Identification of an Intermediate in Autoxidation of Carota-1,4-dien-14-al into Rugosal A

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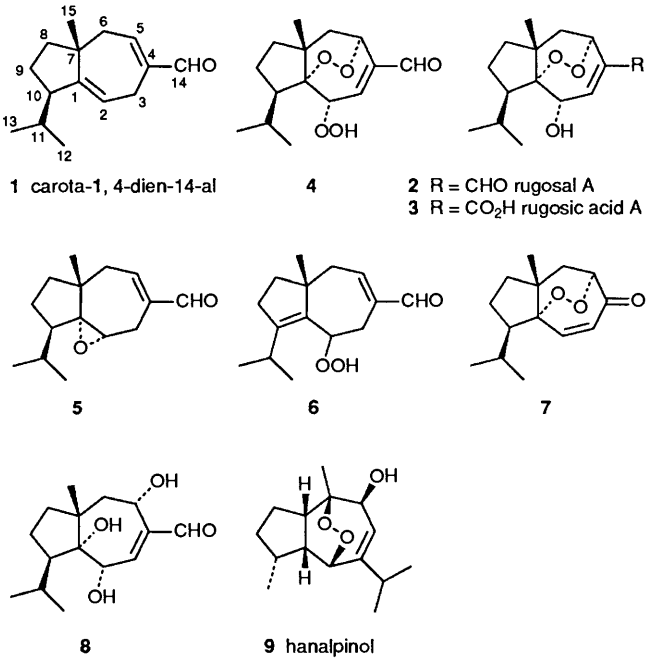
In the autoxidation of (7*R*,10*R*)-carota-1,4-dien-14-al **1**, which is a precursor of an antifungal compound rugosal A **2**, in *Rosa rugosa* leaves, an intermediate possessing a 1,5-endoperoxy-2-hydroperoxy system **4** was isolated as a major product. The structures of compound **4** and other by-products including rugosal A **2** and rugosic acid A **3** were determined. These autoxidation products indicated the conversion pathway of compound **1** into rugosal A **2**, similar to the radical reaction pathway in the autoxidation of polyunsaturated fatty acids.

As we have recently reported,[1] *Rosa rugosa* leaves contain a carotane sesquiterpene diene, carota-1,4-dien-14-al **1**, which is regarded as a precursor of rugosal A **2**, a major antifungal carotane peroxide of *R. rugosa*.[2] Conversion of compound **1** into rugosal A **2** was confirmed by its spontaneous oxygenation;[1] however, the oxygenation pathway has not been established. This peroxidation was expected to be initiated by hydrogen abstraction at the bisallylmethylene portion,[3-6] as observed in the autoxidation of polyunsaturated fatty acids having a 1,4-diene moiety. Guaia-6,9-diene has successfully been converted into hanalpinol **9** having a similar peroxide system to that of rugosal A **2**, through a radical reaction pathway.[7] Autoxidation of compound **1** gave various products, some of which were characteristic of the particular reaction conditions. Analysis of the autoxidation products of compound **1** to compound **2** confirmed a similar reaction pathway for the peroxidation which involved some radical formation steps (Scheme 1). In this paper the isolation, identification and further discussions on the oxygenation pathway and stereoselectivity of compound **1** are reported.



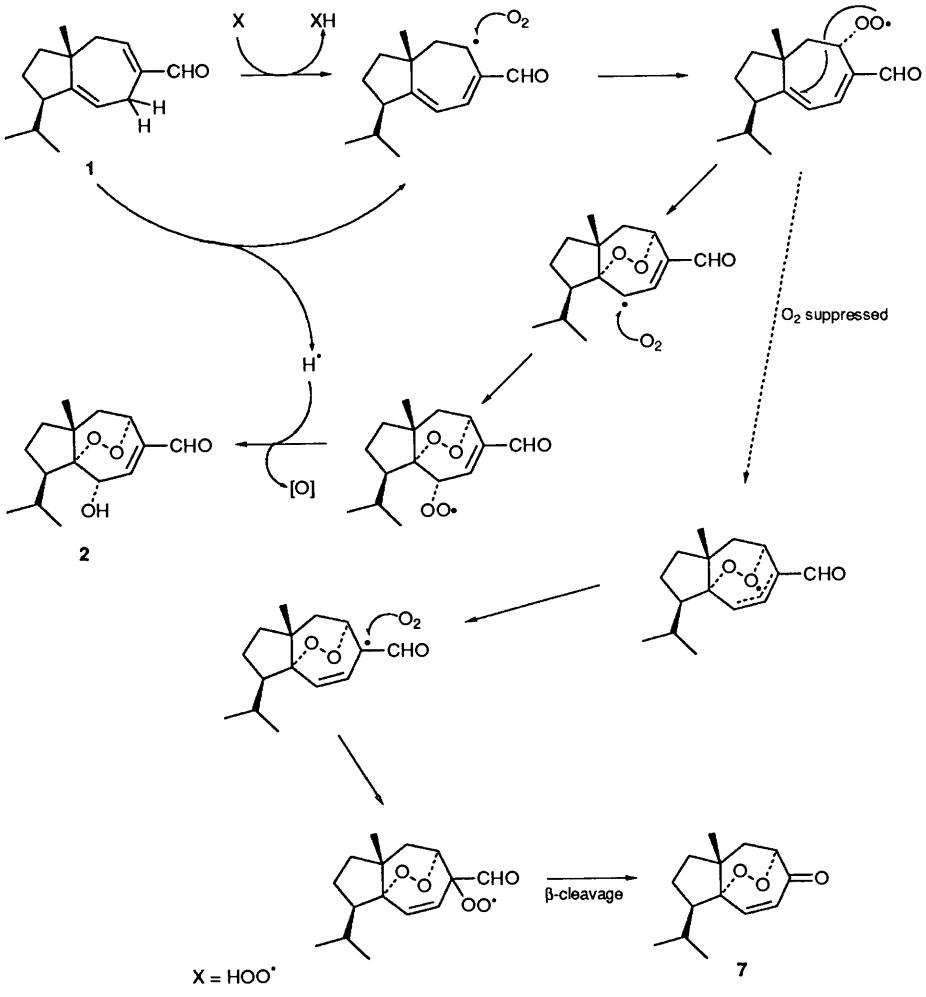
Results and Discussion

The precursor of rugosal A **2**, carota-1,4-dien-14-al **1**, is unstable on exposure to air, and is converted into some peroxy compounds. Two of the oxidation products have been identified as rugosal A **2** and rugosic acid A **3**, and their stereochemistry has been elucidated. To elucidate the peroxidation pathway, oxygenation of **1** was performed quantitatively. A hexane solution of pure compound **1** was made and a thin film of this substance on a glass wall was then obtained by removal of the solvent. The thin film was kept for 3 h at 40 °C to afford a syrup. The unknown product (A-2, 60% yield) detectable on TLC was successfully separated by preparative TLC (PTLC) (n-hexane–EtOAC, 4:1), and the structure of A-2 was analysed by the usual spectroscopic methods.

The product A-2, with a molecular ion at *m/z* 282 in its FDMS, showed quite similar EIMS, ^{1}H NMR and ^{13}C NMR spectra to rugosal A **2**. In the ^{1}H NMR spectrum, most of the signal patterns were in accord with those of compound **2**, indicating that the substitution pattern of A-2 was basically the same as that of **2**. An exchangeable signal assignable to a hydroperoxy proton was uniquely detected at δ_H 8.94, instead of the 2-OH proton in rugosal A **2**. In the ^{13}C NMR spectrum, all carbons were also essentially the same as those of rugosal A **2**, except for the C-2 carbon whose chemical shift was markedly deshielded by substitution with the hydroperoxy group.

The reduction of A-2 with thiourea[8] gave compound **2** as the major product, which confirmed the structure of A-2 as 1,5-endoperoxy-2-hydroperoxycarot-3-ene-4-carbaldehyde **4**. The stereochemistry of rugosal A **2** obtained by reduction of the hydroperoxide moiety of A-2 **4** agreed with that of the naturally occurring product **2**, which suggests A-2 **4** might be a possible intermediate in the peroxidation of compound **1** into rugosal A **2**.[7,9] In fact, A-2 **4** was found to be unstable in most solvents and was converted into rugosal A **2** spontaneously. This intermediate thus confirmed the hypothetical scheme[1] for the peroxidation of compound **1** (Scheme 1). In the first process, hydroperoxyl radical (HOO•) probably initiates this chain reaction through hydrogen abstraction.[6,10] Since the C-5 hydroperoxy intermediate was not isolated on TLC, it may be a compound with a short half-life and quite unstable on silica gel.[7]

Owing to the steric hindrance of the bridgehead methyl and the isopropyl groups, the stereoselectivities in the peroxidations



Scheme 1 Autoxidation pathway of carota-1,4-dien-14-al **1** into rugosal A **2** and compound **7**

at C-5 and C-2 were noticeable. A similar steric effect was observed in the epoxidation of compound **3**,[1] whose formation was highly stereoselective and whose structure was elucidated as 1*R*,2*S* from an NOE analysis. This example also showed the high steric hindrance of these groups during the epoxidation of C-1/C-2.

Compounds **5** and **6** together with **2** and **3** were also found as minor by-products. The epoxidation product from compound **1** was in accord spectroscopically with compound **5**.[1] The structure of compound **6**, M^+ 250, was elucidated by decoupling experiments. As is known in the self-conversion of hydroperoxy polyunsaturated fatty acids,[11] derivative **6** may be a precursor of compound **5**. Although compounds **6** and **4** must release active oxygen to be converted into products **5** and **2**, respectively, carotane aldehydes are oxygen acceptors and are converted into the corresponding carboxylic acids.

In the autoxidation of compound **1** dissolved in an organic solvent, the substrate **1** characteristically gave compound **7**, together with the oxygenated derivatives obtained on treatment with air. Compound **7** also supported the hypothetical peroxidation scheme for rugosal A **2**. When O_2 attack at the C-2 radical is blocked due to low partial pressure of dissolved O_2, the allyl radical is expected to be delocalized and equilibrated to stabilize as a tertiary radical at C-4. When O_2 reacts with the newly formed radical, an *exo* peroxyl radical should be formed at C-4, and the unstable intermediate should immediately rearrange into compound **7** through deformylation of C-14 with β-cleavage.[12]

In the formation of hanalpinol **9**, Morita *et al.* suggested that the guaiane peroxide was formed through autoxidation of guaia-6,9-diene in rhizomes of *Alpinia japonica*.[7] In *R. rugosa* leaves, however, compound **1** is possibly converted into A-2 **4** by a lipoxygenase- or a cycloxygenase-like enzyme.[9,13] The product **4** may then be reduced enzymatically (glutathione peroxidase, for example)[14] or non-enzymatically into rugosal A **2**. It is a matter of conjecture that the peroxidation process in *R. rugosa* tissues is thus regulated enzymatically. However, the suggestion that the peroxidation process results in possible membrane damage with bisulphite put forward by Peiser *et al.*[15] would mean an unregulated process leading to gross structural damage in plant tissues.[16] In *R. rugosa* leaves, such cell damage seems not to have been observed so far, therefore enzymatic regulation of the peroxidation system on compound **1** is possible. The hypothetical peroxidation enzyme in the leaf-tissues of *R. rugosa* is now a target for further research.

Experimental

General.—^{1}H NMR and ^{13}C NMR spectra were recorded at 500 MHz on a Bruker AM-500 spectrometer using $SiMe_4$ as internal standard. Coupling constants are given in Hz. Merck silica gel $60F_{254}$ precoated on glass plate was used for analytical or preparative TLC (PTLC); R_f-values refer to spots which quench the impregnated fluorescing agent under UV λ_{254} nm light or give red colour reactions with a peroxide test using *N*, *N*-dimethyl-*p*-phenylene diamine sulphate reagent. UV–visible spectra were recorded on a Hitachi U-3210, and mass spectra

Table 1 Comparison of proton and carbon chemical shift values of compound **4** with those of rugosal A **2** (500 and 125 MHz; in C_6D_6; $SiMe_4$ as internal standard)

A-2 **4**			Rugosal A **2**	
Proton (*J*/Hz)	Carbon	Carbon no.	Proton (*J*/Hz)	Carbon[a]
	95.4	C-1		94.8
4.634 d (5.7)	81.5	C-2	4.198 dd (11.7, 6.4)	69.1
6.257 d (5.7)	146.3	C-3	6.031 dd (6.4, 1.1)	149.4
	148.3	C-4		146.5
5.342 dd (5.2, 2.4)	69.5	C-5	5.277 ddd (5.1, 2.6, 1.1)	70.1
1.920 dd (14.1, 5.2)	41.5	C-6	1.942 dd (13.9, 5.1)	42.0
1.352 dd (14.1, 2.4)			1.384 dd (13.9, 2.6)	
	40.0	C-7		39.6
1.642 ddd (12.5, 11.5, 7.4)	38.7	C-8	1.630 ddd (12.8, 12.5, 7.3)	38.5
1.227 br dd (12.5, 7.1)			1.275 br dd (12.5, 6.6)	
1.277 br ddd (12.9, 9.0, 7.4)	20.4	C-9	1.295 m	20.2
1.078 dddd (12.9, 11.5, 10.6, 7.1)			1.105 dddd (12.8, 10.6, 10.3, 6.6)	
2.032 ddd (10.6, 9.0, 2.6)	55.1	C-10	1.912 ddd (10.6, 9.7, 2.6)	54.6
2.641 d sept. (6.8, 2.6)	26.2	C-11	2.739 d sept. (6.8, 2.6)	24.8
0.801 d (6.8)	23.2	C-12	0.845 d (6.8)	22.8
0.689 d (6.8)	17.9	C-13	0.732 d (6.8)	18.2
9.153 s	190.7	C-14	9.046 s	190.7
0.359 s	24.8	C-15	0.393 s	25.9
ca. 8.94 br s		exchangeable proton	2.758 d (11.7)	

[a] 78 MHz.

were recorded on JEOL JMS DX-300 and JEOL JMS 015G-2 instruments.

Air Oxidation and Product Isolation.—Carota-1,4-dien-14-al **1** used as substrate for the autoxidation was of *R. rugosa* origin, which has been isolated in the previous work [1] and preserved in a cold room in the dark as a mixture with β-carotene in n-hexane. Before use, the substrate was chromatographed once (TLC) and developed in n-hexane–EtOAc, 20:1. Spectroscopically pure compound **1** (5.1 mg) was dissolved in n-hexane (~10 cm^3) in a 30 cm^3 flask, and the solvent was evaporated off. The flask on whose wall a thin film of compound **1** had been prepared was then kept at 40 °C in the dark. After 3 h the film was recovered by washing with EtOAc and the mixture was analysed by TLC. Purification was carried out by rapid PTLC. By this method, compounds **2**, **3**, **4**, **5** and **6** (R_f 0.42, 0.02, 0.33, 0.69 and 0.56, respectively in EtOAC–n-hexane, 1:3), all comparatively stable on TLC plates, were obtained. From this reaction, a quantity (0.7 mg, 13%) of the reactant was recovered.

Compound **4**.—A syrup (2.9 mg, 57%); *N,N*-dimethyl-*p*-phenylenediamine sulphate test was positive (clear pink); FDMS *m/z* 283 (M^+ + 1, 50%), 282 (M^+, 100), 268 (38), 266 (65) and 265 (78); FI-HRMS (Found: M^+, 282.144. Calc. for $C_{15}H_{22}O_5$: M, 282.147); EIMS *m/z* 266 (0.4%), 264 (0.3), 248 (0.6), 237 (0.7), 233 (1.2), 220 (1.4), 219 (1.3), 205 (1.7), 203 (1.9), 191 (3.8), 177 (2.1), 165 (2.5), 139 (10), 137 (5.9), 121 (9.2), 109 (11), 97 (21), 83 (17), 81 (17), 70 (12), 69 (100), 55 (54), 43 (34) and 41 (68). ^1H and ^{13}C NMR data are shown in Table 1.

Peroxide Reduction of Compound **4**.—To a solution of compound **4** (2.1 mg) in MeOH (2 cm^3) was added thiourea (1.2 mg) and the mixture was stirred for 1 h at room temperature. Substrate **4** completely disappeared, and two products were detected on TLC. Major product (R_f 0.43 in hexane–EtOAc, 3:1) (1.2 mg, 50%) was identical with compound **1** by TLC, EIMS, ^1H NMR and CD. The minor product **8** (R_f 0.12), (0.5 mg, 25%) was indistinguishable from the diol obtained by treatment of compound **1** with thiourea, in TLC, EIMS and ^1H NMR comparisons. Formation of this minor product is explicable by reduction of the C-2 *exo* peroxy and successive *endo* peroxy groups of compound **4**.

Product of the Peroxide Reduction Identical with Compound **1**.—Obtained as needles; CD (nm) 343 (Δε +6.8), 261 (Δε −1.8) and 230 (Δε + 0.4); *N,N*-dimethyl-*p*-phenylenediamine sulphate test was positive (orange/pink); vanillin–H_2SO_4 test: dark brown; EIMS *m/z* 266 (M^+, 0.6%), 248 (4.7), 237 (3.4), 220 (5.2), 205 (5.2), 191 (4.8), 177 (9.5), 165 (14), 149 (13), 135 (22), 121 (17), 109 (34), 91 (25), 81 (28), 69 (71), 67 (26), 55 (77), 44 (39), 43 (44) and 41 (100); δ_H(500 MHz; C_6D_6) 4.186 (1 H, dd, *J* 11.4 and 5.0, 2-H), 6.001 (1 H, dd, *J* 6.2 and 0.9 Hz, 3-H), 5.281 (1 H, ddd, *J* 5.0, 2.4 and 0.9 Hz, 5-H), 1.935 (1 H, dd, *J* 14.2 and 5.0 Hz, 6-H), 1.375 (1 H, dd, *J* 14.2 and 2.4 Hz, 6-H^b), 1.626 (1 H, ddd, *J* 12.9, 12.5 and 7.1 Hz, 8-H^a), 1.273 (1 H, dd, *J* 12.5 and 6.6 Hz, 8-H^b), 1.291 (1 H, m, 9-H^a), 1.095 (1 H, dddd, *J* 12.9,

12.9, 10.9 and 6.6 Hz, 9-H^b), 1.912 (1 H, ddd, *J* 10.9, 8.9 and 2.3 Hz, 10-H), 2.739 (1 H, double sept., *J* 6.8 and 2.3, 11-H), 0.840 (3 H, d, *J* 6.8, 12-H_3), 0.725 (3 H, d, *J* 6.8, 13-H_3), 9.047 (1 H, s, 14-H) and 0.378 (3 H, s, 15-H_3).

Product of the Peroxide Reduction, **8**.—This was obtained as a syrup; *N,N*-dimethyl-*p*-phenylenediamine sulphate test was negative; EIMS *m/z* 250 ($M^+ - H_2O$, 0.9%), 248 (2.2), 232 ($M^+ - 2H_2O$, 5.7), 217 (6.1), 216 (5.9), 201 (7.1), 189 (13), 173 (16), 161 (17), 140 (54), 121 (22), 105 (31), 97 (100), 91 (48), 81 (39), 77 (29), 69 (52), 55 (62) and 41 (99); δ_H(500 MHz; C_6D_6) 4.066 (1 H, incomplete dd, *J* 9.7 and 3.1, 2-H), 2.097 (1 H, d, *J* 9.7, 2-OH), 5.809 (1 H, d, *J* 3.1, 3-H), 4.600 (1 H, dd, *J* 8.1 and 6.8, 5-H), 2.150 (1 H, dd, *J* 14.6 and 8.1, 6-H^a), 1.732 (1 H, dd, *J* 14.6 and 6.8, 6-H^b), *ca.* 1.64 (1 H, m, 8-H^a), 1.055 (1 H, dd, *J* 12.2 and 7.2, 8-H^b), 1.428 (1 H, m, 9-H^a), *ca.* 1.28 (1 H, m, 9-H^b), 1.923 (1 H, ddd, *J* 9.0, 9.0 and 3.5, 10-H), 2.550 (1 H, double sept., *J* 6.8 and 3.5, 11-H), 0.877 (3 H, d, *J* 6.8, 12-H_3), 0.772 (3 H, d, *J* 6.8, 13-H_3), 8.918 (1 H, s, 14-H) and 0.412 (3 H, s, 15-H_3).

Compound **5**.—The product (0.1 mg) was identical (NMR) with that obtained by treatment of compound **1** with *m*-chloroperbenzoic acid. The ^{13}C NMR spectra (C_6D_6) of compound **5**, obtained from a decomposed mixture in a partly purified solution of compound **1**, and of the epoxidation product of **1** were both measured. These two ^{13}C NMR spectra were identical in their carbon chemical shifts. Thus, compound **5** was identical with the epoxide of compound **1**, whose stereostructure was elucidated by NOE measurements as being 1*R*,2*S*. Compound **5** was obtained as a syrup; *N,N*-dimethyl-*p*-phenylenediamine sulphate test was negative (pale yellow); EIMS *m/z* 234 (M^+, 7.0%), 216 (49), 201 (21), 187 (18), 173 (48), 160 (25), 159 (24), 152 (32), 149 (28), 145 (50), 131 (49), 119 (30), 109 (40), 107 (36), 105 (40), 91 (56), 81 (33), 79 (33), 71 (91), 69 (32), 55 (36), 43 (100) and 41 (80); δ_H(500 MHz; C_6D_6) 3.004 (1 H, d, *J* 5.1, 2-H), 3.342 (1 H, br dd, *J* 19.8 and 5.1, 3-H^a), 2.294 (1 H, br d, *J* 19.9, 3-H^b), 5.873 (1 H, ddd, *J* 7.1, 2.3 and 2.3, 5-H), 2.393 (1 H, br d, *J* 17.3, 6-H^a), 1.826 (1 H, br dd, *J* 17.3, 7.1, 6-H^b), 1.486 (1 H, m, 8-H^a), 1.192 (1 H, dd, *J* 11.4 and 6.4, 8-H^b), 1.415 (1 H, m, 9-H^a), 1.266 (1 H, ddd, *J* 11.7, 11.5 and 6.4, 9-H^b), 2.117 (1 H, ddd, *J* 11.7, 8.0 and 4.5, 10-H), 1.475 (1 H, m, 11-H), 0.729 (3 H, d, *J* 6.8, 12-H_3), 0.612 (3 H, d, *J* 6.8, 13-H_3), 9.152 (1 H, s, 14-H) and 0.620 (3 H, s, 15-H_3); δ_C(125 MHz; C_6D_6) 51.9 (C-2), 25.4 (C-3), 139.3 (C-4), 151.5 (C-5), 40.2 (C-6), 43.8 (C-7), 40.3 (C-8), 22.2 (C-9), 57.9 (C-10), 26.7 (C-11), 20.6 (C-12), 17.5 (C-13), 194.5 (C-14) and 21.6 (C-15). The C-6 and C-8 signal assignments may be interchanged. The C-1 carbon signal was invisible. The sample used for the ^{13}C NMR spectrum was prepared from an oxidized mixture of a fraction (containing *ca.* 100 mg of compound **3**) by HPLC (Unisil Q 100-5; 2.5% Pr^{i}OH–n-hexane). C-1 of synthetic compound **5** was detected at δ_C 71.5. Furthermore, hydrogenation values of all the carbons were revealed by a DEPT experiment, with compatible results for the carbon assignments of compound **5** shown above. NOEs between 2-H and 15-H_3, C-2 and 12-H_3 and 2-H and 13-H_3 in compound **5** indicated the absolute configuration of the epoxy ring as 1*R*,2*S* (*cis*).

Compound **6**.—Obtained as a semisolid (<0.1 mg); *N,N*-dimethyl-*p*-phenylenediamine sulphate test was positive (pinkish red); λ_{max}(MeOH) 229 nm; FIMS *m/z* 251 (M^+ + 1, 22%), 250 (M^+, 100), 234 (15) and 232 (21); FI-HRMS (Found: M^+, 250.160. Calc. for $C_{15}H_{22}O_3$: M, 250.157); EIMS *m/z* 232 ($M^+ - H_2O$, 2.4%), 216 (12), 203 (6.9), 201 (7.0), 187 (4.7), 173 (15), 160 (8.8), 159 (9.9), 149 (14), 145 (19), 131 (20), 117 (15), 107 (28), 105 (19), 91 (36), 79 (22), 77 (23), 55 (22), 44 (57), 41 (48) and 40 (100); δ_H(500 MHz; C_6D_6) 5.027 (1 H, br dd, *J* 5.6 and 1.3, 2-H), 3.452 (1 H, br dd, *J* 14.5 and 5.6, 3-H^a), 1.949 (1 H, br d, *J* 14.5, 3-H^b), 6.205 (1 H, br dd, *J* 8.2 and 6.3, 5-H), 2.049 (1 H, dd, *J* 13.4 and 6.3 Hz, 6-H^a), 1.905 (1 H, br dd, *J* 13.4 and 8.2, 6-H^b), 1.436 (1 H, ddd, *J* 11.8, 7.6 and 1.6, 8-H^a), 1.316 (1 H, ddd, *J* 11.8, 9.4 and 9.1, 8-H^b), 2.083 (1 H, ddd, *J* 16.8, 9.4 and 7.6, 9-H^a), 1.970 (1 H, ddd, *J* 16.8, 9.1 and 1.6, 9-H^b), 2.747 (1 H, sept., *J* 6.9, 11-H), 0.885 (3 H, d, *J* 6.9, 12-H_3), 0.824 (3 H, d, *J* 6.9, 13-H_3), 9.268 (1 H, s, 14-H), 0.865 (3 H, s, 15-H_3) and 9.126 (1 H, s, exchangeable, 2-OOH).

Compound **7**.—The product **7**, found only in the dilute EtOAc solution of compound **3** (*ca.* 50 mg/200 cm^3 solvent), was isolated by PTLC (R_f 0.67 in hexane–EtOAc, 4:1) as syrup (6.7 mg); *N,N*-dimethyl-*p*-phenylenediamine sulphate test was positive (clear pink); FDMS *m/z* 473 ($2M^+$ + 1, 10%), 472 ($2M^+$, 7), 237 (M^+ + 1, 100), 236 (M^+, 98) and 110 (24); FI-HRMS (Found: M^+, 236.144. Calc. for $C_{14}H_{20}O_3$: M, 236.141); EIMS *m/z* 192 (7.4%), 177 (2.8), 150 (33), 149 (7.6), 135 (7.5), 121 (4.1), 108 (11), 107 (100), 91 (14), 79 (23), 44 (67) and 41 (14); δ_H(500 MHz; C_6D_6) 6.310 (1 H, d, *J* 11.2, 2-H), 6.372 (1 H, dd, *J* 11.3 and 2.1, 3-H), 4.458 (1 H, ddd, *J* 5.4, 2.8 and 2.1, 5-H), 1.790 (1 H, dd, *J* 14.0 and 5.4, 6-H^a), 1.489 (1 H, dd, *J* 14.0 and 2.8, 6-H^b), 1.463 (1 H, ddd, *J* 12.9, 12.0 and 6.1, 8-H^a), 1.258 (1 H, dd, *J* 12.0 and 5.7, 8-H^b), 1.292 (1 H, m, 9-H^a), 0.847 (1 H, dddd, *J* 12.9, 12.4, 11.7 and 5.7 Hz, 9-H^b), 1.562 (1 H, 11.7, 8.1 and 8.0 Hz, 10-H), 1.314 (1 H, double sept., *J ca.* 8 and 6.5, 11-H), 0.773 (3 H, d, *J* 6.5, 12-H_3), 0.591 (3 H, d, *J* 6.5, 13-H_3) and 0.341 (3 H, s, 15-H_3); δ_C(125 MHz; C_6D_6; COM and DEPT) 92.7 (C-1), 141.9 (C-2), 135.7 (C-3), 203.3 (C-4), 82.9 (C-5), 39.6 (C-6), 44.7 (C-7), 36.6 (C-8), 25.0 (C-9), 55.2 (C-10), 28.8 (C-11), 23.0 (C-12), 20.0 (C-13) and 25.0 (C-15).

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