Antimicrobial Diterpenes of *Croton sonderianus*. II. *ent*-Beyer-15-en-18-oic Acid

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Fractionation of the antimicrobially active acidic portion of the hexane extract of the roots of *Croton sonderianus* Muell. Arg. (Euphorbiaceae) allowed the characterization of a new diterpene acid, *ent*-beyer-15-en-18-oic acid.

KEY WORDS: *Croton sonderianus;* Euphorbiaceae; diterpene; *ent*-beyerene; antimicrobial.

INTRODUCTION

Croton sonderianus Muell. Arg. is a shrub widespread in the Brazilian Northeast and known in the region as "marmeleiro preto." The bark is used in folk medicine for treatment of gastric diseases. Hexane or benzene extracts of its heartwood and roots have shown antifungal and antibacterial activity (Tables I and II). From the benzene extract of the heartwood was isolated a new clerodane diterpene possessing a spirolactone ring system and named sonderianin, whose structure and stereochemistry were determined by X-ray crystallography (1), a known coumarin, and two new cleisthantane diterpenes (2).

Extraction of the dried, ground entire roots of the plant with hexane and concentration under pressure provided a golden brown viscous extract from which sonderianin precipitated during storage at 5°C. The sonderianin was filtered, and the remaining viscous oil was partitioned between benzene and 20% NaOH in water to provide neutral and acidic fractions. The acidic fraction showed somewhat greater antimicrobial activity (3) and was further fractionated by chromatography over silica (Tables I and II).

MATERIALS AND METHODS

General

Melting points were determined in either a Fisher-Johns digital melting-point analyzer Model 355 or a Thomas–Hoover Uni-Melt capillary apparatus and were not corrected. Microanalyses were performed on a Hewlett-Packard 185B CHN analyzer at the Department of Medicinal Chemistry, University of Kansas, Lawrence. Infrared spectra (IR) were taken as KBr pellets on a Perkin–Elmer 281B spectrometer. Specific rotations ($[\alpha]_D$) were obtained on a Perkin–Elmer 141 automatic polarimeter using chloroform solutions. Mass spectra (MS) were obtained on a Finnigan 3200 GC/MS mass spectrometer coupled to an INCOS data system operating in electron impact mode at 70 eV. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Varian EM-390 (90 MHz) operating in a CW mode, using $CDCl_3$ solutions and tetramethylsilane (TMS) as internal standard. The carbon-13 nuclear magnetic resonance experiments (^{13}C NMR) were performed on a JEOL JNM-FX60 (15.03 MHz), which was operated in Fourier transform (FT) mode. For both ^{1}H and ^{13}C NMR spectra, chemical shifts are expressed as parts per million (ppm) relative to TMS as the internal standard (δ units). For both ^{1}H and ^{13}C NMR spectra the descriptions are as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; and br s, broad singlet. Silica gel column chromatography employed MN silica gel 60 (70–270 mesh, 230–400 mesh) or MN silica gel G/UV_{254} for thin-layer chromatography. Thin layer chromatography (TLC) analysis was performed utilizing MN precoated plates and detection of compounds was achieved by spraying with a prepared solution of EtOH/*p*-anisaldehyde/AcOH (90:5:1) to which 5% (by volume) of concentrated H_2SO had been added immediately before use.

Biological Screening Procedures

Qualitative and quantitative antimicrobial screening was performed at the Department of Pharmacognosy, University of Mississippi. The assay is based on the general qualitative and quantitative antimicrobial activity bioassay described by Clark *et al.* (3). The qualitative antimicrobial screening procedure consisted of testing the extract, fractions, and pure compounds against the following microorganisms, obtained from the American Type Culture Collection (ATCC): *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 10536), *Pseudomonas aeruginosa* (ATCC 15442), *Mycobacterium smegmatis* (ATCC 607), *Candida albicans* (ATCC 10231), *Saccharomyces cerevisiae* (ATCC 9763), *Aspergillus niger* (ATCC 16888), *Trichophyton mentagrophytes* (ATCC 9972), *Polyporus sanguineus* (ATCC 16422), and *Helminthosporium* species (ATCC 4671).

Plates for the assay were prepared by dispersing 25 ml of sterile agar medium in 100 × 15-mm sterile petri dishes. The sterile agar plates were streaked with a dilution of the test organism (1 ml of broth culture in 9 ml of sterile water) cultured in eugon broth (for bacteria) and cophil broth (for fungi). Wells were created in the agar by removal of cylindrical plugs (11-mm ϕ) from the solidified agar plates using a sterile cork borer. To the wells were added 100 μl of solution or suspension of an extract, fraction, or pure compound. The extracts and fractions were tested at a concentration of 20 mg/ml, while pure compounds were tested at 1 mg/ml. The plates prepared as described were incubated at 37°C (for bacteria) and 30°C for fungi or yeasts. The antimicrobial activity was recorded as the width (millimeters) of the inhibition zone (average radius) measured from the edge of the agar well to the edge of the inhibition zone after 24 and 48hr of incubation for bacteria and 48 and 72hr of incubation for

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Table I. Qualitative Antibacterial Activity[a]

Sample[b]	*B. subtilis*		*E. coli*		*S. aureus*		*M. smegmatis*		*Ps. aeruginosa*	
Hexane extract	+ + + +	+ + +	–[c]	–	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
Acid fraction	+ + + +	+ + +	–	–	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
Neutral fraction	+ +	+ +	–	–	+ +	+	+ + +	+ + +	–	–
I	+ + +	+ + +	–	–	+ + +	+ +	+ + +	+ + +	–	–
Streptomycin sulfate	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + + +	+ + + +	+ + +	+ + +

[a] Code for expression is detailed under Materials and Methods.

[b] All substances were prepared as solutions in ethanol at concentrations of 20 mg/ml, except for I and streptomycin sulfate, which were tested at 1 mg/ml.

[c] No observed zone of inhibition.

fungi and the *Mycobacterium*. Zones of inhibition are reported using the following codes: (–) no activity; (+) 1–2 mm; (+ +) 3–6 mm; (+ + +) 7–12 mm; and (+ + + +) greater than 13 mm. A standard antibacterial agent, streptomycin sulfate, and a standard antifungal agent, amphotericin B, were included in each assay as positive controls.

Quantitation of antimicrobial activity was accomplished using the twofold serial broth dilution assay (pure compounds which showed significant relative activity in the qualitative screen) to establish the minimum inhibitory concentration (MIC) of those active compounds (3). The MIC was taken as the concentration of compound in the first test tube demonstrating no visible growth after 24- and 48-hr periods for *B. subtilis* and 48- and 72-hr periods for *T. mentagrophytes*.

Plant Material

The entire roots of *Croton sonderianus* Muell Arg. were used in this study. The whole plant was collected in Sobral, Ceara, Brazil, and was identified by Dr. Afranio G. Fernandes. The voucher specimens representing the collection are deposited at herbarium of the Departmento de Botanica, Universidade Federal do Ceará, Fortaleza-CE, 60.021, Brazil.

Plant Extraction

The roots of "marmeleiro preto" were air-dried at room temperature and ground. The ground material (1.6 kg dry weight) was extracted by percolation with hexane. The hexane solution, upon evaporation under low pressure at 40°C, yielded 85.0 g of a yellowish resinous extract which showed antimicrobial activity. Upon standing in the refrigerator for a short time, a crystalline precipitate began to form. A first crop of sonderianin was obtained by filtration.

Partitioning of Hexane Extract: Acidic and Neutral Fractions

The resinous filtrate remaining after recovery of sonderianin (75 g) was solubilized in 200 ml of benzene and partitioned in 20% NaOH/H_2O solution to yield, after usual workup, 25.0 g of a clear yellowish oily neutral fraction and 47.8 g of a clear, brownish resinous acidic fraction. Both fractions showed antimicrobial activity (Tables I and II).

Isolation of ent-*Beyer-15-en-18-oic acid (I) (Scheme I).* The crude acid fraction was coarsely fractionated in the following manner: 30 g of the acidic fraction was absorbed onto 30 g of silica gel (70–230 mesh) and packed onto the top of a silica gel (150-g) layer in a large diameter column. Elution of the column with a mixture of hexane:AcOEt:AcOH (87.5:10.0:2.5) gave 20 fractions of 100 ml. [Four additional 150-ml fractions were obtained by elution with a mixture of Hexane:AcOEt:MeOH (4:3:3).] Comparison of the collected fractions by TLC and ^1H NMR allowed us to combine them into eight pooled fractions: F1/1–F1/8. Successive chromatography of fraction F2/3 (1.4 g) on silica gel for flash chromatography (230–400 mesh) yielded 125 mg of I, which was crystallized from hexane, m.p. 138–141°C, $[\alpha]_D^{23}$ + 3.6° (c. 2.3, $CHCl_3$), IR ν max (KBr) 3300–3000 (br sh), 2650, 1695, 1450, 1270 cm^{-1}; MS m/z (%RA) 302 (12.9, M^+), 287 (2.2, M^+–Me), 257 (2.3), 256 (1.9), 241 (3.8), 135 (100), 119 (49.5), 106 (49), 105 (73), 91 (69.5); ^1H NMR ($CDCl_3$), δ 11.85 (1H, br s, H), 5.76 (1H, d, J = 6.0 Hz, H-16), 5.53 (1H, d, J = 6.0 Hz, H-15), 1.20 (3H, s, H-19), 1.00 (3H, s, H-17), 0.80 (3H, s, H-20). *Anal.* Calcd. for $C_{20}H_{30}O_2$; C, 79.42; H, 10.00. Found: C, 79.07; H, 10.19.

Table II. Qualitative Antifungal Activity[a]

Sample[b]	*C. albicans*		*S. cerevisiae*		*A. niger*		*T. mentagrophytes*		*P. sanguineus*		*Helminthosporium* sp.	
Hexane extract	–[c]	–	+ + +	+ + +	–	–	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
Acid fraction	–	–	+ + +	+ + +	–	–	+ +	+	+ +	±	+ + + +	+ + +
Neutral fraction	+	+	+ +	+ +	–	–	+ +	+ +	+ + +	+ +	+ + +	+ +
I	–	–	+ +	+ +	–	–	+ + +	+ +	–	–	–	–
Amphotericin B	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + + +	+ + +	+ + + +	+ + + +	+ + + +	+ + + +

[a] Code for expression is detailed under Materials and Methods.

[b] All samples were prepared as solutions in ethanol at concentrations of 20 mg/ml, except for I and amphotericin B, which were tested at 1 mg/ml.

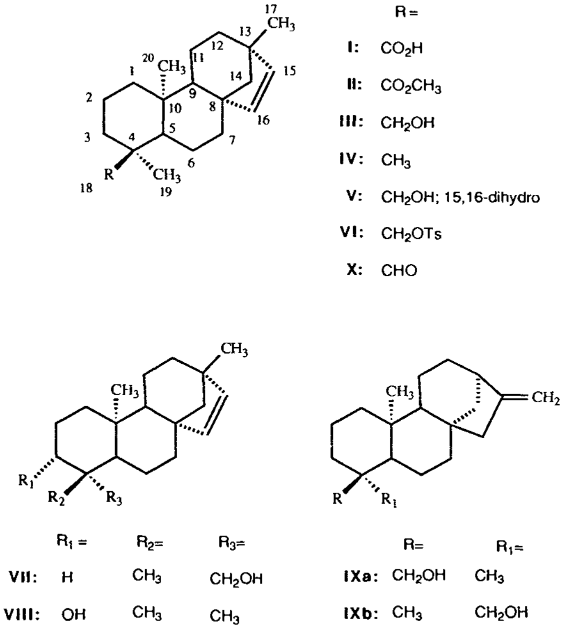
[c] No observed zone of inhibition.

Table III. Minimal Inhibitory Concentrations (MIC) of *ent*-Beyer-15-en-18-oic Acid (I) (μg/ml)

Compound	*B. subtilis*		*T. mentagrophytes*	
	24 hr	48 hr	48 hr	72 hr
I	6.25	6.25	25.0	25.0
Streptomycin	3.12	3.12	—	—
Amphotericin B	—	—	0.78	0.78

Ent-*Beyer-15-en-18-oic acid methyl ester (II).* Methylation of 1.2 g of acid I with excess ethereal diazomethane gave 1.1 g of product, which was purified by filtration through a small silica gel column (hexane, AcOEt, 4:1) to give *ent*-beyer-15-en-18-oic acid methyl ester (II), m.p. 70–73°C, IR ν KBr/max 1720, 1450, 1250, 750 cm^{-1}; MS *m/z* (%RA) 316 (28.1, M$^+$), 301 (6.9, M$^+$–Me), 273 (4.6), 257 (1.9), 241 (11.0), 229 (35), 201 (9.8), 187 (18.6), 135 (100.0), 91 (28); ^{1}H NMR (CDCl$_3$), δ 3.70 (3H, s, OCH$_3$), 5.73 (1H, d, J = 6.0 Hz, H-16), 5.50 (1H, d, J = 6.0 Hz, H-15), 1.20 (3H, s, H-19), 1.03 (3H, s, H-17), 0.80 (3H, s, H-20).

Ent-*Beyer-15-en-18-ol (III).* Acid I, 0.425 g, was dissolved in ether (20 ml), and 100 mg LiAlH$_4$ in 10 ml ether was added dropwise with stirring. The reaction mixture was stirred for 3hr at room temperature, excess AcOET was added, and the resultant mixture was partitioned between ether and water. The ether layer was separated and dried (Na$_2$SO$_4$) and the solvent evaporated. The residue (0.45 g) was adsorbed onto 3 g of silica gel and chromatographed over a column (2.5-cm diameter) of 80 g of silica (230–400 mesh). Elution with 0.5% MeOH in CHCl$_3$ afforded 320 mg of *ent*-beyer-15-en-18-ol, III. Crystallization from hexane gave III as white crystals, m.p. 104–106°C, [α]$_D$^{23} + 9.9° (c. 0.7, CHCl$_3$), IR ν max (KBr) 3520 (sharp), 3450 (broad), 1450, 1385, 1365, 1040, 1020 cm^{-1}; MS *m/z* (%RA) 288 (28.8, M$^+$), 273 (2.5, M$^+$–Me) 257 (50.2), 229 (20.5), 135 (34.5), 134 (28.5), 133 (29.7). 105 (100); ^{1}H NMR (CDCl$_3$), δ 5.70 (1H, D, J = 6.0 Hz, H-16), 5.45 (1H, d, J = 6.0 Hz, H-15), 3.40 (1H, d, J, = 11.0 Hz, H-18), 3.07 (1H, d, J = 11.0, H-18), 1.90 (1H, br s, OH), 1.00 (3H, s, H-17), 0.78 (6H, br s, H-19, H-20).



	R=
I:	CO$_2$H
II:	CO$_2$CH$_3$
III:	CH$_2$OH
IV:	CH$_3$
V:	CH$_2$OH; 15,16-dihydro
VI:	CH$_2$OTs
X:	CHO

	R$_1$ =	R$_2$=	R$_3$=
VII:	H	CH$_3$	CH$_2$OH
VIII:	OH	CH$_3$	CH$_3$

	R=	R$_1$=
IXa:	CH$_2$OH	CH$_3$
IXb:	CH$_3$	CH$_2$OH

Scheme I

Ent-*Beyer-15-en-18-al (X).* The alcohol III (80.0 mg) was dissolved in 15.0 ml of CH$_2$Cl$_2$, 50.0 mg of pyridinium chlorochromate (PCC) was added, and the reaction mixture was stirred for 2hr. The reaction mixture was diluted with ether, the precipitated salts were filtered, and the ether solution was filtered through a small column of silica gel to yield 50.0 mg of aldehyde as a colorless oil which crystallized when stored in the refrigerator; white crystals, m.p. 59–62°C, [α]$_D$^{23} + 11.7° (c. 3.4, CHCl$_3$), IR ν max (KBr) 3020, 2800, 2710, 1725, 1440, 1385, 710 cm^{-1}. MS *m/z* (%RA) 286 (29.2, M$^+$) 271 (4.0 M$^+$–Me), 258 (17.3), 257 (41.3), 243 (9.9), 201 (91.1), 187 (19.7), 105 (100); ^{1}H NMR (CDC13), δ 9.40 (1H, s, H-18), 5.77 (1H, d, J = 6.0 Hz, H-16), 5.57 (1H, d, J = 6.0 Hz, H-15), 1.13 (3H, s, H-19), 1.05 (3H, s, H-17), 0.83 (3H, s, H-20).

*Dihydro-*ent*-beyer-15-en-18-ol (V).* Alcohol III (100 mg was dissolved in 15 ml of MeOH, 15 mg of platinum oxide (PtO$_2$) was added, and the mixture was stirred for 2hr at room temperature under an atmosphere of hydrogen held in a balloon. The reaction mixture was then filtered over a small silica gel column to remove the catalyst, giving a residue (88.0 mg) of the dihydroderivative, V, as a white solid, m.p. 112–114°C, [α]$_D$^{23} − 132.2° (c. 8.0, CHCl$_3$), IR ν max (KBr) 3380, 1440, 1380, 1050, 1020, 1000 cm^{-1}; MS *m/z* (%RA) 290 (0.6, M$^+$), 259 (52.4), 243 (2.0), 203 (6.3), 189 (9.0), 163 (41.9), 123 (100), 109 (47.2); ^{1}H NMR (CDCl$_3$), δ 3.42 (1H, d, J = 11.0, H-18), 3.10 (1H, d, J = 11.0, H-18), 0.98 (6H, br s, H-17, H-20), 0.80 (3H, s, H-19). *Anal.* Calcd. for $C_{20}H_{34}O$: C, 82.69; H, 11.80. Found: C, 82,80; H, 12.20.

RESULTS AND DISCUSSION

Extensive silica gel chromatography of the acidic fraction of the extract of *Croton sonderianus* allowed the separation of a diterpene acid, whose structure was determined by a combination of chemical derivatization and spectroscopic analyses. The acid, I, M$^+$ (*m/z*) 302 ($C_{20}H_{30}O_2$) showed an IR spectrum indicative of its acidic character: 3300–3000 and 2650 cm^{-1} and 1695 cm^{-1}. The ^{1}H NMR spectrum revealed a broad singlet exchangeable by D$_2$O at δ 11.85 (1H, CO$_2$H), a pair of doublets at δ 5.76 and 5.53 (1H each, J = 6.0 Hz), and three quartenary methyls at δ 1.20, 1.00 and 0.80.

Diazomethane methylation of the acid produced an ester II showing no D$_2$O exchangeable absorption, a pair of doublets at δ 5.73 and 5.50 (1H each, J = 6.0 Hz), a methoxyl at δ 3.70, and three quaternary methyls at δ 1.20, 1.03, and 0.80 in its ^{1}H NMR spectrum. IR absorptions at 1720 and 1250 cm^{-1} were indicative of an equatorial carboxymethyl in a six-membered ring (4). These observations are supported by characteristic ^{13}C NMR absorptions (Table IV).

Reduction (LiAlH$_4$) of either the acid or the ester yielded a solid alcohol III, presenting in ^{1}H NMR a pair of doublets at δ 5.70 and 5.45 (1H each, J = 6.0 Hz), another pair of doublets at δ 3.07 and 3.40 (1H each, J = 11.0 Hz, H-18), a quaternary methyl at δ 1.00 (H-19), and two other

Table IV. ^{13}C NMR Spectral Data of *ent*-Beyer-15-en-18-oic Acid (I) and Derivatives

	Compound					
	I	II	IV	V	VI	X
	Solvent[a]					
	C	B	C	C	C	C
C number[b]	δ	δ	δ	δ	δ	δ
1	$38.2^{d,c}$	38.5^{d}	38.8^{d}	39.3^{d}	38.3^{d}	38.3^{d}
2	17.7	18.1	17.9	17.8	17.6	17.0
3	37.0^{d}	37.4^{d}	35.4^{d}	35.3^{d}	35.3^{d}	36.6^{d}
4	47.4	47.8	37.1	37.5	36.6	48.1
5	50.0	50.6	49.0	49.6	48.8	49.7
6	20.0^{c}	20.3^{c}	19.9^{c}	20.5^{c}	19.8^{c}	20.1^{c}
7	36.6^{d}	37.0^{d}	37.1^{d}	41.0^{d}	37.2^{d}	33.1
8	49.2	49.5	49.0	44.9	48.8	49.2
9	52.8	52.9	52.8	56.8	52.6	52.8
10	36.6	37.4	37.5	37.5	36.9	36.6
11	22.9^{c}	23.3^{c}	20.2^{c}	20.1^{c}	22.7^{c}	23.3^{c}
12	33.0	33.5	33.2	40.0	33.1	32.4
13	43.6	43.8	43.6	39.3	43.6	43.6
14	61.0	61.4	61.2	57.7	61.1	61.1
15	136.5^{e}	136.5^{e}	136.4^{e}	37.5	136.5^{e}	136.7^{e}
16	135.0^{e}	135.4^{e}	135.2^{e}	33.8	134.5^{e}	134.8^{e}
17	24.9	25.1	24.9	27.1	24.8	24.9
18	186.0	178.6	72.2	72.2	78.2	206.4
19	15.3^{f}	15.9^{f}	15.6^{f}	17.9^{f}	15.4^{f}	14.4^{f}
20	16.5^{f}	17.2^{f}	17.8^{f}	15.6^{f}	17.4^{f}	15.3^{f}

[a] C, $CDCl_3$; B, C_6D_6.

[b] See structure I for numbering system. Carbon frequency assignments were made by comparison with model compounds (see Table V).

[c] Superscripts c–f, interchangeable within the same column.

[d] 144.5 (s), 133.3 (s), 129.7 (d), 127.8 (d) aromatic portion of VI.

quaternary methyls as an intense and broad singlet at δ 0.78 (6H, H-19 and H-20).

An attempt to transform III to hibaene (IV), its hydrocarbon derivative, via $LiAlH_4$ reduction of the tosylate VI was unsuccessful. Catalytic hydrogenation of III gave the corresponding saturated alcohol V showing no pair of doublets around δ 5.50 but only the pair of doublets at δ 3.10 and 3.42 (2H, J = 11.0 Hz, H-18), a broad and intense singlet at δ 0.98 (6H, H-17 and H-20), and a singlet at δ 0.80 (3H, H-19). The downfield shift of an angular methyl group (H-20) following hydrogenation of the double bond is indicative that these two groups must be on the same side of the molecule. Hydrogenation of the dextrotatory alcohol III ($[\alpha]_D^{23}$ + 9.9°) to levorotatory dihydroalcohol ($[\alpha]_D^{23}$ − 13.2°), as well as the unsuccessful attempt to convert the tosylate to the corresponding hydrocarbon, is similar to results reported for erythroxylol A (VII), also referred to as monogynol (5) and previously obtained from *Erythroxylum monogynum* (Erythroxylaceae) (6). Erythroxylol A is also known as beyeren-19-ol and is obtained from *Helipterum craspedioides* (Compositae) in the succinate ester form (7).

These data suggest the (+)-beyer-15-ene [or its synonymous (+)-stach-15-ene, isostevene, or (+)-hibaene)] (6–9) type of structure for the acid and its derivatives. The IR absorption at 1720 and 1250 cm^{-1} (C–CO–O) attributed to an equatorial carbomethoxy in II and the chemical shift of the pair of doublets centered at δ 3.25 for III were in good agreement for the structure of I as *ent*-beyer-15-en-18-oic acid. Indeed III had a different melting point and TLC behavior when compared with an authentic sample of erythroxylol A generously provided by Dr. R. D. H. Murray (6). Comparison of the ^{13}C NMR absorptions of carbons 6–17 of III with a model compound, stachenol (VIII), described in the literature (9), and carbons 1–5, 18, and 19 with the C-18 and C-19 epimers of hydroxykaur-16-ene (XI) (10) offered confirming evidence for the equatorial disposition of the CH_2OH in III and, consequently, for an equatorial carboxyl in its acid precursor I.

Stach-15-en-19-oic acid has been reported numerous times previously (11–14,20,21) but no mention of its C-18 epimer has been made. Although C-19 oxygenation is probably the most common feature of the tetracyclic diterpenes, the C-18 isomers have been found in *Sideritis* (Labiatae) species (15–17). However, *ent*-beyer-15-en-19-ol (erythroxylol A or monogynol) and *ent*-beyer-15-en-18-ol were isolated from the same plant, *Baccharis tola* (Compositae) (18), in which a labdane diterpene and scopoletin were also found. Scopoletin has previously been isolated from "marmeleiro preto" (2).

Table V. Comparison of the ^{13}C NMR Data of *ent*-Beyer-15-en-18-ol to Reported Diterpenes

	Compound					
	III	III[c]	IV[d]	VIII[e]	IXa[f]	IXb[f]
	Solvent[a]					
	C	C	C	C	C	C
C number[b]	δ	δ	δ	δ	δ	δ
1	38.8	38.8	39.7	37.5	39.9	40.5
2	17.9	18.0	18.5	27.4	18.0	18.3
4	35.4	35.4	42.0	79.1	35.3	35.6
5	*49.0*	*49.1*	56.5	55.4	*49.3*	*56.8*
6	19.9	19.9	20.4	19.9	20.0	20.5
7	37.1	37.0	41.2	37.3	39.9	41.6
8	49.0	48.6	44.9	48.9	44.2	44.2
9	52.8	52.8	56.8	52.8	56.0	56.2
10	37.5	37.1	37.6	37.2	39.2	39.2
11	20.2	20.3	20.2	20.3	18.2	18.2
12	33.2	33.2	40.0	33.2	33.2	33.2
13	43.6	43.6	39.2	43.7	44.0	44.0
14	61.2	61.2	57.7	61.2	40.9	39.9
15	136.4	135.0	37.6	136.5	49.3	49.1
16	135.2	136.0	33.6	135.1	155.8	155.8
17	24.9	24.9	27.1	24.9	103.0	103.0
18	*72.2*	*72.3*	33.7	28.4	*71.1*	27.1
19	15.6	17.7	21.9	15.7	17.5	65.4
20	17.8	15.6	15.1	15.1	18.2	18.5

[a] C, $CDCl_3$.

[b] See structure I for number system.

[c] As described in Ref. 18.

[d] As described in Ref. 19.

[e] As described in Ref. 9.

[f] As described in Ref. 10.

Finally, comparison of the physical and spectral data of III with those reported for *ent*-beyer-15-en-18-ol (Table V) provided a complete match for the ^1H and ^{13}C NMR, IR spectra and mass spectral fragments as well as comparable values for the specific rotation (lit., $[\alpha]_D^{23}$ 29.7°; this is not comparable to $[\alpha]_D$ 9.9 $CHCl_3$) and melting point (lit., 112°C) (18). No authentic sample of *ent*-beyer-15-en-18-ol was available for comparison.

The new *ent*-beyer-15-en-18-oic acid (I) showed strong antibacterial activity against gram-positive bacteria with a minimal inhibitory concentration against *Bacillus subtilis* (ATCC 6633) of 6.25 μg/ml. It showed little antifungal activity (Table III).

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