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Isolation and synthesis of a new clerodane from *Echinodorus grandiflorus*

Marta Costa [b], Clara M. A. Tanaka [c], Paulo M. Imamura [a], Anita J. Marsaioli [a,*]

[a] *Instituto de Química, UNICAMP, CP 6154, Campinas, 13081-970, SP, Brazil*
[b] *Departamento de Química, UFMS, MS, Brazil*
[c] *Departamento de Química, UEM, Maringa, PR, Brazil*

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Abstract

A new clerodane derivative, (−)-16-hydroxycleroda-3,13-dien-16,15-olide-18-oic acid, (4aα,6α,8aα)1-carboxy-5(*S*)-[2(2,5-dihydro-5-hydroxy-2-oxo-4-furanyl)ethyl-5,6,8a-trimethyl-3,4,4a,5,6,7,8,8a-octahydro-naphthalene was isolated as a minor component of the polar fractions of the leaves of *E. grandiflorus*. The structure was determined by spectroscopy (IR, MS, 1D and 2D ^{1}H and ^{13}C NMR). The absolute configuration was determined by a hemisynthesis using (+)-hardwickiic acid methyl ester, from commercial copaiba oil, as starting material.

Keywords: Echinodorus grandiflorus; Alismataceae; Clerodane; Absolute configuration; Hemisynthesis of a clerodane

1. Introduction

Among the Brazilian aquatic plants we were particularly attracted to *Echinodorus grandiflorus* (Tanaka, Sarragiotto, Zuckerman-Schpector & Marsaioli, 1997) mainly due to its application as a diuretic, in natural medicine. Our exploratory work led to the isolation of a new cembrane, echinoic acid (**1**), as the major component of the ethanol extract of the leaf petioles, while echinodol (**2**) was present in the *Echinodorus grandiflorus* reported by (Manns & Hartmann, 1993).

Intrigued by this apparent inconsistency and conscious that our previous study remained unachieved, due to the unstability of several metabolites, prompted us to better monitor the extracts of *Echinodorus grandiflorus* now using GC/MS. From these analyses we could obtain the profile of the fresh extracts and detect the presence of clerodane derivatives. In this paper we report the details of the detection and isolation of two novel clerodanes (−)-**3** and (−)-**4** and of the hemisynthesis of (+)-**3a**. (Fig. 1).

2. Results and discussion

2.1. Detection and isolation of the clerodanes (−)-3 and (−)-4

The GC/MS analyses was chosen as a rapid analytical tool to obtain the profiles of the ethanol extract of the leaves and leaf petioles of *Echinodorus grandiflorus*. The analyses were performed using a fused silica capillary column (J&W Scientific, DB-5) and whole extracts previously derivatized. We have treated the extracts with either bis(trimethylsilyl)-trifluoroacetamide or with diazomethane followed by the silylating agent. The latter furnished better results with gas chromatography and we could observe that cembrane **1** (methyl ester derivative) was indeed the major acidic component present in the leaf petioles extract. We have also detected long chain acids (octadecanoic acid and hexadecanoic acid as methyl esters and as ethyl esters) and phytol (silyl derivative). The analysis of the ethyl acetate crude extracts revealed again the presence of ethyl ester derivatives which led us to the conclusion that ethyl ester derivatives should be artifacts due to the solvent which was used during the extraction. As

* Author to whom correspondence should be sent.

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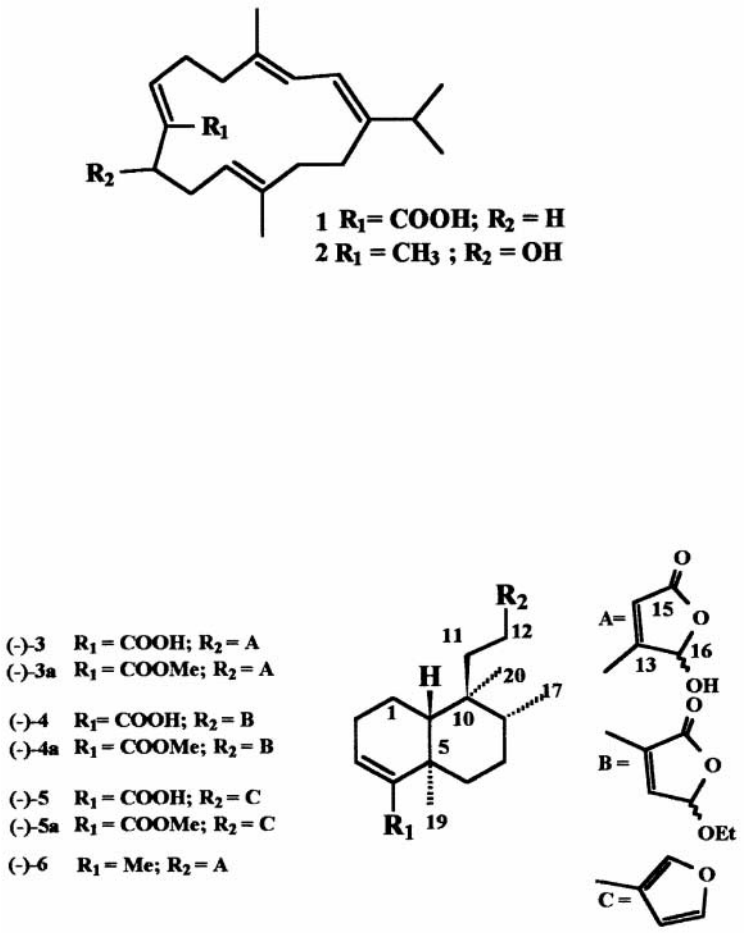


Fig. 1.

minor components we have additionally detected hardwickiic acid (−)-(**5**) (methyl ester derivative) and other carboxylic terpenes (methyl, silyl derivatives) showing a fragmentation pattern compatible with hardwickic acid derivatives. The analyses of the leaves revealed a predominance of the hardwickiic acid (−)-(**5**) (higher than 50%) and a composition analogous to that of the leaf petioles, although the same components were present in different relative abundances.

The chromatographic separation of the leaf crude extract led to the isolation of (−)-hardwickiic acid, (−)-(**5**). This compound was better isolated from the methylated polar fraction as its methyl ester derivative (−)-**5a** (in Section 3 the complete assignment of the NMR signals is reported, based on 1D and 2D NMR spectra, because the literature data are incomplete), which showed spectral data identical to those reported in the literature (Bandara, Wimalasari & Bandara, 1987).

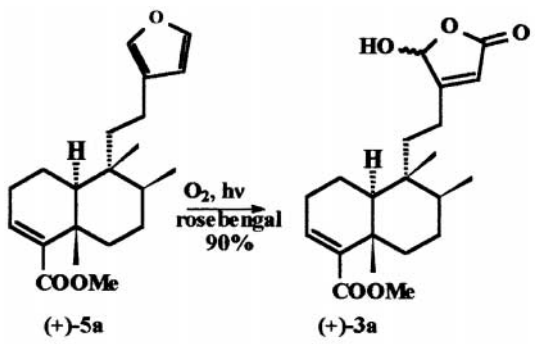
From the same crude extract (non-methylated) a new clerodane derivative, compound (−)-**3** was isolated as crystals and from its spectral data (1D and 2D, ^{1}H and ^{13}C NMR Table 1) we concluded that we were dealing with an epimeric mixture (1:1) of clerodane derivatives possessing rings A and B identical to those of (−)-hardwikiic acid, (−)-(**5**). The major differences between the NMR signals of (−)-**5** and (−)-**3** concerned ring C. We should point out that compound (−)-**3** was only soluble in deuteroacetone and therefore some minor chemical shift differences (corresponding to signals of A/B rings) were observed between the carbon spectra of (−)-**5** (soluble in deuterochloroform) and (−)-**3**. From the signals at δ ^{1}H 5.93 (*s*) and 6.11 (*s*) and at δ ^{13}C 171.7/171.8 (C=O), 117.5/117.6 (CH), 168.5 (C) and 100.0/100.1 (CH) it was evident that we had a hydroxy-lactone moiety with a β-alkyl substituent. Comparison of the NMR chemical shifts of (−)-**3** with those reported for ring C of *6* (Hara et al., 1995) confirmed the presence of the β-butenolide and the proposed structure for (−)-**3**.

A search in the literature revealed that (−)-**3** was a new clerodane derivative. Attempts to determine the absolute configuration of this novel clerodane by X-ray diffraction analysis were frustrated by the poor quality of the crystals and instability of the compound. A comparison of the optical rotation of several clerodane derivatives (Hara et al., 1995) have indicated that within an enantiomeric clerodane series the optical rotation signs depend on the substituents (see Fig. 2).

Table 1
^{1}H (300 MHz) and ^{13}C (75.5 MHz) chemical shift of compound (–)-**3** obtained by 1D and 2D NMR spectroscopy ($(CD_3)_2CO$, TMS)

C		^{13}C (δ)	^{1}H (δ), $^{n}J_{H,H}$ (Hz) (from 1D and 2D H, C-correlations)	H–H COSY
1	CH_2	18.1	1.46 (*m*), 1.13 (*m*)	–
2	CH_2	27.7, 27.8	2.18–2.28 (*m*)	–
3	CH	138.3/138.3	6.71 (*t*, *J* = 3.1)	6.71 × 2.18 H_2
4	C	143.1/143.2	–	–
5	C	38.4	–	–
6	CH_2	36.7	1.10 (Hax, *dt* *J* = 12.9, 3 Hz), 2.46 (m)	–
7	CH_2	28.0	1.38–1.44 (*m*)	1.38–1.44 × 2.46 H_6
8	CH	37.1	1.58 (*m*)	–
9	C	39.5/39.6	–	–
10	CH	47.7	1.38 (*dd*, *J* = 12.0, 1 Hz)	–
11	CH_2	35.7, 35.8	1.52, 1.64–1.78 (*m*)	1.52, 1.64–1.78 × 2.26–2.32 H_{12}
12	CH_2	21.7, 21.8	2.26–2.32 (*m*)	–
13	C	168.5	–	–
14	CH	117.5/117.6	5.93 (*s*)	5.93 × 2.26–2.32 H_{12}
15	C	171.7, 171.8	–	–
16	CH	100.0/100.1	6.11 (*s*)	–
17	CH_3	15.2	0.84, 0.85 (*d*, *J* = 6.3)	0.84, 0.85 × 1.58 H_8
18	C	171.8	–	–
19	CH_3	20.9	1.28 (*s*)	–
20	CH_3	18.6	0.82 (*s*)	0.82 × 1.58 H_8

Thus in order to unquestionably establish the absolute configuration of (–)-**3** we proposed the comparison of the optical rotation of the natural (–)-**3** with a homochiral synthetic standard, preferably obtained in a few steps using a starting material of known absolute configuration. The use of the (–)-**5** was our first choice, but difficulties in the isolation of homochiral starting material, prevented this strategy. Our second best choice relied on the (+)-hardwickiic acid, which is readily available from commercial copaíba oil (Cocker, Moore & Pratt, 1965; delle Monache, d'Albuquerque, delle Monache & Marini-Bettólo, 1970). The absolute configuration of (+)-**5** was determined by Dev et al. (1968) and its isolation (as a methyl ester) from copaiba oil has been optimized in our laboratories some years ago (Araujo, 1991). Finally, to obtain **3** from (+)-**5a** depended on a suitable reaction to transform the furane moiety into a butenolide. The appropriate methodology for this purpose was the regioselective photooxygenation of furanes developed by Kernan & Faulkner, 1988). Thus the photooxidized (+)-hardwickiic acid methyl ester, (+)-**5a** furnished (+)-**3a** in 90% yield, that produced identical ^{1}H NMR and ^{13}C NMR spectra to those of (–)-**3**, but with an opposite optical rotation (Scheme 1). Methylation of (–)-**3** was performed with diazomethane but an excess of diazomethane led to the methylation of the carboxy group and to the addition of a diazomethane molecule to the butenolide moiety preventing the isolation of the (–)-**3a**. We have, therefore, restricted our comparison to the optical rotation of (–)-**3** and (+)-**3a** to determine the absolute configuration of the new clerodane (–)-**3**.



Scheme 1. Regioselective photo-oxygenation of (+)-**5a**.

Compound (–)-**4a** was isolated as an oil from the acidic fraction treated with diazomethane. Comparison of the NMR signals (Table 2) with those of (–)-**3** revealed that major differences between these two compounds were located in ring C, which in (–)-**4a** was an ethoxy derivative of a β-alkyl-butenolide. We found in the literature (Singh, Jain & Jakupovic, 1988) the 15-methoxy-derivative, but the 15-ethoxy derivative was never mentioned before. This compound was suggested to be an artefact arising from the use of ethanol or ethyl acetate during the extraction. GC/MS monitoring of the methyl and silyl derivatized crude extracts showed the presence of **4a** in all extracts.

The analyses of the *Echinodorus grandiflorus* can be easily performed by GC/MS on the previously derivatized extract which allows determination of the distribution profile of the compounds. We have also isolated two novel clerodane derivatives (–)-**3** and (–)-**4** and determined the absolute configuration of (–)-16-hydroxycleroda-3,13-dien-16,15-olide-18-oic acid (–)-**3**,

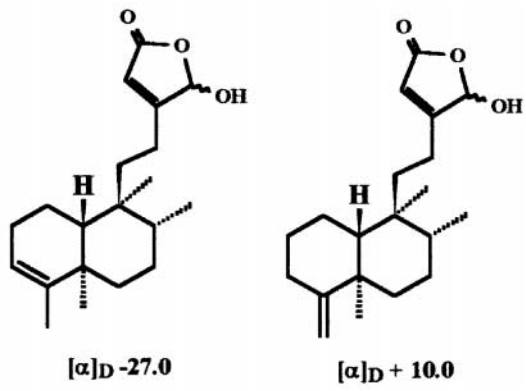


Fig. 2.

{(4aα,6α,8aα)1-carboxy-5(*S*)-[2(2,5-dihydro-5-hydroxy-2-oxo-4-furanyl)ethyl-5,6,8a-trimethyl-3,4,4a,5,6,7,8,8a-octahydronaphthalene]}.

3. Experimental

M.p.'s were recorded with a Kofler hot plate set up in a microscope Thermopan model (C. Reichert Optische Werke A G). Optical rotation angles were measured in a Polamat A Rutina Carl Zeiss polarimeter. ^{1}H NMR spectra were recorded with a GEMINI 300 (300.1 MHz, Varian) or Bruker AC 300P spectrometer. $CDCl_3$ was used as the solvent, with Me_4Si (TMS) as internal standard. ^{13}C NMR spectra were obtained with a GEMINI 300 (75.5 MHz, Varian) or a Bruker AC 300P spectrometer. $CDCl_3$ (77.0 ppm) was used as solvent and internal standard. The number of hydrogens attached to the carbon atoms were obtained from the DEPT-135 and DEPT-90 and 2D NMR spectra were performed using standard pulse sequences provided by Varian.

3.1. GC/MS

Analyses were carried out using a HP-5990/5970 system equipped with J&W Scientific DB-5 fused silica capillary column (30 m × 0.25 mm × 0.25 μm). The carrier gas was helium and the temperature program was 80°C–10° min^{-1} to 280°. The MS were obtained at 70 eV. Scanning speed was 0.84 scan/s from *m*/*z* 40 to 550. The compound identification was made on the basis of standard compound coinjection (commercial and synthetic, characterized by IR, ^{1}H NMR and ^{13}C NMR spectra) and comparison by computerized matching of the acquired mass spectra with those stored in the Wiley/NBS mass spectral library of the GC/MS data system.

3.2. Plant material and isolation

Leaf petioles and leaves (10 kg) of *E. grandiflorus* were collected in January 1996 near the town of Curitiba, PR, Brazil. A voucher specimen is deposited at the IB/UNICAMP Herbarium # UEC 807481. The

Table 2
^{1}H (300 MHz) and ^{13}C (75.5 MHz) chemical shifts of compound (–)-**4a** obtained by 1D and 2D NMR spectroscopy ($CDCl_3$, TMS)

#		^{13}C (δ)	^{1}H (δ), $^nJ_{H,H}$ (Hz) (from 1D and 2D C,H correlations)	H, H COSY	C, H long range correlations
1	CH_2	17.3	1.50 (*m*)–1.10 (*m*)	–	1.30 (H-10)
2	CH_2	27.0	2.23	–	1.30 (H-10)
3	CH	137.2	6.60 (*t*, *J* = 3.6 Hz)	6.60 × 2.23 (H-2)	
4	C	142.4	–	–	1.25 (H-19)
5	C	37.6	–	–	1.25 (H-19), 2.30 (H-6)
6	CH_2	35.7	1.06–2.30	–	1.25 (H-19)
7	CH_2	27.1, 27.2	1.42		0.82 (H-17)
8	CH	36.3	1.48	–	0.76 (H-20)
9	C	38.8	–	–	0.76 (H-20),
10	CH	46.6	1.30	–	2.23 (H-2)
11	CH_2	35.5, 35.6	1.08 (*m*)	1.08 × 2.24(H-12)	0.76 (H-20)
12	CH_2	18.7, 18.8	2.24 (*m*)	–	–
13	C	139.3	–	–	2.24 (H-12), 6.76 (H-14)
14	CH	141.6	6.76 (*d*, *J* = 1.2)	6.76 × 5.80 (H-15)	2.24 (H-12)
15	CH	101.6	5.80 (*d*, *J* = 1.2)	–	
16	C	171.6	–	–	6.76 (H-14)
17	CH_3	15.7	0.82 (*d*, *J* = 6.1)	0.82 × 1.48 (H-8)	–
18	C	167.9	–	–	6.60 (H-3), 3.68 (OCH_3)
19	CH_3	20.5	1.25 (*s*)	–	
20	CH_3	18.0	0.76 (*s*)		
	OCH_2	66.0	3.95 (*m*) –3.75 (*m*)		
	CH_3	14.9	1.30 (*t*, *J* = 6 Hz)		
	OCH_3	51.0	3.68 (*s*)		

leaf petioles were separated and divided in small portions. The fresh material was blended with ethanol and filtrated. The solvent was evaporated and the residue containing 10% of water was extracted with EtOAc yielding 35.0 g of leaf extract and 14.0 g of leaf petiole extract.

Successive silica gel CC of the leaf petiole extract, and its fractions, eluted with hexane and hexane with increasing amounts of EtOAc produced echinoic acid (**1**) (390 mg). A small sample of the polar fractions eluted with hexane and hexane–EtOAc (30–50%) was methylated and submitted to silica gel CC yielding compound (−)-**4a** (47 mg).

Successive silica gel CC of the leaf extract and its fractions, eluted with hexane and hexane with increasing amounts of EtOAc led to the isolation of (−)-**3** (50 mg) as crystals. A small sample (100 mg) of the polar fractions eluted with hexane and hexane–EtOAc (30–50%) was methylated and submitted to prep. TLC producing (−)-**5a** (hardwickiic acid methyl ester, 37 mg).

3.3. (−)-16-Hydroxycleroda-3,13-dien-16,15-olide-18-oic acid (−)-**3**, *{(4aα,6α,8aα)1-carboxy-5(S)-[2(2,5-dihydro-5-hydroxy-2-oxo-4-furanyl) ethyl-5,6,8a-trimethyl-3,4,4a,5,6,7,8,8a-octahydronaphthalene}*

m.p.: 146–147°C; $[\alpha]_D^{25}$ −39.4 ($CHCl_3$; *c* 4.0); IR ν_{max}^{KBr} cm^{-1}: 2948, 2911, 1716, 1363, 1165, 767; ^1H NMR and ^{13}C NMR (deuteroacetone): Table 1; EIMS (70 eV) *m/z* (rel. int.): 330 (M^{+}–H_2O, 25), 315(22), 203(30), 125(78), 105 (33), 91 (52), 55 (71), 41(100).

*3.4. (−)-16-Ethoxycleroda-3,13-dien-15,16-olide-18-oic acid, methyl ester (−)-***4a**, *{(4aα,6α,8aα)1-carboxy-5(S)-[2(2,5-dihydro-5-ethoxy-2-oxo-4-furanyl)ethyl-5,6,8a-trimethyl-3,4,4a,5,6,7,8,8a-octahydro-naphthalene}*

Oil, $[\alpha]_D^{25}$ −80 ($CHCl_3$; *c* 1.0); IR ν_{max}^{film} cm^{-1}: 1769, 1712, 1116; ^1H NMR and ^{13}C NMR ($CDCl_3$) Table 2; GC/EIMS (70 eV) *m/z* (rel. int.): 359(M + –OMe, 40), 343 (100), 253 (5), 207 (31), 173 (18), 139 (18), 105 (22), 55 (26).

3.5. (−)-Hardwickiic acid methyl ester (−)-**5a**

Oil, $[\alpha]_D^{25}$ −113 ($CHCl_3$; *c* 2.0); IR ν_{max}^{film} cm^{-1}: 2953, 2871, 1714, 1496, 1439, 1250, 1196, 786; ^1H NMR ($CDCl_3$): δ 0.78 (3H, *s*, Me-20), 0.83 (3H, *d*, J = 6 Hz, Me-17), 1.25 (3H, *s*, Me-19), 1.00–1.80 (8H, *m*), 2.00–2.40 (6H, *m*), 3.69 (3H, *s*, -OCH_3), 6.25 (1H, *bs*, H-14), 6.60 (1H, *t*, J = 3.6 Hz, H-3); 7.19 (1H, *bs*, H-16); 7.34 (1H, *bs*, H-15).; ^{13}C NMR ($CDCl_3$): δ 15.8 (C-17), 17.4 (C-1), 18.0 (C-12), 18.1 (C-20), 20.6 (C-19), 27.0 (C-2), 27.1 (C-7), 35.8 (C-5), 36.1 (C-8), 37.5 (C-5), 38.5 (C-11), 38.7 (C-9), 46.4 (C-10), 51.1(OMe), 111.0 (C-14), 125.7 (C-13), 136.9 (C-3), 138.5 (C-16), 142.6 (C-4), 142.8 (C-15), 168.0 (C-18); GC/EIMS (70 eV) *m/z* (rel. int.): 330 (M^{+},8), 283 (30), 235 (60), 203 (75), 139 (100), 96 (65), 81 (80).

3.6. Isolation of (+)-*hardwickiic acid methyl ester* (+)-(**5a**)

Commercial Copaiba oil (46.0 g) was dissolved in Et_2O (100 ml) and was extracted with 5% KOH (4 × 50 ml). The aq. layer was acidified with HCl (pH ~3), extracted with Et_2O (4 × 60 ml). The organic layers were washed with brine until neutral, dried over anhydrous sodium sulfate and the solvent was evaporated under vacuum yielding a crude acidic fraction (30.8 g). This fraction was dissolved in NaOH (5.9 g, 147.5 mmol) aq. solution (120 ml) and treated with dimethyl sulfate (21.3 ml, 126.8 mmol) and refluxed for 2 h. Upon cooling the reaction mixture was extracted with Et_2O (3 × 60 ml). The organic layers were washed with brine (3 × 50 ml), dried over anhydrous Na_2SO_4 and concentrated under vacuum to give a viscous oil (20.6 g). Silica gel CC of the crude material eluted with *n*-hexane/Et_2O (99:1) produced (+)-**5a**, 5.4 g, oil $[\alpha]_D^{25}$ +113 ($CHCl_3$, *c* 4.0); Calculated for $C_{21}H_{31}O_3$: 9.15% H, 76.33% C. Found: 9.10% H, 76.51% C. Other spectroscopic data were identical to (−)-**5a**.

3.7. Photooxygenation of (+)-*hardwickiic acid methyl ester*

A soln of compound (+)-**5a** (34.65 mg, 0.105 mmol) in CH_2Cl_2 (15 ml), diisopropylethylamine (0.159 ml) and rose bengal on polystyrene (1.60 mg) was irradiated with a tungsten lamp (250 W) at −78°. Oxygen was bubbled through the reaction for 5 h. The reaction mixture was filtered through a celite pad at room temp. and the residue washed several times with hexane–EtOAc (70:30). The solvent was evaporated and the residue purified by silica-gel column chromatography eluted with hexane–EtOAc (90:10), yielding 34.21 mg (90%, 0.095 mmol) of (+)-**3a**: oil, $[\alpha]_D^{25}$ +55 ($CHCl_3$, *c* 0.6); IR ν_{max}^{film} cm^{-1}: 3381, 2954, 1760, 1712 1447. ^1H NMR ($CDCl_3$): δ 0.79 (3H, *s*, Me-20), 0.81 (3H, *d*, J = 6 Hz, Me-17) 1.26 (3H, *s*, Me-19), 3.69 (3H, *s*, -OMe), 5.83 (1H, *s*, H-14), 6.01 (1H, *bs*, H-16), 6.59 (1H, *t*, J = 3 Hz, H-3); ^{13}C NMR ($CDCl_3$): δ 15.9 (C-17), 17.4 (C-1), 18.1 (C-20), 20.6 (C-19), 21.3/21.4 (C-12), 26.8 (C-2), 27.1 (C-7), 34.8 (C-11), 35.8 (C-6), 36.7 (C-8), 37.5 (C-5), 38.7 (C-9), 46.5 (C-10), 51.3 (OMe), 99.0/99.1 (C-16), 117.0/117.1 (C-14), 136.9/136.8 (C-3), 142.3 (C-4), 168.1 (C-13), 170.3/170.4 (C-15), 171.5 (C-18); GC/EIMS (70 eV) *m/z* (rel. int.) 362 (M^{+}).

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