

Article

Synthetic Studies with *Pinus elliotii*' Rosin Derivatives. Oxidation of Maleopimaric Anhydride Methyl Ester and Trimethyl Fumaropimarate

Sonia C. Hess^a, Maria I. S. Farah^b, Silvia Y. Eguchib^b, and Paulo M. Imamura^{c*}

^aDepartamento de Morfofisiologia/CCBS, Universidade Federal de Mato Grosso do Sul, CP 549, CEP 79070-900, Campo Grande - MS, Brazil

^bFundação Tropical de Pesquisas e Tecnologia André Tosello, R. Latino Coelho, 1301, CEP 13087-010, Campinas - SP, Brazil

^cInstituto de Química, Universidade Estadual de Campinas, CP 6154, CEP 13083-970, Campinas - SP, Brazil

A ozonólise do éster metílico do anidrido maleopimárico na presença de tetracianoetileno produziu um epóxido e um ozonídeo, e a ozonólise do fumaropimarato de trimetila, seguida por tratamento com Me₂S, forneceu um epóxido, um dieno, um ceto-ácido e um produto de oxidação alílica. Alguns dos compostos obtidos apresentaram atividade antibacteriana contra *Staphylococcus aureus*, *Bacillus subtilis* e *Micrococcus luteus*.

Ozonolysis of maleopimaric anhydride methyl ester in the presence of tetracyanoethylene led to an epoxide and an ozonide. Ozonolysis of the trimethyl fumaropimarate, followed by treatment with Me₂S, led to an epoxide, a diene, a keto-acid and an allylic oxidation product. Some of the compounds obtained were active against *Staphylococcus aureus*, *Bacillus subtilis* and *Micrococcus luteus*.

Keywords: *Pinus elliotii*' rosin, oxidation, maleopimaric anhydride methyl ester, trimethyl fumaropimarate.

Introduction

In the search for biologically active substances, we have envisioned that maleopimaric anhydride methyl ester (**1**) and fumaropimaric monomethyl ester (**2**), easily prepared through Diels-Alder reaction of abietane acids present in *Pinus elliotii*' rosin¹ with maleic anhydride and fumaric acid, respectively, would be potential starting materials mainly for the synthesis of some C-17 oxygenated naturally occurring polycyclic systems such as **4**, **5** and **6**.^{2,3} (Figure 1), if a way could be found to cleave the Δ¹³ double bond in C-ring. Oxidation of **1** and **2** with KMnO₄^{4,5}, RuO₄ and O₃⁶⁻⁸ has been already studied during the establishment of the correct stereochemistry of the Diels-Alder products. As part of our research program on the use of *Pinus elliotii*' rosin as chiral synthons⁹, we prepared **1** and **2** in order to cleave the Δ¹³ double bond using different conditions. In the present paper, we describe the results of our work on the oxidative transformations of **1** and trimethyl fumaropimarate (**3**), from which two new compounds were isolated and characterized by

spectroscopic data. Some products obtained during our investigation were submitted to biological assays and shown to be active against *Staphylococcus aureus*, *Bacillus subtilis* and *Micrococcus luteus*.

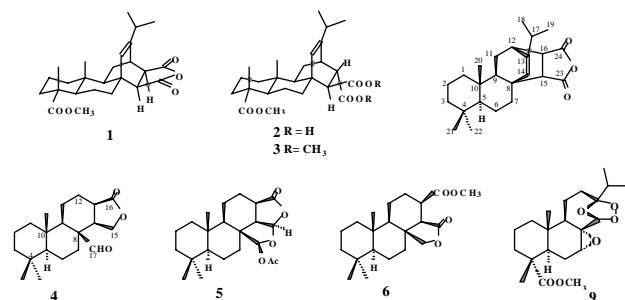


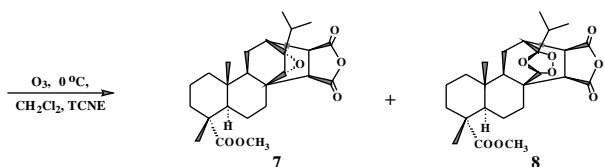
Figure 1

Results and Discussion

It is well known that the product of the ozonolysis of olefins depends on the solvent medium, i.e., protic or aprotic¹⁰. Zalkow^{6,7} and Halbrook⁸ reported that ozonolysis of **1** and **2** carried out in acetic acid leads to a mixture of many products.

*e-mail: imam@iqm.unicamp.br

Thus we decided to follow the literature suggestion¹¹ to carry out the ozonolysis of **1** at 0°C in CH₂Cl₂ solution in the presence of tetracyanoethylene (TCNE) in attempt to minimize the formation of the undesired products obtained previously by Zalkow^{4,6,7} and Halbrook⁸. Although in this case the mechanism is not clear, the catalytic action of TCNE on the alcoholysis of epoxides is well known due to its π -acid and one-electron acceptor properties¹². Carrying out the reaction under these conditions we isolated, after purification, a known epoxide **7** in 20% yield and the ozonide **8** in 7% yield (Scheme 1). This ozonide proved to be stable at low temperature¹³ and has not been observed before; this is not surprising since there are reports in the literature concerning the isolation of many stable ozonides^{14,15}. The ozonide was characterized by careful analysis of ¹H and ¹³C NMR data. The hydrogen H-14 appeared at δ 3.19 as a singlet and the carbons C-13 and C-14 of ozonide appeared, respectively, at δ 112.5 and 106.6. These chemical shifts are in good agreement with those observed for the ozonide of methyl abietate (**9**) previously prepared in our laboratory⁹.

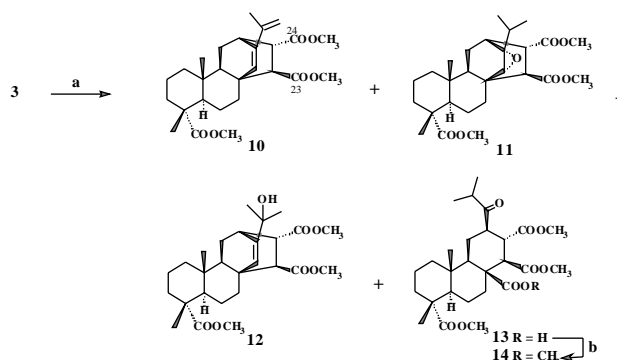


Scheme 1

On the other hand, epoxide **7** was the only product isolated from the ozonolysis of **1**, in the absence of tetracyanoethylene (-78°C; CH₂Cl₂, 20% yield), after treatment with Me₂S. An intractable mixture was obtained when the reaction mixture was treated with NaBH₄ or Zn/HOAc. The reaction of **1** with RuO₄ (25°C; CH₂Cl₂; NaIO₄/RuCl₃)^{16,17} also gave the epoxide **7** in 10% yield and did not cleave the Δ^{13} double bond to the corresponding keto-acid.

In contrast, ozonolysis of **3** (0°C; CH₂Cl₂), followed by treatment with Me₂S furnished, as expected, the known diene **10** (10% yield)⁶, epoxide **11** (19% yield), alcohol **12**⁶ (18% yield) and the desired keto-acid **13** which was characterized as tetramethyl ester **14** after methylation with diazomethane (32% yield) (Scheme 2). The ¹³C NMR spectrum of **14** showed two new carbonyl carbons [at δ 214.6 (C-13, ketone) and at δ 171.8 (C-14, ester)]¹⁸. The ¹H NMR spectrum showed four carbomethoxyl groups (at δ 3.57; 3.62; 3.64 and 3.66) which was confirmed by ¹³C NMR data (at δ 51.7, 51.8 and 51.9 (2x)).

Although Zalkow^{4,5} reported the oxidation of **3** with KMnO₄ in basic medium leading to a mixture of products, our protocol was carried out using recent and improved conditions described in the literature: a)¹⁹ KMnO₄ with dibenzo-

Scheme 2. a) O₃, CH₂Cl₂, 0°C; b) CH₂N₂

18-crown-6; CH₂Cl₂; 25°C; 24 h; b)²⁰ KMnO₄ supported on silica gel; CH₂Cl₂; 25°C; c)^{21,22} KMnO₄ (cat.) with NaIO₄; K₂CO₃; H₂O/*t*-BuOH; 70°C; 160 h, in hope to obtain a better yield of **13**. Nevertheless, in all cases, the reaction was incomplete and led to an intractable mixture of products.

The desired keto-acid **13** was obtained in only moderate yield and the present result showed that ozonation seems to be the best way to oxidize the hindered Δ^{13} double bond of **3**. The easiest ozonolysis of **3**, in comparison with **1**, is probably due to the less hindered α -orientation of the carbomethoxyl group at C-24.

The compounds obtained from the oxidation of **1** and **3** were evaluated for antibacterial activity by means of biotographic tests, following the methodology previously described²³⁻²⁴ using chloramphenicol as standard. Compounds **1**, **2**, **12** and **14** proved to be active against gram-positive bacteria (*Bacillus subtilis*, *Micrococcus luteus*, *Staphylococcus aureus*) and were submitted to tests for the determination of the Minimal Inhibitory Concentration (MIC) following the cylinder-cup method²⁵. MIC values are presented in Table 1 and as can be seen, compound **12** was the most active against the gram-positive bacteria. In contrast, *Salmonella choleraesuis*, a gram-negative bacteria, was resistant to every substance tested.

Further investigation of the synthesis of some C-17 oxygenated polycyclic systems are underway in our laboratory.

Experimental

All melting points were determined on Kofler block and are uncorrected. TLC was performed on silica gel with fluorescent indicator on glass plates (Silica gel GF₂₅₄, Merck). Column chromatography was carried out on silica gel (Silica gel 60, 0.06-0.2 mm, Merck). NMR spectra were measured on Varian Gemini - 300 and Bruker ACP - 300 (¹H NMR at 300 MHz, ¹³C at 75.6 MHz) instruments, in deuterated chloroform with tetramethylsilane as internal standard. IR spectra were measured on FT IR Perkin-Elmer 16 PC. Optical

measurements were run in chloroform, on a polarimeter Carl Zeiss Jena Polamat A. Mass spectra were measured on a high resolution Micromass Autospec (Manchester, UK) spectrometer, using the EI (electron energy 70 eV), source temperature 200°C and resolution 10,000.

Maleopimaric anhydride methyl ester (1). *Pinus elliotii*' rosin was esterified with dimethylsulfate (NaOH, Na₂CO₃/H₂O, Me₂SO₄). After purification by column chromatography with hexane-ethyl acetate mixture (95:5), the mixture of methyl esters was reacted with maleic anhydride according to the literature⁴ to give compound **1** (38 % yield) as a colorless crystals: [α]_D²⁰ +27.4 (c 2.5, CHCl₃); mp 211-213°C; IR (KBr, cm⁻¹): 2927, 1781, 1720, 1462, 1242, 1222, 1082, 918; ¹H NMR (CDCl₃, δ ppm): 5.53 (s, 1H), 3.67 (s, 3H), 3.09 (m, 1H), 3.08 (dd, *J* = 3; 11 Hz, 1H), 2.71 (d, *J* = 11 Hz, 1H), 2.51 (dt, *J* = 3; 14 Hz, 1H), 2.23 (m, 1H), 1.80-1.35 (m, 11H), 1.30-1.15 (m, 2H), 1.15 (s, 3H), 1.00 (d, *J* = 6,8 Hz, 3H),

0.99 (d, *J* = 6,8 Hz, 3H), 0.59 (s, 3H); ¹³C NMR: see Table 2; HRMS Calcd. for C₂₅H₃₄O₅ 414.2406, Found 414.2407 (M⁺).

Trimethyl fumaropimarate (3). Compound **2** was prepared according to the literature procedure²⁶ in 46 % yield. Treatment of **2** with ethereal diazomethane at 0°C led to product **3** (99 % yield) as a viscous liquid: [α]_D²⁰ +27.4 (c 2.5, CHCl₃); IR (KBr, cm⁻¹): 2951, 1730, 1710, 1435, 1385, 1267, 1199, 1177; ¹H NMR (CDCl₃, δ ppm): 5.33 (s, 1H), 3.68 (s, 3H), 3.64 (s, 3H), 3.57 (s, 3H), 2.86 (m, 1H), 2.78 (d, *J* = 6 Hz, H-22), 2.57 (dd, *J* = 3; 6 Hz, 1H), 2.38 (m, 1H), 1.10 (s, 3H), 1.80-1.20 (m, 12H), 1.04 (d, *J* = 7 Hz, 3H), 1.03 (d, *J* = 7 Hz, 3H), 1.10-0.80 (m, 2H), 0.56 (s, 13H); ¹³C NMR: see Table 2; HRMS Calcd. for C₂₇H₄₀O₆ 460.2824, Found 460.2822 (M⁺).

Ozonolysis of maleopimaric anhydride methyl ester (1). A rapid stream of ozone containing approximately 3% of O₃ in O₂ was passed through a solution of **1** (250 mg;

Table 1. Minimal Inhibitory Concentration (MIC) in mg/mL for compounds **1**, **2**, **12** and **14**.

Compound	<i>Bacillus subtilis</i> CCT 0089	<i>Micrococcus luteus</i> CCT 2720	<i>Staphylococcus aureus</i> CCT 4295
1	100	>250	200
2	100	>250	100
12	50	100	50
14	75	100	100

Table 2 - ¹³C-NMR Chemical Shifts of **1**, **3**, **7**, **8**, **10-12**, **14** (δ in ppm from TMS, CDCl₃)

Carbon	1	3	7	8	10	11	12	14 ^d
1	38.0	37.8	37.8	37.6	37.5	37.7	37.8	38.1
2	16.9	17.1	16.9	17.2	17.1	17.3	17.1	17.7
3	36.5	36.9	36.5	36.5	36.9	36.6	36.8	36.6
4	47.0	47.3	46.8	46.9	47.0	47.0	47.3	47.4
5	49.4	49.6	49.1	50.0	49.6	49.1	49.5	50.7
6	21.6	21.9	21.7	20.4	21.9	21.4 ^b	21.9	22.3 ^b
7	34.7	34.8	33.3	36.5	34.4	33.6	34.7	36.5
8	40.4	41.4	40.7	47.4	41.4	39.5	41.6	46.6
9	53.3	54.7	52.7	52.6	55.1	52.8	54.3	57.5
10	37.8	37.8	36.5	37.5	37.4	37.3	37.7	37.3
11	27.2	23.7	24.2	24.1	23.1	21.9 ^b	23.9	22.6 ^b
12	35.7	35.9	33.3	40.1	33.3	34.3	34.1	50.2
13	148.1	148.8	65.7	112.5	139.5	63.5	149.0	214.6
14	125.1	124.7	59.5	106.6	130.0	57.5	125.2	171.8
15	53.1	54.2	52.7	53.3	54.1	51.6	54.1	57.2
16	45.7	48.8	43.7	42.2	49.0	44.9	49.0	42.8
17	32.7	32.8	26.9	34.1	142.5	27.4	72.2	38.9
18	19.9 ^a	20.5	15.0 ^a	16.1 ^a	20.0	15.0 ^a	27.8 ^a	18.0 ^a
19	20.5 ^a	20.5	16.9 ^a	16.4 ^a	110.0	17.6 ^a	28.0 ^a	18.8 ^a
20	15.5	16.0	14.7 ^a	15.6 ^a	15.7	15.5 ^a	16.1	14.3
21	16.7	16.7	16.9	16.5	16.7	16.6	16.7	16.2
22	179.0	179.9	179.1	179.1	179.6	179.0	179.9	178.8
23	171.0	174.8	171.8	171.5	174.6	172.8	174.6	173.2
24	172.7	175.4	173.4	173.2	175.7	175.2	175.7	174.1
OMe	52.0	52.1 ^c	51.9	52.0	52.1 ^c	51.9 ^c	51.7 ^c	51.9 ^c
OMe		51.5 ^c			51.7 ^c	51.4 ^c	52.1 ^c	51.7 ^c
OMe		52.1 ^c			52.3 ^c	52.2 ^c	52.3 ^c	51.9 ^c
OMe								51.8 ^c

^{a, b, c} May be interchanged; ^d the original numbering for carbons was used

0.60 mmol) and tetracyanoethylene (102 mg; 0.79 mmol) in 20 cm³ of dichloromethane at 0°C for 2h until the blue color of excess of ozone was present. The solvent was removed in a rotary evaporator and residue was purified by column chromatography with a hexane-ethyl acetate mixture (7:3-1:1) obtaining two products:

Compound **7**: (51 mg; 0.12 mmol; 20%) as an amorphous solid: $[\alpha]^{20}_{\text{D}} -11.6$ (c 3.3 CHCl₃); mp 285-287°C; IR (KBr, cm⁻¹): 2923, 1776, 1718, 1467, 1384, 1261, 1231, 1095, 920; ¹H NMR (CDCl₃, δ ppm): 3.67 (s, 3H), 3.19 (s, 1H), 2.82 (dd, *J* = 3; 11 Hz, 1H), 2.80 (m, 1H), 2.64 (dt, *J* = 3; 14 Hz, 1H), 1.80-1.36 (m, 11H), 1.30-1.18 (m, 2H), 1.24 (m, 1H), 2.43 (d, *J* = 11 Hz, 1H), 1.95 (m, 1H), 1.18 (s, 3H), 1.04 (d, *J* = 6.8 Hz, 3H), 0.82 (s, 3H), 0.69 (d, *J* = 6.8 Hz, 3H); ¹³C NMR: see Table 2; HRMS Calcd. for C₂₅H₃₄O₆ 430.2355, Found 430.2357 (M⁺); Compound **8**: (20 mg; 0.04 mmol; 7%) as an amorphous solid: $[\alpha]^{20}_{\text{D}} -22.4$ (c 3.0 CHCl₃); mp 193-195°C; IR (KBr, cm⁻¹): 2940, 1776, 1719, 1461, 1255, 1233, 1105, 942; ¹H NMR (CDCl₃, δ ppm): 5.62 (s, 1H), 3.67 (s, 3H), 3.07 (t, *J* = 5 Hz, 1H), 3.03 (dd, *J* = 4.7; 11 Hz, 1H), 2.75 (d, *J* = 11 Hz, 1H), 2.50 (dt, *J* = 3; 14 Hz, 1H), 2.05 (m, 1H), 1.85-1.40 (m, 11H), 1.57 (m, 1H), 1.35-1.20 (m, 2H), 1.18 (s, 3H), 1.05 (s, 3H), 1.04 (d, *J* = 6.8 Hz, 3H), 0.98 (d, *J* = 6.8 Hz, 3H); ¹³C NMR: see Table 2; HRMS Calcd. for C₂₅H₃₄O₈ 462.2253, Found 462.2254 (M⁺).

Ozonolysis of trimethyl fumaropimarate (3). A rapid stream of ozone containing approximately 3% of O₃ in O₂ was passed through a solution of **3** (300 mg; 0.65 mmol) in 20 cm³ of CH₂Cl₂ at 0°C for 2h, until the blue color of excess of ozone was present. Then, oxygen was passed through the solution for removal of excess ozone, dimethyl sulfide (10 drops) was added, and the mixture was stirred 12h at room temperature. After removal the solvent, the residue was purified through column chromatography with hexane-ethyl acetate mixtures (5:5 - 3:7) giving two products:

Compound **10**: (29 mg; 0.06 mmol; 10%) as a viscous liquid: $[\alpha]^{20}_{\text{D}} + 50.2$ (c 2.0 CHCl₃); IR (KBr, cm⁻¹): 2949, 1728, 1434, 1387, 1254, 1188, 1172, 737; ¹H NMR (CDCl₃, δ ppm): 5.81 (s, 1H), 5.14 (s, 1H), 4.90 (s, 1H), 3.73 (s, 3H), 3.68 (s, 3H), 3.60 (s, 3H), 3.36 (m, 1H), 2.88 (d, *J* = 6 Hz, 1H), 2.58 (dt, *J* = 2; 6 Hz, 1H), 1.91 (s, 3H), 1.80-1.30 (m, 14H), 1.20-0.80 (m, 2H), 1.13 (s, 3H), 0.52 (s, 3H); ¹³C NMR: see Table 2; HRMS Calcd. for C₂₇H₃₈O₆ 458.2668, Found 458.2667 (M⁺); Compound **11**: (59 mg; 0.12 mmol; 19%) as a viscous liquid: $[\alpha]^{20}_{\text{D}} -10.3$ (c 3.0 CHCl₃); IR (KBr, cm⁻¹): 2948, 1727, 1434, 1386, 1254, 1195, 1175, 736; ¹H NMR (CDCl₃, δ ppm): 3.74 (s, 3H), 3.66 (s, 3H), 3.62 (s, 3H), 3.13 (s, 1H), 3.04 (m, 1H), 2.88 (dt, *J* = 3; 14 Hz, 1H), 2.70 (d, *J* = 6 Hz, 1H), 2.47 (m, 1H), 1.98 (m, 1H), 1.80-1.10 (m, 13H), 1.17 (s, 3H), 1.09 (d, *J* = 6.8 Hz, 3H), 0.80 (s, 3H), 0.76 (d, *J* = 6.8 Hz, 3H); ¹³C NMR: see Table 1; HRMS Calcd. for C₂₇H₄₀O₇ 476.2774, Found 476.2774 (M⁺);

Compound **12**: (50 mg; 0.11 mmol; 18%) as a viscous liquid: $[\alpha]^{20}_{\text{D}} + 18.3$ (c 3.2 CHCl₃); IR (KBr, cm⁻¹): 3490, 2950, 1724, 1435, 1387, 1254, 1194, 1175, 737; ¹H NMR (CDCl₃, δ ppm): 5.62 (s, 1H), 3.71 (s, 3H), 3.67 (s, 3H), 3.60 (s, 3H), 3.15 (brs, 1H), 2.86 (d, *J* = 6 Hz, 1H), 2.59 (dt, *J* = 3; 6 Hz, 1H), 1.90-0.90 (m, 15H), 1.39 (s, 6H), 1.13 (s, 3H), 0.58 (s, 3H); ¹³C NMR: see Table 2; HRMS Calcd. for C₂₇H₄₀O₇ 476.2774, Found 476.2775 (M⁺);

Compound **13**: (110 mg; 0.21 mmol; 32%). This compound was esterified with ethereal diazomethane at 0°C to give product **14** (111 mg; 0.21 mmol; 100%) as an amorphous solid: $[\alpha]^{20}_{\text{D}} + 37.0$ (c 2.5 CHCl₃); mp 45-46°C; IR (KBr, cm⁻¹): 2959, 1726, 1435, 1240, 1200, 1150; ¹H NMR (CDCl₃, δ ppm): 3.73 (s, 3H), 3.65 (s, 3H), 3.63 (s, 3H), 3.57 (s, 3H), 2.94 (dt, *J* = 4.5; 12 Hz, 1H), 2.80 (m, 1H), 2.41 (d, *J* = 12 Hz, 1H), 2.30 (d, *J* = 12 Hz, 1H), 2.05 (q, *J* = 12 Hz, 1H), 1.95-1.50 (m, 10H), 1.40-1.10 (m, 5H), 1.13 (s, 3H), 1.11 (d, *J* = 6.8 Hz, 3H), 1.08 (d, *J* = 6.8 Hz, 3H), 0.73 (s, 3H); ¹³C NMR: see Table 2; HRMS Calcd. for C₂₈H₄₂O₉ 522.2828, Found 522.2826 (M⁺).

Acknowledgements

We thank "Fundação de Amparo à Pesquisa do Estado de São Paulo" (FAPESP) for financial support and Drs. M. Nogueira, A. L. M. Porto, E. G. Magalhães and A. J. Marsaioli for their help and information on bioautographic tests. We also thank Dr. L.H.B. Baptistella for helpful discussion and Dr. F.Y. Fujiwara for reviewing the article.

References

- Seebacher, W.; Hüfner, A.; Haslingeer, E.; Weis, R. *Monatsh. Chem.* **1995**, *129*, 697 and references cited therein.
- Ksabati, M.B.; Schimtz, F.J. *J. Org. Chem.* **1987**, *52*, 3766.
- Miyamoto, T.; Sakamoto, K.; Arao, K.; Komori, T.; Higuchi, R.; Sasaki, T. *Tetrahedron* **1996**, *52*, 8187.
- Zalkow, L. H.; Ford, R. A.; Kutney, J. P. *J. Org. Chem.* **1962**, *27*, 3535.
- Zalkow, L. H.; Brannon, D. R. *J. Org. Chem.* **1964**, *29*, 1296.
- Zalkow, L. H.; Girotra, N. N. *J. Org. Chem.* **1963**, *28*, 2033.
- Zalkow, L. H.; Kulkarni, M. V.; Girotra, N. N. *J. Org. Chem.* **1965**, *30*, 1679.
- Halbrook, N. J.; Lawrence, R. V.; Dressler, R. L.; Blackstone, R. C.; Herz, W. *J. Org. Chem.* **1964**, *29*, 1017.
- Santos, C.; Rosso, C. R. S.; Imamura, P. *M. Synth. Commun.* **1999**, *29*, 1903.
- Bailey, P. S. In *Ozonization in Organic Chemistry*, Academic Press, New York, vol. I, 1978.
- Herz, W.; Blackstone, R. C. *J. Org. Chem.* **1969**, *34*, 1257. See also ref. 10; p.135.

12. Masaki, Y.; Miura, T.; Ochiai, M. *Bull. Chem. Soc. Jpn.* **1996**, *69*, 195 and references cited therein.
13. The ozonide could be stored in a freezer without decomposition at -5°C for few weeks.
14. Shiojima, K.; Masuda, K.; Ageta, K. *Chem. Pharm. Bull.* **1990**, *38*, 79.
15. Li, T.; Yang, Y.; Li, Y. *J. Chem. Res. (S)* **1993**, 30.
16. Carlsen, P. H. J.; Katsuki, T.; Martin, V. S.; Sharpless, K. B. *J. Org. Chem.* **1981**, *46*, 3936.
17. Funk, R. L.; Abelman, M. M. *J. Org. Chem.* **1986**, *51*, 3247.
18. For convenience, the numbering of carbons for **14** was used the same given for **3**.
19. Sam, D. J.; Simmons, H. F. *J. Am. Chem. Soc.* **1972**, *94*, 4024.
20. Ferreira, J. T. B.; Cruz, W. O.; Vieira, P. C.; Yonashiro, M. *J. Org. Chem.* **1987**, *52*, 3698.
21. Mitra, R. B.; Muljiani, Z.; Deshmukh, A. R. A. *S. Synth. Commun.* **1982**, *12*, 1063.
22. Aristoff, P. A.; Johnson, P. D.; Harrison, A. W. *J. Am. Chem. Soc.* **1985**, *107*, 7967.
23. Homans, A. L.; Fuchs, A. *J. Chromatogr.* **1970**, *75*, 327.
24. Hamburger, M. O.; Cordell, G. A. *J. Nat. Prod.* **1987**, *50*, 19.
25. Bruch, M. K. In *Methods of Testing Antiseptics: Antimicrobials Used Topically in Humans and Procedures for Hand Scrubs, in Disinfection, Sterilization and Preservation*, 4th. Ed., Lea & Febinger, Philadelphia, 1991.
26. Halbrook, N. J.; Lawrence, R. V. *J. Am. Chem. Soc.* **1958**, *80*, 368.

Received: November 25, 1998

FAPESP helped in meeting the publication costs of this article.