of the Me ester (120 mg, EIMS: $[M]^+$ 338, 100%). The former (**3**) was crystallized from hexane–Et_2O as colourless fine needles, mp 150–151°. $[\alpha]_D^{23}+154°$ (Me_2CO; *c* 0.02). EIMS *m/z* (rel. int.): 296 $[M]^+$ (0.8), 278 $[M-H_2O]^+$ (2.3), 264 (2.7), 246 (3.1), 235 (3.5), 221 (8.9), 218 (4.9), 203 (5.8), 175 (7.0), 153 (10), 140 (13), 139 (31), 109 (20), 97 (27), 83 (24), 69 (100), 55 (54), 43 (34), 41 (66). UV λ_{max}^{MeOH}: 218 nm. IR ν_{max}^{KBr} cm^{-1}: 3460, 2960, 1730, 1440, 1380, 1250, 1230, 1080, 980. ^1H NMR, ^{13}C NMR and ^{13}C–^{13}C correlated 2D-NMR data shown in Table 1 and Fig. 5.

Antimicrobial assay. Extracts of *R. rugosa* leaves and diffusates were subjected to TLC on silica gel. Test compounds were applied in Me_2CO soln (15 μl) to give a 10 mm diameter zone on the adsorbent layer. Plates were then sprayed with a spore suspension of *C. herbarum* and incubated at 25° in moist conditions until fungal growth was apparent (2–3 days) [1, 6].

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