Chemical Composition and Antitumor Activities from *Givotia madagascariensis*

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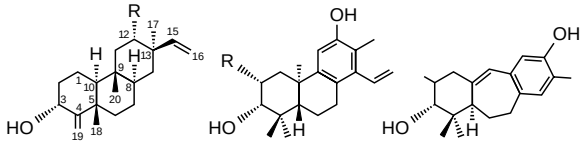
Two new erythroxylane diterpenes, named givotin A (**1**) and givotin B (**2**) were isolated from the bark of *Givotia madagascariensis*. Their structures have been established as 3α,12α-dihydroxy-4(19),15-erythroxyladiene (**1**) and 3α-hydroxy-4(19),15-erythroxyladiene (**2**), respectively, on the basis of one and two-dimensional NMR spectroscopic studies (^{1}H, ^{13}C, COSY, HMQC, HMBC, NOESY, NOE difference spectra) as well as on mass spectral analysis. In addition six known compounds (**3 – 8**) have been isolated and identified. Cleistanthol (**3**), spruceanol (**4**) and 1,2-dihydroheudelotinol (**5**) demonstrated significant antitumor activities against three tumor cell lines (HM02, Hep G2, MCF7).

Key words: Givotia madagascariensis, Euphorbiaceae, Erythroxylane Diterpenoids, Givotin, Antitumor Activity

Introduction

Givotia madagascariensis (Euphorbiaceae) is an endemic species distributed in a large part of Southwestern Madagascar [1]. It is a large tree, has a very thick trunk and is used locally to carve out a pirogue due to its water resistant wood. The plant is commonly known as "farafatsy" and used in folk medicine for the treatment of skin diseases and leprosy [2]. However, this species hat not been subject of a phytochemical and pharmacological investigation before.

We now report on the isolation and structure elucidation of two new diterpenoids, named givotin A (**1**) and givotin B (**2**), together with six known compounds, cleistanthol (**3**) [3], spruceanol (**4**) [4], 1,2-dihydroheudelotinol (**5**) [5], 3,4-seco-sonderianol (**6**) [6], scopoletin (**7**) [7, 8] and psoromic acid (**8**) [9, 10]. The antitumor activities of compound **3**, **4** and **5** against three tumor cell lines (HM02, Hep G2 and MCF7) are also described.



1 R = OH
2 R = H

3 R = OH
4 R = H

5

Results and Discussion

Dried bark of *G. madagascariensis* was extracted with ethanol, and the EtOH extract was partitioned between CH_2Cl_2 and H_2O. The aq. Phase was extracted with n-BuOH. Repeated chromatography on silica gel and sephadex LH-20 of the CH_2Cl_2 and n-BuOH extracts led to the isolation of two novel diterpenes, **1** and **2**, together with six known compound **3 – 8**.

The molecular formula of **1** was determined as $C_{20}H_{32}O_2$ from its HREIMS ([M^+], m/z 304.4721) as well as from its ^{13}C NMR and DEPT spectra. The IR spectrum indicated the presence of hydroxyl group(s)

Table 1. ^{1}H and ^{13}C NMR data of givotin A (**1**) in $CDCl_3$.

Atom	δ_C	δ_H, mult. (J in Hz)	HMBC (H → C)	NOESY
1	16.3	1.57, m, 1α	3, 5	2α, 10
		1.80, m, 1β	10	2β
2	34.7	2.02, m, 2α	1	1α
		1.56, m, 2β	1, 3	3
3	74.6	4.33, t (2.9)	4, 5, 19	2β
4	160.7			
5	39.9			
6	38.7	1.54, m, 6α	5, 10	7α
		1.75, m, 6β	5, 7, 10	18, 7β
7	24.6	1.46, m, 7α	6, 8	6α, 14α
		1.28, m, 7β		6β
8	42.0	1.34, m, α	7, 9	17
9	38.6			
10	56.3	0.95, m, α	1, 5, 9, 18, 20	11α, 1α
11	42.7	1.88, dd (11.7, 4.7), 11α	9, 12, 13, 20	10α, 17
		0.98, dd (11.7, 2.1), 11β	9, 12, 13, 20	12, 20
12	71.7	3.70, dd (11.7, 4.7), β	13, 15, 17	11β
13	42.8			
14	39.8	1.09, dd (12.3, 1.7), 14α	8, 13, 17	7α
		1.40, m, 14β	8, 15	
15	147.9	5.82, dd (17.6, 10.8)	12, 13, 14, 17	16a,b
16	112.5	5.02, dd (10.8, 1.3), 16a	12, 13, 15	15
		5.08, dd (17.6, 1.3), 16b	12, 13, 15	15
17	15.0	1.01, s (3H)	12, 13, 14, 15	8, 11α
18	23.8	1.25, s (3H)	4, 5, 6, 10	20, 6β
19	109.1	4.79, d (1.0), 19a	3, 4, 5	
		4.84, d (1.0), 19b	3, 4, 5	
20	14.0	0.88, s (3H)	8, 9, 10, 11	18, 11β

($\nu = 3370$ cm^{-1}). In the ^{13}C NMR spectrum 20 carbon signals were observed, including three methyls, eight methylenes, five methines and four quaternary carbons. The presence of four sp^2 hybridized carbon atoms in the molecule, as deduced from DEPT spectra, corresponding to two carbon-carbon double bonds, indicated compound **1** to be tricyclic.

The ^{1}H NMR spectrum, in combination with the HMQC data, showed three signals of an ABX system corresponding to vinyl protons from a monosubstituted double bond at δ 5.82 (1H, dd, $J = 17.6$, 10.8 Hz, H-15), 5.08 (1H, $J = 17.6$, 1.3 Hz, H-16a) and 5.02 (1H, $J = 10.8$, 1.3 Hz, H-16b), indicating the C-15 and C-16 position of this double bond [11, 12]. In addition, two signals corresponding to an AB system of an olefinic methylene group at δ 4.84 (d, $J = 1.0$ Hz, H-19a) and 4.79 (d, $J = 1.0$ Hz, H-19b), two oxygenated methine groups at δ 4.33 (t, $J = 2.9$ Hz, H-3) and 3.70 (dd, $J = 11.7$, 4.7 Hz, H-12), and three tertiary methyl groups at δ 1.25 (3H, s, CH_3-18), 1.01 (3H, s, CH_3-17) and 0.88 (3H, s, CH_3-20), were observed.

The ^{1}H-^{1}H COSY spectrum, in combination with HMQC data, showed cross-peaks for H-1/H-10, H-1/H-2, H-2/H-3, H-6/H-7, H-7/H-8, H-8/H-13, H-11/H-12 and H-15/H-16, thus confirming the following structural fragments: $CHCH_2CH_2CHOH$ (C_{10}-C_1-C_2-C_3), $CH_2CH_2CHCH_2$ (C_6-C_7-C_8-C_{14}), CH_2CHOH (C_{11}-C_{12}) and $CH{=}CH_2$(C_{15}-C_{16}). These fragments are connected with tertiary methyl groups and the olefinic methylene group to yield the erythroxylane skeleton by virtue of the HMBC correlations shown in Table 1. The position of the two hydroxy groups were confirmed on basis of the observed HMBC correlations from H-3 to C-4, C-5 and C-19, from H-2 to C-3, from H-12 to C-13, C-15 and C-17, and from H-11 to C-12. The long-range correlations observed between H_3-17 (δ 1.01) and carbons C-13 (δ 42.8) and C-15 (δ 147.9) as well as olefinic protons H-15 (δ 5.82) and H-16 (δ 5.08 and 5.02) and carbon C-13, confirmed the location of the methyl group CH_3-17 and the vinyl group at C-13.

The relative stereochemistry of **1** was defined by analysis of NMR chemical shifts, coupling constant values and by NOESY correlations (Table 1) as well as by selective 1D NOE experiments. The small coupling constant of the carbinol proton at δ 4.33 (t, $J = 2.9$ Hz) indicated an equatorial position for H-3 on the β-face from an examination of the Dreiding model. The ^{1}H-^{1}H coupling constants ($J = 11.7$, 4.6 Hz) observed between H-12 and H-11, as well as

Table 2. ^{1}H and ^{13}C NMR data of givotin B (**2**) in $CDCl_3$.

Atom	δ_C	δ_H, mult. (*J* in Hz)	HMBC (H → C)
1	16.1	1.54, m, 1α	10
		1.77, m, 1β	
2	34.8	2.03, m, 2α	1
		1.57, m, 2β	3
3	74.8	4.33, t (3.4)	1, 5, 19
4	161.0		
5	40.3		
6	38.7	1.54, m, 6α	
		1.76, m, 6β	7
7	25.5	1.47, m, 7α	
		1.22, m, 7β	6
8	42.4	1.32, m, α	9
9	37.2		
10	56.3	0.94, m, α	5, 20
11	35.3	1.60, m, 11α	
		1.03, m, 11β	10
12	32.0	1.23, m	
		1.57, m	
13	36.3		
14	39.1	0.98, dd (12.3, 1.7), 14α	
		1.35, m, 14β	8
15	151.3	5.80, dd (17.5, 10.7)	12, 13, 17
16	109.0	4.84, dd (10.7, 1.0), 16a	13
		4.90, dd (17.4, 1.0), 16b	13, 15
17	23.0	1.00, s (3H)	12, 14, 15
18	23.6	1.25, s (3H)	4, 6, 10
19	108.5	4.79, d (1.2), 19a	3, 4, 5
		4.84, d (1.2), 19b	3, 4, 5
20	12.4	0.78, s (3H)	8, 9, 10, 11

NOE correlations between H-12 and H-11β , H-11β and H_3-20, and H_3-20 and H_3-18, indicated the β-orientation of H-12, CH_3-18 and CH_3-20. The configuration of the methyl group (CH_3-17) at C-13 was assigned to be α on the basis of the absence of the correlations from CH_3-17 to H-12 and H-14α as well as the correlations from CH_3-17 to H-8 and H-11α in the NOESY experiment. Thus, compound **1** was identified as 3α,12α-dihydroxy-4(19),15-erythroxyladiene, a new natural diterpenoid.

Compound **2** was isolated as colorless oil, showing the molecular ion peak at m/z 288 (M^+) in the EIMS. Its molecular formula $C_{20}H_{32}O$ was deduced from the HREIMS ([M^+], m/z 288.2450) and DEPT spectra. The fragmentation pattern in the mass spectrum as well as the IR spectrum was nearly identical to compound **1**. The ^{1}H NMR and ^{13}C NMR spectra of compound **2** were also very similar to those of **1** except for up field shift of the signal of C-12 at δ 32.0, suggesting the replacement of the hydroxyl group at C-12 by a proton. The confirmation for this was obtained by the observation of cross-peaks between the methylene carbon C-12 (δ 32.0) and proton signals H_3-17 and H-15 (δ 5.80) in the HMBC spectrum (Table 2). Furthermore, the ^{13}C NMR spectrum, analyzed with the aid of DEPT spectra, showed the only presence of one oxygenated methine (δ 74.8), and in contrast to **1**, the ^{1}H NMR spectrum of **2** displayed only one proton within the range 4.5 to 3.0 ppm.

Table 3. Antitumor activity measured toward HMO2, Hep G2 and MCF7 cells (values in *n*M/ml).

Com-	HMO2			Hep G2			MCF7		
pound	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}
3	3.5	7.6	19.0	4.1	15.8	> 32	5.7	15.8	23.7
4	2.2	18.3	> 33	3.0	> 33	> 33	5.0	16.7	28.3
5	8.8	> 36	> 36	8.1	21.7	> 36	8.1	20.6	29.4

GI50: drug concentration causing 50% growth inhibition; TGI: drug concentration causing 100% growth inhibition; LC50: drug concentration causing 50% reduction of the cells present at time zero, *i.e.* at 24 h.

A combination of COSY, HMQC and HMBC experiments enabled us to determine the structure of **2**, and allowed the assignment of all ^{1}H and ^{13}C NMR signals (Table 2).

Analysis and comparison of NMR chemical shifts and coupling constants of **2** with that of **1** suggested an identical relative stereochemistry of the chiral centers at C-3, C-5, C-8, C-9, C-10 and C-13. The structure of **2** was, therefore, determined to be 3α-hydroxy-4(19),15-erythroxyladiene, a new natural compound.

Six known compounds, 8,11,13,15-cleistanthatetraene-2,3,12-triol (**3**), 8,11,13,15-cleistanthatetraene-3,12-diol (**4**), 3,12-dihydroxy-9(10→20)-abeo-16,17-dinor-abieta-8,10(20),11,13-t etraene (**5**), 12-hydroxy-3,4-seco-4(18),8,11,13,15-cleistanthapentaene-3-oic acid (**6**), 7-hydroxy-6-methoxy-cumarin (**7**) and psoromic acid (**8**), were isolated along with compounds **1** and **2**. No ^{13}C NMR data of compounds **3** and **4** have been reported previously. Full ^{1}H and ^{13}C NMR data of compounds **3**–**5** are reported in the experimental section for the first time. Compounds **6**–**8** were identified by comparison of their physical and spectroscopic data with those reported in literature [6–9].

Antitumoral tests against three human cancer cell lines, HMO2 (stomach adenocarcinoma), Hep G2 (hepatocellular carcinoma) and MCF 7 (breast adenocarcinoma), showed that compounds **3**, **4** and **5** were significantly active in the growth inhibition (Table 3). However, compounds **1**, **2**, **7** and **8** had no inhibition on survival of the three tested tumor cells. Compound **6** has not been tested.

Experimental Section

General comments

Melting points were determined on a Büchi 510 melting point apparatus and are uncorrected. IR spectra were taken on a Perkin-Elmer 341 spectrometer. ^{1}H, ^{13}C NMR and 2D NMR spectra were recorded using a Brucker AM 400 spectrometer at 400 and 100.5 MHz, respectively, with TMS as internal standard. EIMS and HR-EIMS data were obtained using a Finnigan MAT 312 and Autospec (Firma VG) spectrometer, respectively. TLC were carried out on precoated silica gel 60F_{254} plates (0.2 mm, Merck). CC were also performed on Merck silica gel 60 (0.063 – 0.040 mm or 0.04 – 0.02 mm).

Plant material

Givotia madagascariensis was collected around Morondava, Madagascar, in March 2000, and was identified by Prof. A. Rakotozafy. A voucher specimen has been deposited at the Institut Malgaches de Recherches Appliquées of Antananarivo.

Extraction and isolation

The air dried and powdered bark (850 g) of *G. madagascariensis* was exhaustively extracted by repeat maceration with EtOH at room temperature, and the extract was concentrated in vacuo to a syrup, dilued with H_2O, and partitioned successively with CH_2Cl_2 and n-BuOH. The CH_2Cl_2 and n-BuOH layers were evaporated in vacuum to afford 12.1 g and 4.5 g of residue, respectively. The CH_2Cl_2 extract was subjected to silica gel column chromatography and eluted with petroleum ether with increasing amount of EtOAc (0 – 100%) to give ten fractions. Fraction III was rechromatographed on silica gel using the same gradient system of petroleum ether/EtOAc. The combined fractions, eluted with petroleum ether/EtOAC (4:1), were submitted to exclusion chromatography on sephadex LH-20 with MeOH/CH_2Cl_2 (1:1) to afford compound **2** (35 mg), while the combined fractions, eluted with 35% EtOAc, treated in the same manner (exclusion chromatography) afforded a crystalline material upon standing, witch was recrystallized from petroleum ether/acetone to yield the pure compound **1** (115 mg). Fraction V was purified by CC on silica gel using petroleum ether/EtOAc (2:1) as solvent, and by chromatography on sephadex LH-20 and by preparative TLC on silica gel with petroleum ether/EtOAc (1:1) to isolate compound **5** (19 mg). Fraction VI and VII were further purified by a combination of silica gel CC (petroleum ether/EtOAc, 1:1), sephadex LH-20 CC (CH_2Cl_2/MeOH, 1:1), preparative TLC (CH_2Cl_2/MeOH, 9:1 or 19:1) and recrystallization, respectively. Fraction VI yielded compounds **3** (98 mg), **4** (47 mg) and **7** (8 mg, crystalline), and fraction VII furnished compound **6** (14 mg).

By chromatography on silica gel with petroleum ether/EtOAc (1:4) from n-BuOH extract, followed by CC on sephadex LH-20, preparative TLC (CH_2Cl_2/MeOH, 4:1) and crystallization from n-hexane/acetone, compound **8** (10 mg) was obtained.

Methods of the biological assay

The antitumor activity of the test compound was determined in three human cancer cell lines, according to the NCI guidelines [13]. The cell lines used were HM02 (stomach carcinoma), Hep G2 (human hepatocellular carcinoma) and MCF 7 (breast adenocarcinoma). Cells were grown in 96-well microtitre plates of RPMI tissue culture medium supplemented with 10% fetal calf serum at 37 °C in a humidified atmosphere. After 24 h incubation the test compounds (0.1 – 20 μg/ml) were added. Stock solutions of the test compounds were prepared in MeOH (concentration of 0.1%). After 48 hours incubation in the presence of the test drugs the cells were fixed by addition of trichloroacetic acid and cell protein was assayed with sulforhodamine B [14]. For each compound tested the GI_{50}, TGI and LC_{50} values were determined.

*3α, 12α-Dihydroxy-4(19),15-erythroxyladiene (**1**)*

M. p. 76 – 78 °C (colorless crystals). – UV (CH_3OH): λ_{max} = 231 nm. – IR (KBr): ν = 3370, 2976, 2920, 2800, 1630, 1390, 905 cm^{-1}. – ^{1}H and ^{13}C NMR see Table 1. – MS (EI, 70 eV): *m/z* (%) = 304 (13) [M$^+$], 289 (18), 287 (47), 260 (42), 271 (19), 235 (79), 219 (32), 202 (29), 201 (100), 199 (29), 191 (20), 187 (19), 174 (26), 173 (28), 161 (23), 159 (36), 147 (33), 145 (30), 133 (33), 122 (38), 121 (43), 119 (46), 107 (54), 105 (41), 93 (48), 91 (37), 81 (37), 79 (37), 69 (24), 67 (23). – HREIMS: *m/z* = 304.4721 (calcd. for $C_{20}H_{32}O_2$ 304.4723).

*3α-Hydroxy-4(19),15-erythroxyladiene (**2**)*

Colorless oil. – ^{1}H and ^{13}C NMR see Table 2. – MS (EI, 70 eV): *m/z* (%) = 288 (12) [M$^+$], 271 (10), 255 (17), 205 (21), 204 (100), 192 (11), 190 (11), 149 (10), 135 (12), 133 (10), 123 (10), 122 (14), 120 (12), 109 (12), 107 (21), 105 (14), 95 (19), 93 (19), 91 (15), 81 (22), 79 (13), 67 (11). – HREIMS: *m/z* = 288.2450 (calcd. for $C_{20}H_{32}O$ 288.2453).

*8,11,13,15-Cleistanthatetraene-2,3,12-triol (**3**)*

M. p. 187 – 188 °C (colorless powder). – UV (CH_3OH): λ_{max} = 288, 211 nm. – IR (KBr): ν = 3468, 3360, 2965, 2940, 2864, 1585, 1424, 1396, 1035, 938, 790 cm^{-1}. – ^{1}H NMR (300.13 MHz, CD_3OD): δ = 2.59 (dd, J = 13.5, 3.0 Hz, 1H, 1α-H), 1.62 (dd, 1H, 1β-H), 4.12 (m, 1H, 2β-H), 3.18 (d, J = 3.4 Hz, 1H, 3β-H), 1.31 (dd, J = 14.2, 1.19 Hz, 1H, 5β-H), 1.92 (m, 1H, 6α-H), 1.76 (m, 1H, 6β-H), 2.76

(m, 1H, 7α-H), 2.52 (m, 1H, 7β-H), 6.67 (s, 1H, 11-H), 6.57 (dd, $J = 17.4$, 12.2 Hz, 1H, 15-H), 5.47 (dd, $J = 17.6$, 2.2 Hz, 1H, 16a-H), 5.07 (dd, $J = 11.2$, 2.2 Hz, 1H, 16b-H), 2.09 (s, 3H, 17-H_3), 1.05 (s, 3H, 18-H_3), 1.08 (s, 3H, 19-H_3), 1.40 (s, 3H, 20-H_3). – ^{13}C NMR (75.47 MHz, CD_3OD): $\delta = 44.4$ (C-1), 72.5 (C-2), 79.3 (C-3), 39.3 (C-4), 51.1 (C-5), 19.6 (C-6), 30.0 (C-7), 124.2 (C-8), 149.3 (C-9), 38.2 (C-10), 110.8 (C-11), 154.3 (C-12), 120.4 (C-13), 140.0 (C-14), 137.3 (C-15), 119.3 (C-16), 12.9 (C-17), 29.8 (C-18), 17.2 (C-19), 27.4 (C-20). – MS (EI, 70 eV): *m/z* (%) = 316 (100) [M^+], 298 (2), 283 (11), 265 (13), 241 (4), 227 (4), 200 (6), 199 (9), 185 (7), 173 (8), 149 (5), 147 (6), 69 (2). – HREIMS: *m/z* = 316.2039 (calcd. for $C_{20}H_{28}O_3$ 316.2038).

*8,11,13,15-Cleistanthatetraene-3,12-diol (**4**)*

M. p. 114 – 116 °C (yellow amorphous solid). – UV (CH_3OH): $\lambda_{max} = 278$, 207 nm. – IR (KBr): $\nu = 3360$, 2965, 2940, 2864, 1585, 1454, 1425, 1375, 1296, 1195, 1062, 1030, 925, 854 cm^{-1}. – ^{1}H NMR (300.13 MHz, CD_3OD): $\delta = 2.24$ (m, 1H, 1α-H), 1.48 (m, 1H, 1β-H), 1.78 (m, 2H, 2-H), 3.24 (dd, $J = 10.6$, 5.5 Hz, 1H, 3β-H), 1.27 (dd, $J = 14.4$, 2.3 Hz, 1H, 5β-H), 1.90 (m, 1H, 6α-H), 1.68 (m, 1H, 6β-H), 2.78 (m, 1H, 7α-H), 2.54 (m, 1H, 7β-H), 6.66 (s, 1H, 11-H), 6.57 (dd, $J = 17.4$, 12.2 Hz, 1H, 15-H), 5.47 (dd, $J = 17.6$, 2.2 Hz, 1H, 16α-H), 5.07 (dd, $J = 11.2$, 2.2 Hz, 1H, 16β-H), 2.11 (s, 3H, 17-H_3), 1.06 (s, 3H, 18-H_3), 0.88 (s, 3H, 19-H_3), 1.19 (s, 3H, 20-H_3). – ^{13}C NMR (75.47 MHz, CD_3OD): $\delta = 38.7$ (C-1), 28.7 (C-2), 79.5 (C-3), 39.9 (C-4), 51.1 (C-5), 20.2 (C-6), 30.6 (C-7), 124.7 (C-8), 148.6 (C-9), 38.5 (C-10), 110.5 (C-11), 154.3 (C-12), 120.5 (C-13), 140.0 (C-14), 137.3 (C-15), 119.3 (C-16), 13.2 (C-17), 28.8 (C-18), 16.1 (C-19), 25.3 (C-20). – MS (EI, 70 eV): *m/z* (%) = 300 (100) [M^+], 285 (6), 267 (28), 241 (6), 213 (9), 199 (11), 197 (15), 187 (5), 185 (85), 173 (10), 147 (6), 84 (4). – HREIMS: *m/z* = 300.2090 (calcd. for $C_{20}H_{28}O_2$ 300.2089).

*3β,12-Dihydroxy-9(10 → 20)-abeo-16,17-dinor-abieta-8,10(20),11,13-tetraene (**5**)*

M. p.186 – 187 °C (colorless crystals). – ^{1}H NMR (300.13 MHz, $CDCl_3$): $\delta = 2.34$ (m, 2H, 1-H), 1.82 (m, 1H, 2α-H), 1.59 (m, 1H, 2β-H), 3.37 (dd, $J = 11.3$, 4.3 Hz, 1H, 3β-H), 2.30 (m, 1H, 5-H), 2.20 (m, 1H, 6α-H), 1.55 (m, 1H, 6β-H), 2.62 (m, 2H, 7-H), 6.51 (s, 1H, 11-H), 6.70 (s, 1H, 14-H), 2.12 (s, 3H, 15-H_3), 1.05 (s, 3H, 18-H_3), 0.66 (s, 3H, 19-H_3), 6.25 (s, 1H, 20-H). – ^{13}C NMR (75.47 MHz, CD_3OD): $\delta = 39.3$ (C-1), 33.0 (C-2), 78.2 (C-3), 42.4 (C-4), 54.2 (C-5), 31.1 (C-6), 33.4 (C-7), 135.2 (C-8), 135.5 (C-9), 143.4 (C-10), 117.4 (C-11), 154.1 (C-12), 123.1 (C-13), 131.0 (C-14), 15.7 (C-15), 25.7 (C-18), 14.1 (C-19), 127.0 (C-20). – MS (EI, 70 eV): *m/z* (%) = 272 (100) [M^+], 254 (36), 239 (38), 211 (22), 201 (12), 200 (18), 199 (13), 187 (52), 186 (45), 185 (30), 173 (19), 172 (22), 171 (33), 159 (31), 149 (31), 121 (13), 71 (16).

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