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Marine Terpenes and Terpenoids. I. Structures of Four Cembrane-type Diterpenes; Sarcophytol-A, Sarcophytol-A Acetate Sarcophytol-B, and Sarcophytonin-A, from the Soft Coral, *Sarcophyton glaucum*

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The lipid extract of the soft coral, *Sarcophyton glaucum*, was found to contain significant amounts of cembrane-type diterpenes. The structures of four major components, sarcophytol-A, sarcophytol-A acetate, sarcophytol-B, and sarcophytonin-A were characterized on the basis of spectral data and degradative studies by ozonolysis. Sarcophine and 16-deoxosarcophine, which were reported to be abundant in *S. glaucum* collected in the Red Sea, were not found yet.

Keywords——sarcophytol-A; sarcophytol-A acetate; sarcophytol-B; sarcophytonin-A; cembrane diterpenes; *Sarcophyton glaucum*; Alcyonacea

During the past decade, a number of cembrane-type diterpenes have been found in coelenterates belonging to the orders Corgonacea and Alcyonacea (soft corals), which are common species in tropical and subtropical coral reefs.[2,3] In some cases they have been reported to have biological activities.[4]

A cembrane-type lactone, sarcophine (I), which is believed to be a repellent against predators, and its 16-deoxo derivative (II) had been found in the soft coral, *Sarcophyton glaucum*, in the Red Sea.[5] Considerable variation in the relative contents of these two compounds and related minor constituents was also noted[5] depending on the collection period and location.

This species is common in the coral reefs of the Indian and Pacific Oceans, and the present study on the lipid extract of *S. glaucum* collected in Ishigaki Island, Okinawa Prefecture, revealed that it also contains cembrane-type diterpenes. However, the presence of compounds corresponding to I or II, which were reported to amount to 4% in some cases, has not yet been confirmed.

Repeated chromatography of the lipids on a column of silica gel yielded four major components, sarcophytol-B (IIIa), sarcophytol-A (IVa), sarcophytol-A acetate (IVb), and sarcophytonin-A (V). Only sarcophytol-B (IIIa) was obtained in a crystalline state. At least a half of the lipids is believed to be cembrane-type diterpenes, and sarcophytol-A (IVa) constitutes about one-third of the total lipids. These four cembrane-type diterpenes were found to be extremely susceptible to autoxidation when purified, and in the case of V brief exposure to air converts it to a mixture of degradation products. Sarcophytol-B (IIIa), mp 125—126.5°, $[\alpha]_D$ +164°, corresponded to a molecular formula of $C_{20}H_{32}O_2$ on the basis of its elemental analysis and mass spectrum (MS) (M^+, *m/e* 304), containing five unsaturations.

1) Location: *Kita-12, Nishi-6, Sapporo 060, Japan.*
2) D.J. Faulkner, *Tetrahedron*, **33**, 1421 (1977).
3) J.C. Braekman, "Marine Natural Products Chemistry," ed. by D.J. Faulkner and W.H. Fenical, Plenum Press, New York, 1977, p. 5.
4) *a*) B. Tursch, J.C. Braekman, D. Daloze, M. Herin, R. Karlsson, and D. Losman, *Tetrahedron*, **31**, 129 (1975); *b*) A.J. Weinheimer, J.A. Matson, *Tetrahedron Lett.*, **1977**, 2923.
5) *a*) J. Bernstein, U. Shmueli, E. Zadock, Y. Kashman, and I. Neeman, *Tetrahedron*, **30**, 2817 (1974); *b*) Y. Kashman, E. Zadock, and I. Neeman, *Tetrahedron*, **30**, 3615 (1974).

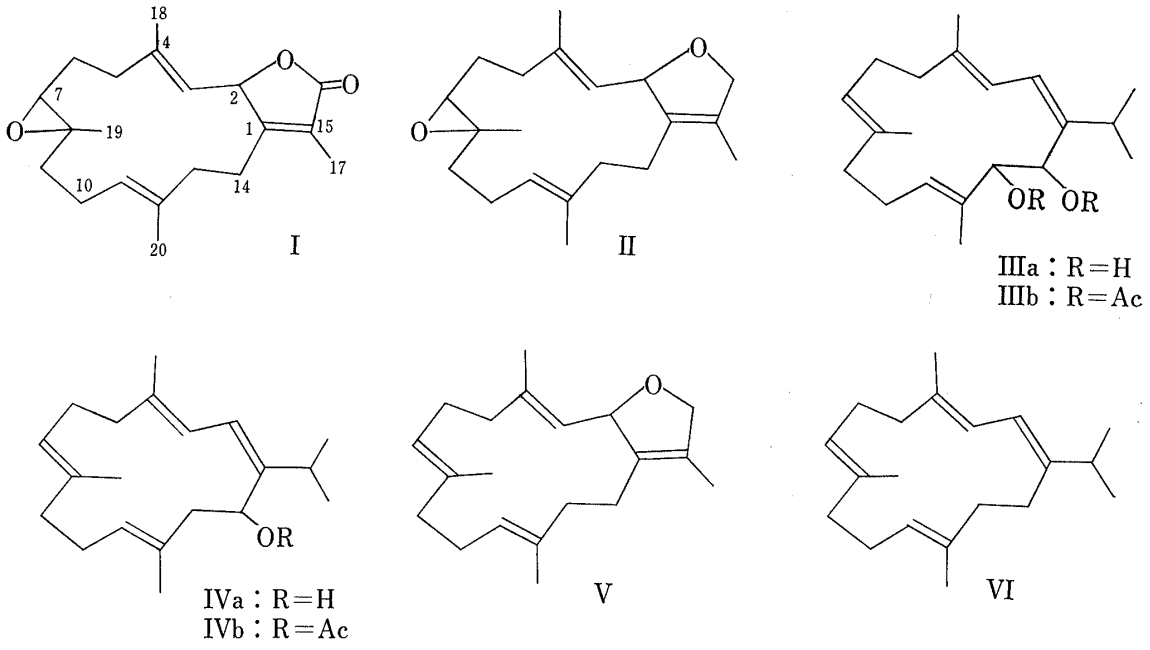

Fig. 1

The proton magnetic resonance (PMR) spectrum of IIIa in $CDCl_3$ indicated the presence of three vinylic methyl groups on quaternary carbons (δ 1.47, 3H, br. s; 1.63, d, J=1.2 Hz; 1.74, 3H, d, J=1.3 Hz) and an isopropyl group (δ 1.07, 3H, d, J=7 Hz; 1.13, 3H, d, J=7 Hz: 2.57, 1H, sept, J=7 Hz).

The isopropyl group was also found to be linked to a quaternary carbon on the basis of the multiplicity of its methine signal at δ 2.57. Irradiation around δ 1.1 and at 2.57 changed the signals of the methine and methyl groups into singlets. The infrared (IR) absorptions at 1600 and 1660 cm^{-1}, and the ultraviolet (UV) absorption at 253 nm (ε 17400) of IIIa indicate the presence of a conjugated diene, present in a medium sized ring. Its olefinic signals appeared at δ 5.90 and 6.20 as an AB system (d, J=11.5 Hz) showing it to be a 1,1,4,4-tetrasubstituted diene. The deshielded vinylic methyl at δ 1.74 is linked to the conjugated diene and the slightly broadened nature of the signal at δ 5.90 suggests allylic coupling with the methyl at δ 1.74. The oxygen atoms of IIIa were attributed to a glycol moiety, whose hydroxy-methine signals were observed as two doublets at δ 3.93 (J=8 Hz) and 4.73 (J=8 Hz) as an AB system. Irradiation of the signal at δ 3.93 changed the doublet at δ 4.73 into a singlet and, similarly, irradiation at δ 4.73 changed the doublet at δ 3.93 into a singlet. Acetylation of IIIa gave a diacetate (IIIb), mp 74—75°, which showed two acetoxy-methine signals at δ 5.34 and 6.15 (each doublet, J=10 Hz). Since the ^{13}C-nuclear magnetic resonance (^{13}C–NMR) spectrum of IIIa (Table I) shows no signal due to sp^3 quaternary carbons, both of the quaternary centers adjacent to the glycol are unsaturated.

Compound IIIa bears two additional isolated trisubstituted double bonds, each of which carries a methyl group, and their olefinic signals were observed at δ 4.94 and 5.40 as degenerated triplets (J=7 Hz).

The remaining PMR signals are those of allylic protons which appear between δ 1.9 and 2.3 as a sharp envelope, integrating as 8H, so that IIIa is a monocyclic compound, based on the unsaturation number. The ^{13}C–NMR spectrum (Table I) suggests that eight allylic protons are ascribable to four vinylic methylenes. These chemical shifts account for all 20 carbons in IIIa. Combination of the four vinylic methylenes with a 1,1,4,4-terasubstituted diene, a secondary glycol which is surrounded by quaternary centers, and two isolated trisubstituted double bonds would result in a structure having a 14-membered ring with three methyls and one isopropyl group on double bonds.

Table I. ^{13}C-NMR Chemical Shifts (ppm) in Sarcophytol-B (IIIa), Sarcophytol-A (IVa), and Sarcophytonin-A (V)

Carbons	IIIa	Carbons	IVa	Carbons	V
1	144.1	1	146.8	1	140.3
2, 3	121.3, 121.8	2, 3	121.2, 120.4	2	84.0
4, 8, 12	134.3, 134.3	4, 8	134.5, 135.8	3, 7, 11	124.0, 125.5
	136.2	5, 9	38.8, 39.6		125.8
5, 9	38.2, 39.7	6, 10	24.5, 25.5	4	127.1
6, 10	24.5, 25.3	7, 11	124.5, 125.2	5, 9	39.2, 40.4
7	127.3	12	131.3	6, 10, 14	23.5, 24.8, 25.9
11	123.9	13	44.5	8, 12, 15	133.2, 134.0
13, 14	73.2, 77.4	14	69.8		135.4
15	28.0	15	27.0	13	37.2
16, 17	23.7, 25.9	16, 17	24.4, 25.4	16	78.4
18, 19, 20	15.8, 16.4	18, 19	15.5, 16.3	17	10.2
	16.4	20	18.2	18, 19, 20	15.0, 15.2
					15.6

Ozonolysis of IIIa gave a carboxylic acid mixture, consisting of levulinic, acetic, and isobutyric acids.

Since the diene bears a single vinylic methyl and, accordingly an isopropyl group, the formation of isobutyric acid as well as levulinic acid establishes the position of the glycol group vicinal to isopropyl and also indicated that sarcophytol-B has a cembrane-type skeleton, as shown by IIIa. The geometry of each of the three double bonds which bear a methyl group, was assigned as *trans* from their signals in the ^{13}C–NMR spectrum, which showed a significant shielding in methyl groups, caused by vicinal carbons in the same way as in *trans*-polyisoprene.[6] In *cis*-polyisoprene, the shifts of vinylic methyls, which are *trans* to the vicinal carbons, occur at 23.6 ppm.[6] The conformation of the diene was also deduced to be *s-trans* from the position and intensity of UV absorption in IIIa compared to the *s-trans*-1,1,4,4-tetrasubstituted diene system in cembrene-C (VI, 252 nm, ε 18,400), which was isolated from a soft coral, *Nephthea* sp.[7]

Sarcopytol-A (IVa), $[\alpha]_D$ +141°, $C_{20}H_{32}O$ (M^+, *m/e* 288), was obtained as an oil, and its IR absorption at 3300 cm^{-1} showed it to be a monohydroxy compound. It showed PMR signals of an isopropyl group (δ 1.05, 3H, d, J=6.5 Hz; 1.11, 3H, d, J=6.5 Hz: 2.58. 1H, sept, J=6.5 Hz), three vinylic methyls (δ 1.47, 3H, s; 1.60, 3H, s; 1.74, 3H, d, J=1 Hz), and four olefinic protons (δ 4.9—5.1, 2H, m; 5.96, 1H, d, J=11.5 Hz; 6.16, 1H, d, J=11.5 Hz), suggesting a close structural analogy to sarcophytol-B (IIIa).

The presence of an *s-trans*-tetrasubstituted diene was confirmed by its PMR signals δ 5.96 and 6.16 (each d, J=11.5 Hz), and its UV (252 nm, ε 20000) and IR (1600 and 1660 cm^{-1}) absorptions. A single hydroxy-methine signal was observed at δ 5.0, which was enveloped by olefinic protons, and a sharp envelope of allylic proton signals, integrating as 10 H, was found between δ 2.0 and 2.3. Ozonolysis of IVa also afforded levulinic, acetic and isobutyric acids.

The presence of a diene at C-1 to C-4 in the cembrane skeleton, and the formation of isobutyric acid on ozonolysis restrict the position of the hydroxyl group to C-14. This was supported by the results of LIS–PMR using 0.25 molar equivalent of Eu(dpm)$_3$, which caused

6) J.B. Stothers, "Carbon-13 NMR Spectroscopy," ed. by A.T. Blomquist and H. Wasserman, Academic Press, New York, 1972, pp. 434—436, 453.
7) D.J. Vanderah, N. Rutledge, F.J. Schmits, and L.S. Ciereszko, *J. Org. Chem.*, **43**, 1614 (1978).

a significant down-field shift of the signals at C-16 and C-17 (0.53 and 0.65 ppm) and C-15 (1.65 ppm), as well as the hydroxy-methine (2.5 ppm), and one methylene (1.9 ppm). The shifts of the vinylic methyls were small and the signals which were originally observed at δ 1.47, 1.60, and 1.74 moved to δ 1.56, 1.85, and 1.97 ppm, respectively; the largest shift was less than 1/6 of that found at C-15. The signal at δ 1.47 was affected least, and was believed to be that of C-19 methyl. This relatively high-field vinylic methyl, which also occurs in IIIa, seems to be influenced by a diamagnetic effect of the diene system, though the relative conformations of IIIa and IVa are unknown.

This assignment was supported by a comparison of the ^{13}C–NMR spectra of IIIa and IVa (Table I). Hydroxylation at C-13 in IIIa caused a down-field shift of the β-carbons (C-12 and C-14) and an up-field shift of the γ-carbons (C-1 and C-20) compared with IVa. Sarcophytol-A also occurs as an acetate (IVb), $[\alpha]_D$ +127°, corresponding to about 5% of total lipids in *S. glaucum*; this was confirmed by hydrolysis of IVb and also by acetylation of IVa.

Sarcophytonin-A (V), also an oil, $[\alpha]_D$ −92°, exhibited a molecular ion peak at *m/e* 286. Its IR and UV spectra showed the absence of hydroxyl group and conjugated diene, and suggested the presence of an ethereal linkage. This was also suggested by the signals at δ 78.4 (t) and 84.0 (d) in its ^{13}C–NMR spectrum. The possibility of a trisubstituted epoxide ring, which has frequently been found in the cembrane-type diterpenes from soft coral,[2,3] was excluded by the absence of the corresponding methine or methyl signals around δ 3.0 and 1.3, respectively.[2,3] It showed four vinylic methyl signals at δ 1.59 (3H×2, br. s), 1.64 (3H, br. s), and 1.69 (3H, br. s), while isopropyl signals were absent. Allylic proton signals were observed between δ 2.0 and 2.5 as a broad envelope integrating as 12 H, while the remaining signals were at δ 4.48 (2H, br. d, J=4.5 Hz) 5.48 (1H, dt, J=10, 4.5 Hz), 5.06 (1H, br. d, J=10 Hz), and at δ 4.85 and 5.0 (each m).

The absence of a conjugated diene system and an isopropyl group, and the presence of four methyl groups, suggest that C-16 participates in the formation of the ethereal linkage, and are also reminiscent of 16-deoxosarcophine (II). In fact, the nature of the low-field signals is essentially the same as for II.[4] Irradiation at the vinylic methyl region sharpened most of the low-field signals, indicating allylic and/or homoallylic coupling with the vinylic methyl groups.

Irradiation at δ 5.06 (C-3) collapsed the signal at δ 5.48 (C-2, unresolved). Irradiation at δ 5.48 changed the two doublets at δ 5.06 and 4.48 (C-16) into singlets and, similarly, irradiation at δ 4.48 changed the signal at δ 5.48 into a broad doublet (J=10 Hz). This indicates the presence of a characteristic α,α'long-range dihydrofuran ring coupling in V, which was also reported in 16-deoxosarcophine (II) by Kashman *et al.*[5b] Compounds II and V differ only in the presence of a double bond at C-7 in V; this was confirmed by the detection of levulinic acid on ozonolysis of V and also by its ^{13}C–NMR data (Table I).

Sarcophytonin-A accounted for 5—10% of the total lipids in the material collected in June, 1977. However, it was not found in the material collected in the same place in September, 1978. The absolute configuration of each compound (IIIa to V) is currently under investigation.

Experimental

Melting points were determined on a Kofler hot stage apparatus and are uncorrected. IR spectra were recorded on a Hitachi 215 spectrometer, UV spectra on a Hitachi EPS 3T spectrometer in ethanol solution, and PMR and ^{13}C-NMR spectra on a JEOL FX-100 spectrometer operating at 100 (PMR) and 25.00 (^{13}C-NMR) MHz in $CDCl_3$ solution with tetramethylsilane (TMS) as an internal standard. Mass spectra were determined with a JMS D-300 mass spectrometer.

Isolation and Fractionation of Lipids——Minced and partly dried *S. glaucum* (17 kg), which had been collected at Ishigaki Island in June, 1977, was extracted exhaustively first with MeOH and then with $CHCl_3$–MeOH (2:1). The dried residue amounted to *ca.* 5.5 kg. The combined extract was concentrated and

Table II. Chromatography of Lipids

Fraction	Solvent (1)	Eluted product (g)	Content
A	Hexane–benzene=3:1 (11)	48	Hydrocarbons and esterified sterols
B	Hexane–benzene=3:1 (4) Hexane–benzene=1:1 (26) Benzene (5.5)	173	Compounds IVb, IVa, and V
C	Benzene (5.5)	21	Carboxylic acids, IVa, V, and free sterols
D	Benzene (25.5)	30	Free sterols
E	Chloroform (36)	50	IIIa and other minor unidentified components
F	Chloroform (3)	7	Mainly IIIa

partitioned by Folch's method,[8] giving 1.6 kg of lipids as a viscous dark-brown oil. A part of the lipids (400 g) was dissolved in hexane and passed through a column containing 2.5 kg of silica gel (70—230 mesh, Merck). The less polar portion of the lipids was fractionated roughly into fractions A to F (Table II).

Repeated column chromatography of fraction B with hexane–$CHCl_3$ (5:1) gave a pure sample of IVb, and that with hexane–$CHCl_3$ (1:1) afforded the pure samples of IVa and V as oils. Fraction F was dissolved in $(CH_3)_2CO$ and the crystals were collected. The *Rf* values of the compounds on thin-layer chromatography (TLC) (Silica gel HF_{254}, Merck) were 0.72 (IVb), 0.31 (V), 0.22 (IVa) ($CHCl_3$ as a solvent), and 0.39 (IIIa) ($CHCl_3$–$(C_2H_5)_2O$=9:1). Although complete separation of each of these components was not performed due to their instability, their estimated contents in total lipids, as judged by TLC, were roughly 1% (IIIa), 35—40% (IVa), 5% (IVb), and 5—10% (V) in this study.

Sarcophytol-A (IVa)——Colorless oil, $[\alpha]_D$ +141° (c=1.10, $CHCl_3$). IR ν_{max}^{neat} cm^{-1}: 3330, 1660, 1600, 1100, 840. UV ν_{max}^{EtOH} nm: 252 (ε 20000). *Anal.* Calcd. for $C_{20}H_{32}O$: C, 83.27; H, 11.18. Found: C, 83.18; H, 11.06. MS *m/e*: 288 (M^+), 273 (M^+-CH_3), 270 (M^+-H_2O), 245 ($M^+-C_3H_7$), 137 (base peak), 43. PMR: see the text. ^{13}C-NMR: see Table I.

Sarcophytol-A Acetate (IVb)——Colorless oil, $[\alpha]_D$ +127° (c=0.84, $CHCl_3$). IR ν_{max}^{neat} cm^{-1}: 1740, 1665, 1630, 1240, 1040, 860, 840. *Anal.* Calcd. for $C_{22}H_{43}O_2$: C, 79.95; H, 10.37. Found: C, 79.77; H, 10.58. MS *m/e*: 330 (M^+), 315 (M^+-CH_3), 270 (M^+-AcOH), 109 (base peak), 43. PMR ($CDCl_3$) δ: 1.06 (3H, d, J=7 Hz), 1.07 (3H, d, J=7 Hz), 1.49 (3H, br. s), 1.60 (3H, br. s), 1.75 (3H, d, J=1 Hz), 2.03 (3H, s), 2.15 (8H, sharp envelope), 2.3—2.7 (3H, m), 5.06 (2H, br. m), 6.0—6.3 (3H, unresolved m). Acetylation of IVa (Ac_2O–pyridine, room temp.) gave a compound which was identical with IVb as regards IR, PMR and mass spectra, and behavior on TLC. Its identity was confirmed by hydrolysis of IVb (5% KOH–MeOH, reflux 2 hr) to give IVa, which was identified by IR and TLC.

Sarcophytol-B (IIIa)——Colorless needles, mp 125—126.5° ($(CH_3)_2CO$). $[\alpha]_D$ +164° (c=1.06, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 3430, 3320, 1660, 1600, 830. UV λ_{max}^{EtOH} nm: 253 (ε 17400). *Anal.* Calcd. for $C_{20}H_{32}O_2 \cdot 1/4H_2O$: C, 77.74; H, 10.64. Found: C, 77.61; H, 10.73. MS *m/e*: 304 (M^+), 289 (M^+-CH_3), 261 ($M^+-C_3H_7$), 109 (base peak), 43. PMR: see the text. ^{13}C-NMR: see Table I.

Sarcophytol-B Diacetate (IIIb)——Sarcophytol-B (IIIa) was acetylated in the usual manner (Ac_2O–pyridine, room temp.), and crystallized from MeOH as colorless needles, mp 74—75°. $[\alpha]_D$ +284° (c=1.04, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 1740, 1735, 1250, 1020, 860, 830. *Anal.* Calcd. for $C_{34}H_{36}O_4 \cdot 1/2H_2O$: C, 72.51; H, 9.38. Found: C, 72.61; H, 9.28. MS *m/e*: 388 (M^+), 345 ($M^+-C_3H_7$), 328 (M^+-AcOH), 268 ($M^+-2\times AcOH$), 43 (base peak). PMR ($CDCl_3$) δ: 1.04 (3H, d, J=6.7 Hz), 1.08 (3H, d, J=6.9 Hz), 1.42 (3H, br. s), 1.63 (3H, br. s), 1.74 (3H, d, J=0.8 Hz), 2.01 (3H, s), 2.04 (3H, s), 2.0—2.3 (8H, sharp envelope), 2.53 (1H, sept, J=7 Hz), 5.04 (1H, br. t), 5.34 (1H, d, J=10 Hz), 5.47 (1H, br. t), 6.15 (1H, d, J=10 Hz). ^{13}C-NMR ($CDCl_3$) δ: 16.2 (q), 17.0 (q), 18.1 (q), 21.8 (q), 21.9 (q), 24.2 (q), 25.4 (t), 26.5 (t), 26.6 (q), 29.8 (d), 39.4 (t), 41.3 (t), 74.2 (d), 77.8 (d), 123.7 (d), 125.9 (d), 126.1 (d), 131.4 (d), 132.5 (s), 136.4 (s), 139.7 (s), 140.2 (s), 172.2 (s), 172.6 (s).

Sarcophytonin-A (V)——Colorless oil, $[\alpha]_D$ −92° (c=2.3, $CHCl_3$). IR ν_{max}^{neat} cm^{-1}: 1600, 1450, 1040, 860, 840. Elemental analysis gave no definite values due to its extreme instability. MS *m/e*: 286 (M^+), 271 (M^+-CH_3), 135 (base peak). PMR: see the text. ^{13}C-NMR: see Table I.

Ozonolysis of IIIa, IVa, and V——a) A solution of IIIa (100 mg) in 10 ml of $CHCl_3$ at −78° (dry ice-$(CH_3)_2CO$) was treated with 1.5% O_3 followed by oxidative work-up (H_2O_2 in H_2O, warmed to 60°) as reported by Dauben *et al.*[9]

8) J. Folch, M. Lees, and G.H.S. Stanley, *J. Biol. Chem.*, **226**, 497 (1957).
9) W.G. Dauben, W.E. Thiessen, and P.R. Rensnick, *J. Org. Chem.*, **30**, 1693 (1965).

The product mixture in H_2O was applied directly to GC–MS (Sp-1200, at 110°). Acetic and isobutyric acids were identified by comparison of their molecular ions (M^+ *m/e*: 60 and 88, respectively) and fragmentation patterns with those of authentic samples, and also by co-chromatography in GC. Levulinic acid was identical with an authentic sample on TLC (*Rf* 0.6, solvent, $CHCl_3$–$(C_2H_5)_2O$=5: 1), GC–MS (PEGS, at 130°; M^+ *m/e*: 130), and GC as the methyl ester.

b) Compound IVa was treated in the same way as in (a) and isobutyric, levulinic, and acetic acids were identified similarly. Acetic acid was thought to be derived by α-oxidation and degradation of a double bond or methyl ketone moiety. Such a side reaction has often been observed in the oxidative degradation of ozonides.[10]

c) Compound V was treated in the same way as in (a), and gave levulinic acid, which was identified as its methyl ester by GC and TLC.

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