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CAROTA-1,4-DIENALDEHYDE, A SESQUITERPENE FROM *ROSA RUGOSA*

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Key Word Index—*Rosa rugosa*; Rosaceae; leaves; sesquiterpene; carotanoid; endoperoxide; carota-1,4-dienaldehyde.

Abstract—A new sesquiterpene, (7*R*,10*R*)-carota-1,4-dienaldehyde was identified in *Rosa rugosa* leaves, and its structure elucidated by spectroscopic and chemical methods. The new compound was markedly unstable under air exposure to give several oxidized derivatives, in which rugosal A was found as the main product due to the 1,4-diene structure. The corresponding carboxylic acid, carota-1,4-dienoic acid was found in the leaves.

INTRODUCTION

Rosa rugosa leaves contain a carotanaldehyde, rugosal A (**5**) which possesses an unique *endo*-peroxy bridge in the molecule and shows marked antifungal activity against *Cladosporium herbarum* [1]. This type of sesquiterpenoid has only been found so far in Umbelliferae [2–5], Compositae [6, 7] and Rosaceae [1]. *Rosa rugosa* leaves are particularly rich in carotano-sesquiterpenes which are regio-specifically oxygenated at the C-14 carbon to an α,β-unsaturated aldehyde or a carboxyl group. This regio-specific oxygenation in carotanoids originating from the Rosaceae is an unique property among all the naturally occurring carotano-sesquiterpenes.

Rugosal A (**5**) has a further unique oxygenation system. The endoperoxy linkage in naturally occurring endoperoxides is usually formed by a reaction between singlet O_2 and a 1,3-diene [8, 9]. This peroxidation system is inapplicable to the formation of the endoperoxy bridge in **5**, but another endoperoxide forming system is known in the oxidation of unsaturated fatty acids having a 1,4-diene partial structure. An exoperoxyradical is believed to be the intermediate [10, 11] as shown in Scheme 1. The possible precursor carota-1,4-dienaldehyde was therefore sought in *R. rugosa* leaf extractives.

Compound **1** was successfully isolated, and its structure elucidated. During exposure to air, this gave rugosal A (**5**) and rugosic acid A (**6**) in significant yield, and both were identical in spectroscopic properties with the authentic compounds, proving that **1** is surely one of the precursors for **5**.

RESULTS AND DISCUSSION

The methanol extractives from *R. rugosa* leaves, in which rugosal A (**5**) was a major component, were initially partitioned between *n*-hexane and methanol. The *n*-hexane-soluble fraction was examined for the expected precursor of **5** and attention was focused on some less polar, quenching constituents observed by silica gel F_{254} TLC. The compounds of interest were fractionated by silica gel column chromatography eluting with *n*-hexane–ethyl acetate mixtures. After preparative TLC of a part of the column fraction containing the constituent of interest, the quenching substance with R_f 0.53 in *n*-hexane–EtOAc (10:1) was analysed by GC-MS. The fraction denoted RL gave four peaks showing $[M]^+$ at *m/z* 218. The major compound detected as the third peak (relative intensity 68%; R_t 21 min) was named RL-C.

The RL fraction dissolved in *n*-hexane was subjected to preparative HPLC using an Unisil Q 100–5 column, a UV detector set at 230 nm and *n*-hexane–EtOAc (39:1) as eluent. The major constituent detected as a symmetrical peak was collected to give *ca* 50 mg of syrup. The isolated compound exhibited an EI mass spectrum in good accordance with that of the major constituent (RL-C) on GC-MS, and the molecular formula $C_{15}H_{22}O$ was determined by EI-HRMS (found 218.165, calcd 218.167). The methanolic UV maximum at 230.5 nm characteristic of α,β-unsaturated aldehydes disappeared upon the addition of HCl to the solution, as observed with rugosal A (**5**) which was easily converted to the corresponding dimethyl acetal [1]. The IR spectrum of RL-C (**1**) also showed absorption bands assignable to an α,β-unsaturated aldehyde group (2860 and 2700 cm^{-1}: CHO, and 1680 cm^{-1}: C=O).

The ^1H NMR spectrum of RL-C (**1**) (Table 1) showed the presence of a formyl proton (δ 9.126, 1H, *s*) and an isopropyl group (0.742, 3H, *d*, $J = 6.8$ Hz; 0.888, 3H, *d*, $J = 6.8$ Hz and 1.704, 1H, octet-like signal, $J = ca$ 6.8 Hz) in addition to a bridge-head methyl group (0.847, *s*), all of which were required of a carotane-14-aldehyde skeleton. The octet-like methine proton of the isopropyl group suggested that the isopropyl group is allocated on a methine carbon whose proton shows a vicinal coupling $J = ca$ 6.8 Hz.

The ^{13}C NMR (DEPT and CH-COSY) spectra proved the presence of four sp^2 carbons at δ152.0 (=C<), 150.9 (=CH–), 141.1 (=C<) and 116.0 (=CH–) attributable to two double bonds. Two olefinic protons detected at δ5.122 (1H, *ddd* $J = 6.6$, 3.4 and 2.8 Hz) and 6.065 (1H, *dddd*, $J = 7.9$, 3.3, 1.7 and 0.8 Hz) on C-2 (116.0) and on C-4 (150.9), respectively, did not show any vicinal coupling between them. The coupling constants of the olefinic

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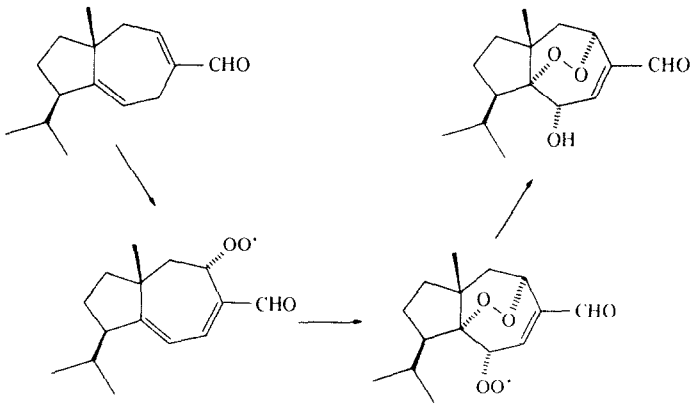
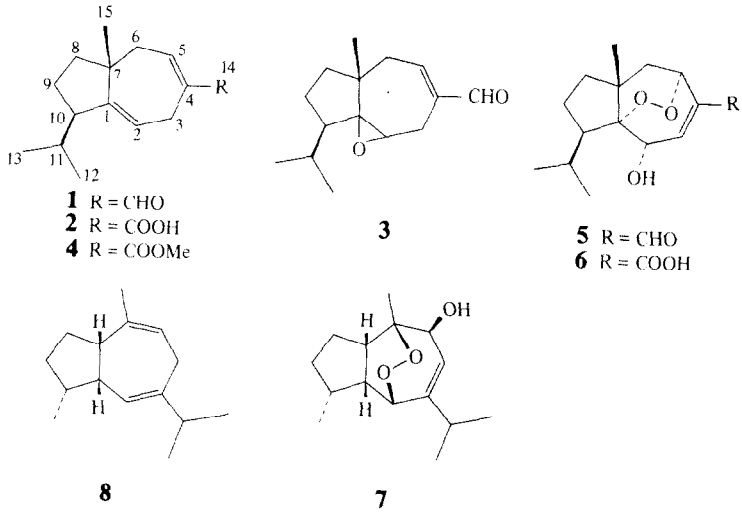


Scheme 1. Possible conversion pathway of carota-1,4-dienaldehyde into rugosal A.



protons suggested that each was vicinally coupled with a pair of methylene protons.

Proton spin–spin decoupling experiments revealed the following proton–proton sequence. By irradiation of the δ5.122 olefinic proton, a pair of methylene protons resonating at δ3.218 (1H, multiply divided broad doublet, $J = 22.2$ Hz) and 3.113 (1H, multiply divided double-doublets, $J = 22.2$ and 6.6 Hz) which were geminally coupled to each other were simplified into two broad doublets (3-Ha and 3-Hb, each $J = 22.2$ Hz). When the δ6.065 olefinic proton at C-5 was irradiated, a clear collapse of signals at δ2.175 (1H, $J = 17.7$ and 3.3 Hz) and 1.983 (1H, $J = 17.7$ and 7.9 Hz) of 6-H_2 was observed (two broad double-doublets⟶two broad-doublets). In addition, the C-3 methylene signals were slightly sharpened by the irradiation. The latter result was indicative of an allyl coupling between the C-5 and the C-3 protons, and consequently identified a partial structure around those olefinic bonds.

On the C-3 and the C-5 protons, a clear deshielding effect was observed. As a carbonyl bond causes a deshielding effect, the α,β-unsaturated aldehyde group of the compound should be located on the C-4 olefinic carbon. In addition, a double bond on the conjugated system is required to take the *cis*-form regarding the formyl group and the C-5 proton. Accordingly, the substructure shown in Fig. 1 became feasible.

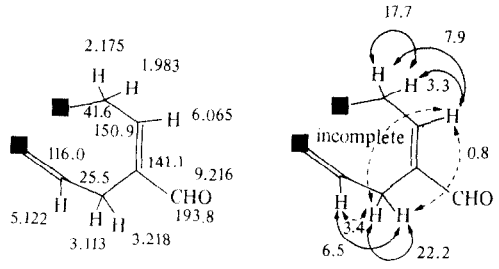


Fig. 1. Partial structure of RL-C. Coupling constants are given by Hz. ◀----▶ indicates a coupling observed during decoupling experiment. ■ shows a non-hydrogen-bearing carbon.

When an unassignable proton signal at δ1.704 (1H, *br m*) was irradiated, the C-11 methine proton which was originally divided into eight peaks became a clear septet ($J = 6.8$ Hz). This result indicated that the δ1.704 proton should be attributed to the C-10 methine proton vicinally coupled with the C-11 methine proton ($J = ca$ 6.8 Hz). By this irradiation, the C-2 olefinic proton at δ5.122 collapsed into a double-doublet ($J = 6.6$ and 3.4 Hz) and the C-3 methylene protons at δ3.218 and 3.113 into a broad doublet ($J = 22.2$ Hz) and a broad double-doublet ($J = 22.2$ and 6.6 Hz), respectively. These results suggested

Table 1. Proton and carbon chemical shift values of carotano-dienes (500 MHz, in C_6D_6)

C	1 δ_H (Hz)	4 δ_C	4 δ_H (Hz)
1	—	152.0	—
2	5.122 *ddd* (6.5, 3.4, 2.8)	116.0	5.164 *ddd* (6.2, 3.7, 2.3)
3	3.218 *br d* (22.2)	25.5	3.470 *br d* (22.2)
	3.113 *br dd* (22.2, 6.5)		3.266 *ddd* (22.2, 6.2, 2.1)
4	—	141.1	—
5	6.065 *dddd* (7.9, 3.3, 2.9, 0.8)	150.9	7.211 *br dd* (8.4, 3.5)
6	2.175 *br. dd* (17.7, 7.9)	41.6	2.230 *br dd* (16.2, 3.5)
	1.983 *br dd* (17.7, 3.3)		2.029 *br dd* (16.2, 8.4)
7	—	45.2	—
8	*ca* 1.40 *m*	41.5	*ca* 1.35 *m*
	1.327 *m*		1.227 *ddd* (13.8, 6.9, 6.9)
9	1.374 *m*	23.4	1.416 *m*
	1.230 *m*		*ca* 2.33 *m*
10	2.274 *br m*	52.6	2.292 *br m*
11	1.704 *br dsept* (6.8, *ca* 6.8)	28.9	1.722 *dsept* (6.8, *ca* 6.8)
12	0.888 *d* (6.8)	21.6	0.898 *d* (6.8)
13	0.742 *d* (6.8)	16.8	0.753 *d* (6.8)
14	9.216 *s*	193.8	—
15	0.847 *s*	23.7	0.964 *s* (14'–COOMe; 3.430 *s*)

that the C-10 methine proton further showed an allylic coupling with the 2-H and a homoallylic coupling with the 3-H_2. Accordingly, the non-conjugated olefinic carbons (–C=CH–) were allocated between C-10 and C-3. Moreover, a pair of multiple signals at δ1.230 (1H) and 1.374 (1H), both showing a cross peak with a 23.4 methylene carbon on the C-H COSY spectrum, showed markedly changed signal patterns by the irradiation. This fact further indicated that the irradiated C-10 methine proton was vicinally coupled with another pair of methylene protons assignable to 9-H_2.

The remaining methylene carbon (δ 41.5) showing cross peaks with two multiplets at 1.327 and *ca* 1.40 (each 1H) was presumed to connect with the C-9 methylene, as no other position can provide a methylene group showing a vicinal proton coupling. Thus, 11 carbons out of total 15 in the compound were eventually assigned to form the partial structure consisting of the α,β-unsaturated aldehyde and the isopropyl groups as shown in Fig. 2.

Among four of the unassigned carbons, the sp^3 quarternary carbon (δ52.6) could only connect with the bridgehead methyl (δ_H 0.847, δ_C 23.7), the C-9 methylene (41.5)

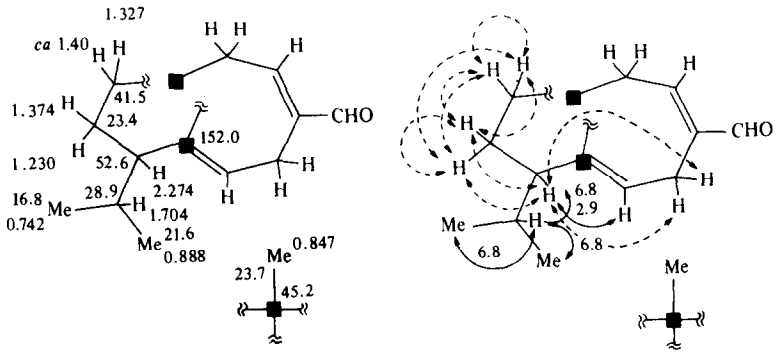


Fig. 2. Partial structure of RL-C elucidated by proton sequences.—55 indicates unassigned C, C-single bond. Proton couplings unlabelled through decoupling experiments are shown by ◄---►

and the C-1 olefinic (152.0) carbons, all possessing an unassigned C, C-single bond. Consequently, all protons and carbons in RL-C were assigned to give a planar structure **1** having a carotane skeleton with a 1,4-diene partial structure, sufficient to be a precursor of **5** (Fig. 2).

The presence of the non-conjugated C-1/C-2-double bond was also proved by epoxidation of **1** with *m*-chloroperbenzoic acid which is known to convert selectively a non-conjugated olefin bond into an epoxy group [12]. From 2.1 mg of **1**, 1.2 mg of a product was obtained and purified by preparative TLC (R_f 0.21 in *n*-hexane–EtOAc, 9:1). The product with M_r 234 (EIMS, $[M]^+$, 2.4%) showed the C-2 methine proton (δ 3.005, *br d*, $J = 5.1$ Hz) locating on the epoxy ring and coupled with one of the C-3 methylene protons (3.341, *br dd*, $J = 19.8$ and 5.1 Hz) in ^{1}H NMR spectrum. Because the non-conjugated olefinic bond causing the allylic or homoallylic coupling in **1** was blocked, complete separation of the two coupling sequences of the epoxy derivative (**3**) became feasible as shown in Fig. 3. In addition, the C-8, C-9 and C-10 protons of **3** showed a better resolution in the lower magnetic field than did those of **1**, and they were all assigned by the decoupling experiments. Accordingly, these proton sequences proved the structure of **3** to have a carotane skeleton. From the ^{1}H NMR spectrum of **3**, stereoselective epoxidation in **1** was presumed; however, the stereochemistry of the epoxide **3** remains unsolved.

The possible precursor (**1**) for **5** found in the *R. rugosa* leaf tissues has been named carota-1,4-dienaldehyde. Young leaf tissues of *R. rugosa* were shown to be rich in compound **1** (*ca* 100 mg/kg fr. leaf) using gas chromatography (GC).

The purified **1** was quite unstable under air, and gave a mixture of more polar compounds, some of which were positive to peroxide reagent (*N,N*-dimethyl-*p*-phenylenediamine). Two of the auto-oxidation products were identified as rugosal A (**5**) and rugosic acid A (**6**) (3.6 mg of **5** and 1.9 mg of **6** from 51.7 mg of the oxidized mixture), respectively. One of the other products showed a peroxide reagent-negative response and was indistinguishable from the epoxy derivative (**3**). These three compounds were much more stable than other auto-oxidation products showing a peroxide nature. Interestingly, some of the unstable products were rapidly converted into **6** during or after preparative TLC. These facts led to an hypothesis that **5** is formed through an exoperoxy-radical intermediate. A similar example has been reported with a guaiano sesquiterpene, hanalpinol (**8**) chemically convertible from guaia-6,9-diene (**7**) [10, 13].

Further analyses of the constituents also revealed the presence of carota-1,4-dienoic acid (**2**) in the *R. rugosa* leaves. A crude mixture containing the acid (**2**) was esterified with CH_2NH_2 to yield the methyl ester. This was purified by TLC and HPLC and identified by comparison with a methoxycarbonyl derivative (**4**) prepared from **1** by Corey's method [14].

In *R. rugosa*, compound **1** may function as an antioxidant through the protection system of the leaf tissues. Also it may constitute a precursor of antifungal carotanoids. Oxidized products, mainly rugosal A (**5**), are possibly stored in the tissues for use as chemical defence agents.

EXPERIMENTAL

General. ^{1}H and ^{13}C NMR spectra were recorded, respectively, at 500 and 125 MHz using TMS as int. std. Coupling constants are given in Hz. UV and IR spectra were recorded for solns in MeOH or for liquid film. Merck silica gel $60F_{254}$ precoated on glass plate or aluminum sheet was used for analytical or prep. TLC; R_f values refer to spots which quench the impregnated fluorescing agent under UV $_{254nm}$ light or give colour reactions with vanillin–H_2SO_4 spray reagent.

Materials. Fresh *R. rugosa* leaves (6 kg) collected at Ishikari near Sapporo in early July were soaked in 95% MeOH (*ca* 50 l) for two months, then the MeOH extracts were collected and directly extracted 2× with *n*-hexane (1/3). The *n*-hexane layer was concd to *ca* 1 l, and washed with an equivalent volume of 5% $NaHCO_3$. The neutral substances (*ca* 12 g) dissolved in a small amount of *n*-hexane were successively chromatographed on silica gel columns. The $NaHCO_3$ washings contained only a small amount (less than 1 g) of acidic constituents.

The *n*-hexane extractives charged onto a silica gel column (500 ml in *n*-hexane, 8 cm diameter) was eluted as follows. After eluting Fr. H-1 with 1.5 l of 2% EtOAc–hexane elution with 4% EtOAc–hexane gave Fr. H-2 and H-3 (each 250 ml) containing quenching substances (R_f 0.65, *n*-hexane–EtOAc, 9:1). The major quenching spot was isolated from Fr. H-2 by prep. TLC (RL fraction), and subjected to GC (5% PGE-20M on Celite 545 AW, 1 m) to give four peaks (RL-A: relative proportion 7%, R_t 17 min, B: 11%, 19 min, C: 68%, 21 min, and D: 14%, 24 min), all showing $[M]^+$ at *m/z* 218. Final purification of carota-1,4-dienaldehyde (**1**) was carried out with HPLC (column; Unisil Q 100–5; solvent 2.5% EtOAc–*n*-hexane; flow rate; 2 ml/min; detector UV 230 nm).

On the other hand, the organic solvent was removed from the MeOH layer to obtain *ca* 1 l of aq. suspension. The extracts were then dissolved in Me_2CO (total 2.5 l), at which point excess NaCl was added to the soln and the mixture stirred overnight to separate Me_2CO and the NaCl solns. The Me_2CO solubles were then decantated washing with fresh Me_2CO. The Me_2CO layer, containing satd. NaCl soln, was directly concd and then dissolved in EtOAc (1.5 l), washed with an equivalent volume of

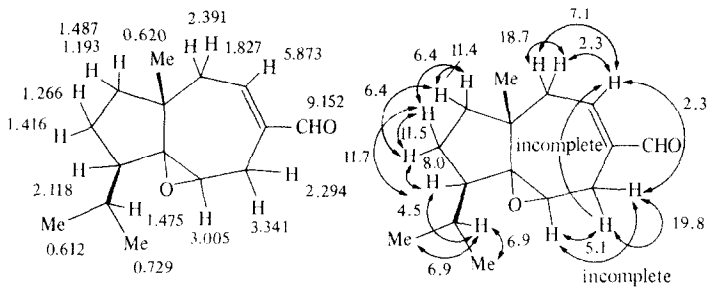


Fig. 3. Proton–proton coupling sequeeuences in epoxy derivative of RL-C.

satd NaCl soln and the organic layer concd. The EtOAc solubles were further dissolved in C_6H_6 (800 ml) and washed with 5% $NaHCO_3$ soln (700 ml), followed by concn after being dried over Na_2SO_4, *ca* 48 g of solute was obtained. The C_6H_6 solubles dissolved in 10% EtOAc–hexane were subjected to silica gel CC (Wako gel C-200, 750 ml in hexane, 4 cm diameter column) to give the following fractions; Fr. B-1 (400 ml, eluted with 10% EtOAc–hexane mixture), Fr. B-2, B-3 (each 400 ml, eluted with 10% EtOAc–hexane), B-4, B-5 (each 400 ml, with 20% EtOAc–hexane), and B-6 (400 ml, with 35% EtOAc–hexane) (eluants, 0, 2.4, 2.5, 2.1, 5.8 and 3.6 g, respectively). From Fr. B-2 and B-3 the remaining RL substances were recovered in good yield.

Carota-1,4-dienaldehyde (**1**). Syrup. Vanillin–H_2SO_4 test; grayish brown. UV λ_{max}^{MeOH} 230.5 nm. EI-HRMS: $[M]^+$ at *m/z* 218.165 ($C_{15}H_{22}O$ requires 218.167). GCMS *m/z* (rel. int.): 218 $[M]^+$ (49), 203 $[M-Me]^+$ (12), 200 (11), 185 (22), 175 $[M-C_3H_7]^+$ (100), 157 (27), 147 (41), 133 (25), 119 (27), 105 (50), 91 (61), 81 (39), 79 (24), 77 (21), 55 (28), 41 (54). IR ν_{max}^{KBr} cm^{-1}: 2940 *br*, 2860 (CHO), 2700 (CHO), 1685 (C=O), 1655 (C=C), 1445, 1420, 1380, 1170, 1140, ^1H NMR data are shown in Table 1.

Epoxidation of compound **1**. Compound **1** (2.1 mg) dissolved in 0.5 ml of Me_2CO was treated with 8.2 mg of *m*-chloroperbenzoic acid overnight at room temp. The reaction mixture was concd *in vacuo* and subjected to prep. TLC (*n*-hexane–EtOAc, 9:1). Together with *ca* 0.8 mg of the starting material (R_f = 0.55), the main product, 1.2 mg of RL-C-OX (**3**, R_f = 0.21, yield 54%) was obtained.

RL-C-OX (**3**). Syrup. Vanillin–H_2SO_4 test: brownish orange. FIMS: $[M]^+$ at *m/z* 234 (100%). EIMS *m/z* (rel. int.): 234 $[M]^+$ (2.4), 219 $[M-Me]^+$ (3.8), 216 $[M-H_2O]^+$ (5.4), 206 (5.4), 205 (30), 191 (26), 173 (21), 163 (10), 151 (13), 145 (30), 135 (24), 123 (42), 121 (26), 109 (34), 107 (32), 105 (25), 95 (100), 91 (35), 81 (62), 69 (35), 55 (42), 43 (42), 41 (72). ^1H NMR (C_6D_6): δ 3.005 (*d*, *J* = 5.1 Hz, 2-H), 3.341 (*br dd*, *J* = 19.8 and 5.1 Hz, 3-Ha), 2.294 (*br d*, *J* = 19.8 Hz, 3-Hb), 5.873 (*ddd*, *J* = 7.1, 2.3 and 2.3 Hz, 5-H), 2.391 (*br d*, *J* = 18.7 Hz, 6-Ha), 1.827 (*br dd*, *J* = 18.7 Hz, 6-Hb), 1.487 (*dd*, *J* = 11.4 and 6.4 Hz, 8-Ha), 1.193 (*dd*, *J* = 11.4 and 6.4 Hz, 8-Hb), 1.416 (*m*, 9-Ha), 1.266 (*ddd*, *J* = 11.7, 11.5 and 6.4 Hz, 9-Hb), 2.118 (*ddd*, *J* = 11.7, 8.0 and 4.5 Hz, 10-H), 1.475 (*m*, 11-H), 0.729 (*d*, *J* = 6.9 Hz, 12-H_3), 0.612 (*d*, *J* = 6.9 Hz, 13-H_3), 9.152 (*s*, 14-H_3), 0.620 (*s*, 15-H_3).

Conversion of **1** *into the methoxycarbonyl derivative* (**4**). Carota-1,4-dienaldehyde (**1**) was converted into the methoxy carbonyl derivative (**4**) according to Corey's method [14]. Compound **1** (5.2 mg) dissolved in 1 ml of MeOH was mixed with 22 mg of HOAc, *ca* 120 mg of active MnO_2 and *ca* 22 mg of NaCN, and stirred overnight at room temp. The reaction mixture was diluted with 50 ml dist. H_2O and extracted with 20 ml EtOAc. After washing 2× with 30 ml dist. H_2O, the organic layer was dried over Na_2SO_4, and subjected to prep. TLC (*n*-hexane–EtOAc, 20:1). The product, RL-C-ME (**4**, R_f = 0.63, 1.5 mg, yield 20%) and recovered **1** (R_f = 0.37, 3.0 mg, 58%) were obtained.

RL-C-ME (**4**). Syrup. Vanillin–H_2SO_4 test: pink. EIMS *m/z* (rel. int.): 248 $[M]^+$ (8.6), 233 $[M-Me]^+$ (4.2), 217 $[M-OMe]^+$ (2.4), 206 (8.6), 205 $[M-C_3H_7]^+$ (52), 189 (5.1), 178 (4.7), 173 (34), 163 (12), 149 (15), 146 (14), 145 (100), 131 (16), 121 (17), 119 (18), 117 (19), 105 (38), 93 (16), 91 (35), 81 (41), 79 (18), 77 (18), 59 (17), 55 (16), 43 (13), 41 (33), 40 (17). ^1H NMR (C_6D_6): δ 5.164 (*ddd*, *J* = 6.2, 3.7 and 2.3 Hz, 2-H), 3.470 (*br d*, *J* = 22.2 Hz, 3-Ha), 3.266 (*ddd*, *J* = 22.2, 6.2 and 2.1 Hz, 3-Hb), 7.211 (*br dd*, *J* = 8.4 and 3.5 Hz, 5-H), 2.230 (*br dd*, *J* = 16.2 and 3.5 Hz, 6-Ha), 2.029 (*br dd*, *J* = 16.2 and 8.4 Hz, 6-Hb), *ca* 1.35 (*m*, 8-Ha), 1.227 (*ddd*, *J* = 13.8, 6.9 & 6.9 Hz, 8-Hb), 1.416 (*m*, 9-Ha), *ca* 1.33 (*m*, 9-Hb), 2.292 (*m*, 10-H), 1.722 (*d sept*, *J* = *ca* 6.8 and 6.8 Hz, 10-H), 0.898 (*d*, *J* = 6.8 Hz, 12-H_3), 0.753 (*d*, *J* = 6.8 Hz, 13-H_3), 3.430 (*s*, 14′-OMe), 0.964 (*s*, 15-H_3).

Auto-oxidation of compound **1**. During exposure to air for 1 day at room temp. in darkness, **1** was easily oxidized to give highly polar and unstable compounds which were positive to peroxide reagent. When the mixtures had been left in the refrigerator for more than 2 weeks, some stable products were found in the mixture. From 51.7 mg of the auto-oxidation products, two constituents were successfully isolated by prep. TLC, and were indistinguishable from rugosal A (**5**, 3.7 mg, R_f 0.41 in *n*-hexane–EtOAc 3:1), and rugosic acid A (**6**, 1.9 mg R_f 0.59 in *n*-hexane–EtOAc–HCOOH 25:25:1) respectively, by chromatographic and spectroscopic (^1H NMR and ORD) properties ($[\alpha]_D$; 125 and 130°, respectively, in MeOH; *c* 0.1) [1].

Partial isolation of carota-1,4-dienoic acid (**2**). In Fr. B-2 to B-5, an unknown component was detected at R_f 0.31 in *n*-hexane–EtOAc (4:1) as a major constituent (*ca* 200 mg/kg leaves), showing an acidic nature and pinkish coloration with vanillin–H_2SO_4 reagent. By isolation using prep. TLC in the same solvent system, *ca* 10 mg of a syrup was obtained, and by successive FIMS and ^1H NMR analyses of the isolate, the unknown substance was found to be a mixture of compounds with $[M]^+$ at *m/z* 234. The ^1H NMR spectrum indicated the major substance to have the approximate structure of carota-1,4-dienoic acid (**2**), showing some features of carotane skeleton having the 1,4-diene structure corresponding to **1**. From the $NaHCO_3$ washings of the C_6H_6 solubles *ca* 8 g of acidic constituents were obtained. However, the acidic fraction was shown by TLC to only contain a trace amount of the compound of interest.

Carota-1,4-dienoic acid (**2**). Syrup. Vanillin–H_2SO_4 test: pink. FIMS: $[M]^+$ at *m/z* 234 (100%). ^1H NMR (C_6D_6): δ 5.124 (*ddd*, *J* = 6.3, 3.7 and 2.3 Hz, 2-H), 3.385 (*br dd*, *J* = 23.7 Hz, 3-Ha), 3.192 (*br d*, *J* = 23.7 Hz, 3-Hb), 7.331 (*br m*, 5-H), 2.270 (*br m*, 10-H), 0.895 (*d*, *J* = 6.6 Hz, 12-H_3), 0.759 (*d*, *J* = 7.0 Hz, 13-H_3), 0.915 (*s*, 15-H_3).

Methylation of compound **2**. Because it was hard to purify **2** under acid-free conditions, esterification of **2** was firstly tried with CH_2N_2 for the purpose of isolation of the acidic compound. *Ca* 51 mg of the mixture containing **2** was added to an excess amount of diluted CH_2N_2 trapped in CH_2Cl_2, and left overnight at room temp. The reaction mixtures were chromatographed on TLC (*n*-hexane– EtOAc, 10:1) to give 30 mg of the methylation products (R_f *ca* 0.8 in *n*-hexane–EtOAc 10:1). Accordingly, the products were separated by HPLC (1% EtOAc–hexane, UV 220 nm) to obtain *ca* 10 mg of the main product. The methyl ester was identical with **4** derived from **1** (TLC, *n*-hexane–EtOAc, 20:1, R_f 0.63; EIMS; ^1H NMR spectrum; optical rotation, both laevorotatory, *ca* −60°).

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