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4,5-Seco-Guaiane and a Nine-Membered Sesquiterpene Lactone from *Holostylis reniformis*

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Abstract: Root extracts of *Holostylis reniformis* (Aristolochiaceae) yielded three new natural sesquiterpenes, a sesquiterpene with an unusual carbon skeleton, 4,5-seco-guaiane (7-*epi*-11-hydroxychabrolidione A, **1**), a nine-membered lactone with new carbon skeleton (holostylactone, **2**), and a new megastigmane [(6*S*,7*E*)-6,9-dihydroxy-10-(2'-hydroxy-ethoxy)-4,7-megastigmadien-3-one, **3**], together with bulnesol and sitosterol-3-*O*-β-D-glucopyranoside. The structures of these compounds were determined by spectroscopic analyses and B3LYP/STO-3G** theoretical studies.
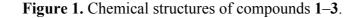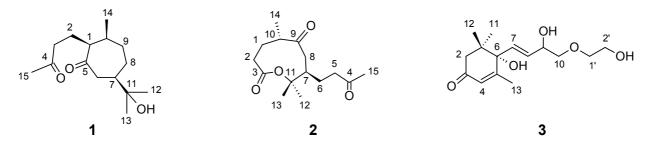
1. Introduction

Holostylis reniformis Duch. (Aristolochiaceae) has been used in traditional Brazilian medicine as an antirheumatic, stomachic, and depurative [1]. The anti-*Plasmodium falciparum* activities and toxicities of compounds and extracts obtained from this species have been demonstrated [2,3]. Since aryltetralone and furan lignans were the main chemical constituents found in the most active extracts, research has been concentrated on their chemical characterization [3–5], biosyntheses [6,7], and microbial transformations [8]. As part of our continuing studies on the chemical constituents of *H. reniformis*, in this paper the terpenoids obtained from the hexane and acetone extracts of its roots are reported.

2. Results and Discussion

The hexane extract of *H. reniformis* roots was subjected to column chromatography and yielded bulnesol, which was identified by comparison of its spectroscopic data with those reported in the literature [9,10], a 4,5-seco-guaiane **1** with an unusual carbon skeleton (Figure 1), and a nine-membered sesquiterpene lactone **2** with a new carbon skeleton (Figure 1). Together with sitosterol-3-*O*-β-D-glucopyranoside, which was identified by comparison of its spectroscopic data with those reported in the literature [11], a new sesquiterpene, megastigmane (**3**), was isolated from the acetone extract of roots (Figure 1). The structures of the new sesquiterpenes were determined by spectroscopic analyses and B3LYP/STO-3G** theoretical studies that were also performed.

Figure 1. Chemical structures of compounds **1**–**3**.

HRMS of compound **1** showed a *quasi*-molecular ion [M+Na]$^+$ at *m/z* 277.1774 ($C_{15}H_{26}O_3$+Na) and the MS-MS of these ions gave a base peak at *m/z* 237.18 [M−H$_2$O+H]$^+$. The ^{13}C-NMR spectra of **1** showed signals for 15 carbons, including two carbonyl groups at δ 213.4 and 208.8, the latter of which corresponded to a methyl ketone ($δ_H$ 2.04; $δ_C$ 29.8), and a quaternary carbinolic carbon (δ 73.0) (Table 1). The presence of carbonyl and hydroxyl groups in the structure was also evidenced by absorption bands observed in the IR spectrum at 1707 and 3440 cm^{-1}, respectively. Moreover, *g*HSQC, *g*HMQC, and DEPT 135° and 90° experiments evidenced the presence of an additional three methyl, five methylene, and three methine groups. The hydrogen chemical shifts of **1** were established with the aid of correlations observed by ^{1}H–^{1}H COSY, selective proton decoupling (HOMODEC), and *g*HMQC experiments. The *g*HMBC spectrum showed correlations between the carbonyl carbon signal at $δ_C$ 208.8 (C-4) and the methyl hydrogens at $δ_H$ 2.04 (3H-15) and the methylene hydrogens at $δ_H$ 2.37 and 2.19 (2H-3), whereas, the carbonyl carbon signal at $δ_C$ 213.4 (C-5) was correlated to H-6β ($δ_H$ 2.13). Both C-7 ($δ_C$ 46.4) and C-11 ($δ_C$ 73.0) were shown to be correlated to the hydrogens of two methyl groups

(δ_H 1.13 and 1.10). ^{1}H–^{1}H COSY experiment showed couplings between H-7 (δ_H 1.84) and H-6α (δ_H 2.52), H-6β (δ_H 2.13), H-8α (δ_H 1.75), and H-8β (δ_H 1.10), as well as between H-3a (δ_H 2.37) and H-3b, (δ_H 2.19), H-2a (δ_H 1.41), and H-2b (δ_H 1.13). The latter (H-2a,b) and H-8β were correlated to C-10 (δ_C 34.9), as well as 3H-14 (δ_H 0.70) were correlated to C-1 (δ_C 53.8) and C-9 (δ_C 37.0) by *g*HMBC experiments. Moreover, selective irradiation at δ_H 2.84 (H-1α) simplified the signals at δ_H 1.41 (H-2a), 1.13 (H-2b), and 1.93 (H-10), and evidenced a *cis* relationship between H-1 and H-10 (*J* = 2.5 Hz). Long-range coupling between H-6α (δ_H 2.52) and H-8α (δ_H 1.75) was also observed (*J* = 1.7 Hz) by the selective irradiation of these hydrogen frequencies. Based on all these data a 4,5-seco-guaiane structure can be suggested for **1**, *i.e.*, a bulnesol-type structure that was oxidized at C-4 and C-5. Spatial interactions between H-1 and H-2a, H-6α, H-7, H-9α, and H-10, as well as between H-8β and H-6β and 3H-14 observed by 1D-NOESY experiments allowed us to determine the conformation and the relative configuration as 1*S*,7*R*,10*S* (Figure 2). The high field chemical shift observed for H-8β (δ_H 1.10) is consistent with an endocyclic hydrogen protected by the carbonyl group at C-5. The spatial interactions between the methyl hydrogens 3H-14 and H-2a, H-8β, and H-9β confirmed this relative configuration.

Table 1. NMR Spectroscopic Data for Compound **1** (CDCl$_3$, 11.7 T).

Position	δ_C, type [a]	δ_H (*J* in Hz) [b]	*g*HMBC [c]
1α	53.8, CH	2.84, ddd (9.5, 4.5, 2.5)	14
2a	24.2, CH$_2$	1.41, dddd (14.5, 8.5, 6.0, 4.5)	
2b		1.13, m	
3a	41.7, CH$_2$	2.37, ddd (17.0, 8.5, 6.0)	
3b		2.19, ddd (17.0, 9.0, 8.5)	
4	208.8, C		3a, 3b, 15
5	213.4, C		6β
6 α	47.0, CH$_2$	2.52, ddd (17.5, 4.5, 1.7)	
6β		2.13, dd (17.5, 12.0)	
7	46.4, CH	1.84, dddd (12.0, 10.0, 4.5, 1.5)	12, 13
8α	25.1, CH$_2$	1.75, m	
8β		1.10, m	
9α	37.0, CH$_2$	1.73, m	8β, 14
9β		2.04, m	
10	34.9, CH	1.93, m	14, 2b and/or 8β
11	73.0, C		12, 13 and/or 8β
12	26.6, CH$_3$	1.13, s	13
13	26.5, CH$_3$	1.10, s	12
14	14.0, CH$_3$	0.70, d (7.5)	
15	29.8, CH$_3$	2.04, s	

[a] Chemical shifts and multiplicities were determined with the assistance of DEPT and *g*HMQC experiments; [b] Multiplicities were determined with the assistance of ^{1}H-^{1}H COSY, ^{1}H-^{1}H TOCSY and HOMODEC experiments; [c] *g*HMBC correlations, optimized for 8 Hz, are from carbon stated to the indicated hydrogen(s).
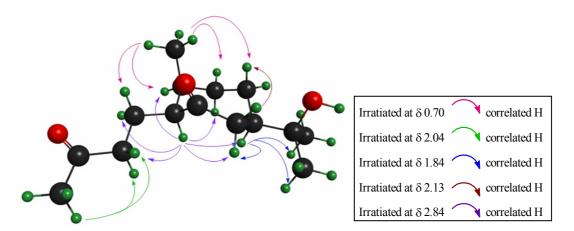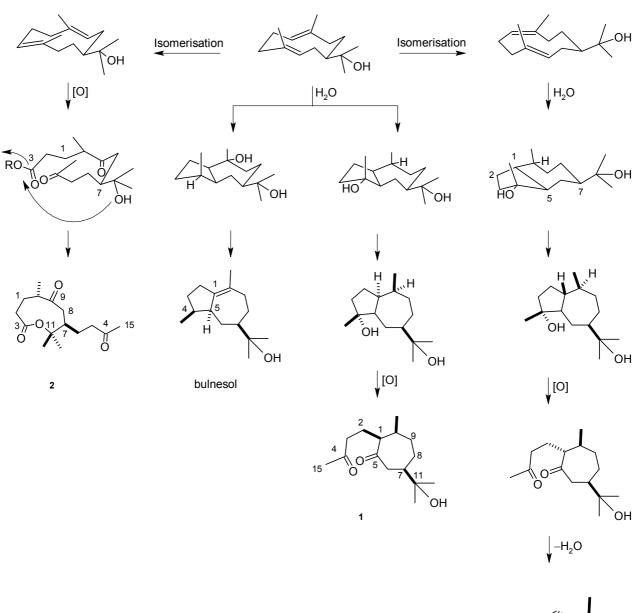
Figure 2. Selected nOe interactions for sesquiterpene **1**.

A compound with this basic structure was previously obtained as a key intermediate in the synthesis of guaipyridine alkaloids from guaiol [12]. To date, however, except for the chemical shift of the methyl ketone hydrogens, no spectroscopic data for this compound have been reported in the literature. Chabrolidione A and its 1-*epi* isomer (**4**, Scheme 1), which have the same carbon skeleton as **1**, were isolated from the soft coral *Nephthea chabrolii* [13] and *Sinularia leptoclados* [14], respectively. However, they showed different relative configurations (at stereocenter C-10). Proposed biogenetic pathways for the formation of sesquiterpenes **1** and **4**, which may involve water addition and the cyclization of hedycaryol and its isomer followed by oxidation, are shown in Scheme 1. Sesquiterpene **1** was named 7-*epi*-11-hydroxychabrolidione A.

The ^{13}C-NMR spectrum of **2** showed signals for 15 carbons, including two carbonyl groups at δ 215.4 and 208.0, and an acyl group at δ 171.0. The IR spectrum showed a broad and intense absorption band of these groups at ~ 1732 and 1707 cm^{-1}, and did not show a characteristic OH absorption band. The HRMS spectrum of compound **2** showed *quasi*-molecular ions [M+Na]$^+$ at *m/z* 291.1567 (calc for C$_{15}$H$_{24}$O$_4$+Na 291.1567) and *m/z*. 269.1745 [M+H]$^+$ (calc for C$_{15}$H$_{25}$O$_4$ 269.1747). From these data, a total of four hydrogen deficiencies were determined for the structure, one of which was a ring, since no additional sp^2 carbon was observed in the ^{13}C-NMR spectrum. In addition, a carbinolic carbon (δ$_C$ 85.0), five methylenes (δ$_C$ 23.8, δ$_H$ 1.57; δ$_C$ 30.9, δ$_H$ 1.51; δ$_C$ 34.6, δ$_H$ ~2.20; δ$_C$ 42.2, δ$_H$ 2.51; δ$_C$ 43.0, δ$_H$ 2.53 and 1.96), two methine groups (δ$_C$ 47.7, δ$_H$ 2.71; δ$_C$ 49.2, δ$_H$ 2.26), and four methyl groups (δ$_C$ 18.8, 20.3, 25.7, 30.2 and δ$_H$ 0.97, 1.26, 1.57, 2.10, respectively) were observed by an analysis of the ^{1}H and ^{13}C-NMR spectra, DEPT 135°, *g*HSQC, and *g*HMQC experiments (Table 2). A detailed analysis of ^{1}H–^{1}H COSY, selective proton decoupling (HOMODEC), and *g*HMBC experiments allowed us to determine the chemical shifts of the remaining hydrogens (H-1α, H-2β, H-6b) (Table 2). The *g*HMBC spectrum showed correlations between the carbonyl carbon at δ$_C$ 208.0 (C-4) and the methyl hydrogens at δ$_H$ 2.10 (3H-15) and the methylene hydrogens at δ$_H$ 2.51 (2H-5). These latter were also correlated to the methine carbon at δ$_C$ 49.2 (C-7). The carbinolic carbon and C-7 correlated with methyl hydrogens at δ$_H$ 1.57 (3H-12) and 1.26 (3H-13) as well as with H-8α (δ$_H$ 1.96). Selective irradiation at δ$_H$ 2.26 (H-7) led to the simplification of the multiplets at δ$_H$ 2.53 (H-8β), 1.96 (H-8α), 1.57 (H-6a), and 1.23 (H-6b). ^{1}H–^{1}H COSY experiment showed couplings between 2H-5 (δ$_H$ 2.51) and H-6a and H-6b, as well as between H-10 (δ$_H$ 2.71) and 3H-14 (δ$_H$ 0.97), H-1α (δ$_H$ 2.06), and

H-1β (δ$_H$ 1.51). 1D-TOCSY experiments evidenced long-distance coupling between H-10 and 2H-2 (δ$_H$ ~2.2). The *g*HMBC spectra also showed correlations between the carbonyl carbon at δ 215.4 (C-9) and H-8α and 3H-14, which, in turn, were correlated to C-1 and C-10. The chemical shifts of the carbinolic carbon and the acyl group suggested the presence of a lactone. Based on all of these data, and with the aid of 1D-TOCSY experiments, a nine-membered lactone structure, with a 3-oxobutyl substituent, was established for **2**. Moreover, the coupling constant values (*J*) for the hydrogens that showed virtual couplings (H-1α, H-1β, H-2α, H-2β, Table 2) were determined by using the ACD/C+H NMR Predictors program (Advanced Chemistry Development, [15]) and confirmed by HOMODEC experiments. In addition, *g*NOESY experiments were used to determine the conformation of this sesquiterpene lactone based on spatial interaction between the hydrogens, as shown in Figure 3. In this conformation, the spatial interactions between H-8β and 3H-13 and H-10β could be justified. Besides, the anisotropic effects of the acyl group C-3 on the hydrogens C<u>H</u>$_3$-12 and of carbonyl group C-9 on H-7 and H-lα, which are in *pseudo*-axial positions, could explain the high chemical shifts observed for these hydrogens. Therefore, the relative configuration for **2** was established as 7*R*,10*S*. Hence, sesquiterpene **2** has a new carbon skeleton and was named holostylactone. Its biosynthesis may involve oxidation at C-3,4, and C-9 of the key precursor hedycaryol, which leads to the breakage of the C-3,4 bound, followed by lactonization involving OH-11 and C-3 (Scheme 1).

HRMS spectrum of compound **3** showed a *quasi*-molecular ion [M+Na]$^+$ at *m/z* 307.1525 (C$_{15}$H$_{24}$O$_5$+Na). The ^{1}H and ^{13}C-NMR spectra of **3** (Table 3) showed signals for two methyl groups linked to a sp^3 quaternary carbon (δ$_H$ 0.91, 0.93; δ$_C$ 23.9, 23.0), a methyl group (δ$_H$ 1.82; δ$_C$ 19.0) linked at the β position of an α,β-unsaturated carbonyl group (δ$_H$ 5.77; δ$_C$ 197.4, 125.5, 164.0), and a *trans* olefin (δ$_H$ 5.75, 5.69; δ$_C$ 129.9, 131.8) linked to carbinolic carbons (δ$_C$ 71.6, 79.1 δ$_C$). These data, together with the results of *g*COSY, *g*HMQC, *g*HMBC, and *g*NOESY experiments, led to a megastigmane carbon skeleton for **3**. Indeed, the ^{1}H and ^{13}C-NMR data of **3** (Table 3) were very similar to those published for cucumegastigmane I and the aglycone of cucumegastigmane II [16]. The main differences between their ^{13}C-NMR spectral data were due to the signals observed for an O-CH$_2$CH$_2$OH group at C-10 of **3** (δ$_C$ 66.1 and 60.2). The absolute configuration of cucumegastigmane I was determined by using a modified Mosher's method and by application of the circular dichroism (CD) helicity rule on analogous compounds for determination of C-6 configuration [16]. Based on a comparison of the optical activities of **3** (CD Δε = +2.3 at 243 nm), cucumegastigmane I (CD Δε = +15.8 at 241 nm), and blumenol A (CD Δε = +9.6 at 243 nm) [17], the same absolute configuration could be inferred for C-6 of **3**, *i.e.*, (6*S*), since they showed positive Cotton effect at λ ~ 242 nm and follow the same helicity rule for π-π* enone Cotton effect [17]. Although the absolute configuration of C-9 remain to be determined, the structure established for **3** is (6*S*,7*E*)-6,9-dihydroxy-10-(2'-hydroxyethoxy)-4,7-megastigmadien-3-one (named holostymegastigmadienone) (Figure 4).

Scheme 1. Proposed biogenetic pathway for sesquiterpenes **1**, **2**, and **4**.

Table 2. NMR Spectroscopic Data for compound **2** (CDCl₃, 11.7 T).

H/C	δ_C, type [a]	δ_H (*J* in Hz) [b, c]	*g*HMBC [d]
1α	30.9, CH₂	2.06, dddd [c] (14.5, 9.8, 9.3, 6.5)	14
1β		1.51, dddd [c] (14.5, 8.5, 2.5, 1.5)	
2α	34.6, CH₂	2.22, ddd [c] (15.0, 6.5, 1.5)	
2β		2.19, ddd [c] (15.0, 9.8, 8.5)	
3	171.0, C		
4	208.0, C		5a, 5b, 15
5a, 5b	42.2, CH₂	2.51 br, t (7.5)	15
6a	23.8, CH₂	1.57, ddt (14.0, 2.0, 7.5)	8β

Table 2. *Cont.*

H/C	δ_C, type [a]	δ_H (*J* in Hz) [b, c]	*g*HMBC [d]
6b		1.23, ddt (14.0, 11.5, 7.0)	
7	49.2, CH	2.26, ddt (11.5, 11.0, 2.0)	5a, 5b, 8α, 12, 13
8α	43.0, CH$_2$	1.96, dd (12.5, 2.0)	
8β		2.53, dd (12.5, 11.0)	
9	215.4, C		8α, 14
10	47.7, CH	2.71, ddq (9.3, 2.5, 7.0)	14
11	85.0, C		8α, 13, 12 and/or 6a
12	25.7, CH$_3$	1.57, s	13
13	20.3, CH$_3$	1.26, s	12
14	18.8, CH$_3$	0.97, d (7.0)	
15	30.2, CH$_3$	2.10, s	

[a] Chemical shifts and multiplicities were determined with the assistance of DEPT and *g*HMQC experiments; [b] Multiplicities were determined with the assistance of ^{1}H–^{1}H COSY, ^{1}H–^{1}H TOCSY and HOMODEC experiments; [c] Calculated values; [d] *g*HMBC correlations, optimized for 8 Hz, are from carbon stated to the indicated hydrogen(s).

Figure 3. Selected nOe interactions for sesquiterpene **2**.

Irratiated at δ 1.26	correlated H
Irratiated at δ 2.10	correlated H
Irratiated at δ 2.53	correlated H
Irratiated at δ 2.26	correlated H
Irratiated at δ 1.51	correlated H
Irratiated at δ 2.71	correlated H

Table 3. NMR Spectroscopic Data for Compound **3** (DMSO-d_6, 11.7 T).

H/C	δ_C, type [a]	δ_H (*J* in Hz) [b]	*g*HMBC [c]
1	40.9, C		11, 12
2	49.4, CH$_2$	2.05, d (16.0)	11, 12
2		2.39, d (16.0)	
3	197.4, C		
4	125.5, CH	5.77, q (2.5)	13
5	164.0, C		13
6	79.1, C		11, 12, 13, 4, 7, O<u>H</u>
7	129.9, CH	5.75, d (17.0)	
8	131.8, CH	5.69, dd (17.0, 5.0)	
9	71.6, CH	4.00, m	7
10	72.2, CH$_2$	3.30–3.40, m	1'
11	23.0, CH$_3$	0.93, s	12

Table 3. *Cont.*

H/C	δ_C, type [a]	δ_H (*J* in Hz) [b]	*g*HMBC [c]
12	23.9, CH_3	0.91, s	11
13	19.0, CH_3	1.82, d (1.5)	4
1'	66.1, CH_2	3.30–3.40, m	
2'	60.2, CH_2	3.30–3.40, m	1'

[a] Chemical shifts and multiplicities were determined with the assistance of DEPT and *g*HMQC experiments; [b] Multiplicities were determined with the assistance of ^1H–^1H COSY experiments; [c] *g*HMBC correlations, optimized for 8 Hz, are from carbon stated to the indicated hydrogen(s).

Figure 4. Conformations for compound **3**, (**a**) or (**b**).
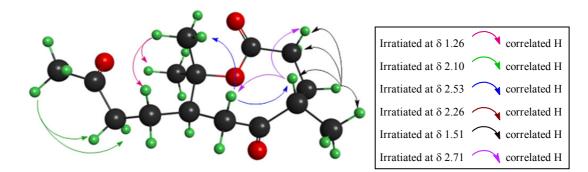

(**a**) (6*S*,9*R*)   (**b**) (6*S*,9*S*)

In addition to the NOESY experiments quantum chemical calculations were carried out at the B3LYP/STO-3G** level as fully described in the supplementary data. In order to have a reliable starting point for the calculations molecular structures with 7- and 9-membered rings were searched in the Cambridge Structural Database [18], basically two different conformations for these rings were found (Figure S8). Unrestricted optimizations have been carried out for these conformers.

The four stationary structures found for the 7-membered ring have similar energies (Table S1 and Figure S9). Nevertheless, the NBO steric analysis (Table 4) indicates that only the **74**-conformer holds all spatial interactions noted by 1D-NOESY experiments. In fact the calculated **74**-conformer was used to draw Figure 2. For sesquiterpene **2** also four conformers were found but in this case one of them, conformer **91**, is much more stable as shown in Table S1. Moreover, the NBO steric analysis shows that this conformer has the strongest spatial interactions for the ones displayed by the 1D-NOESY experiments (Table 4). This conformation was the one used for drawing Figure 3.

From the foregoing it is clear that the molecular modeling studies support the 1D-NOESY experimental results, thus proving that the **74**- and **91**-conformer describe the 3D structures of the 7- and 9- membered ring compounds, respectively.

Table 4. NBO Steric Exchange Energies (kcal.mol^{-1})—Conformation.

7-membered ring				
Spatial Interaction	71	72	73	74
····–C1–H1…. H2a–C2–····	0.79	1.52	1.17	0.68
····–C1–H1… H6α–C6–····	1.00	0.09	----	0.19
····–C1–H1…. H7–C7–····	----	----	0.04	0.59
····–C1–H1…. H9α–C9–····	0.65	----	0.67	0.43
····–C1–H1….. H10–C10–····	1.81	1.74	2.07	0.99
····–C2–H2b… CH$_3$(14)–····	0.99	----	0.89	0.72
····–C8–H8β… H6β–C6–····	----	----	0.63	0.27
····–C8–H8β…. CH$_3$(14)–····	1.39	----	0.78	0.54
····–C8–H8β… O=C5–····	----	0.65	----	0.15
····–C8–H8β….O-H····	2.10	----	----	0.12
····–C9–H9β… .CH$_3$(14)–····	0.30	0.76	0.40	0.75
9-membered ring				
	91	92	93	94
····–C1–H1α… O=C9–····	1.21	----	0.02	0.01
····–C7–H7…. O=C9–····	0.59	----	----	0.03
····–C8–H8β….H10–C10–····	0.97	0.02	----	0.57
····–C8–H8β….CH$_3$(13)– ····	0.62	0.17	0.55	1.91
····–CH$_3$(12)….O=C4–····	11.04	9.24	----	8.18
····–CH$_3$(12)….O=C3–O–····	1.49	1.32	0.59	0.93

3. Experimental

3.1. General Experimental Procedures

One-dimensional (^{1}H, ^{13}C, DEPT, HOMODEC, TOCSY, and *g*NOESY) and two-dimensional (^{1}H–^{1}H *g*COSY, *g*NOESY, *g*HMQC, *g*HSQC, and *g*HMBC) NMR experiments were performed on a Varian INOVA 500 spectrometer (11.7 T) at 500 MHz (^{1}H), and 126 MHz (^{13}C), using deuterated solvents (CDCl$_3$ and DMSO-d_6) (P 99.9% D) as an internal standards for ^{13}C-NMR chemical shifts and residual solvent as an internal standard for ^{1}H-NMR. δ values are reported relative to TMS. Mass spectra (ESI-MS) were obtained on a LCQ *Fleet*–Thermo Scientific, and flow injection into the electrospray source was used for LC-ESI-MS. High-resolution mass spectra (HRMS) were obtained on a Bruker Daltonics ultrOTOFq (ESI-TOFMS). IR spectra were obtained on a Perkin–Elmer 1600 FT-IR spectrometer using KBr discs. Optical rotations were measured on a Perkin Elmer 341-LC polarimeter. Ultraviolet (UV) absorptions were measured on a Perkin Elmer UV-vis Lambda 14P diode array spectrophotometer. HPLC analyses were performed using a Shimadzu liquid chromatograph (SPD-10 Avp), equipped with UV–vis and 341-LC polarimeter detectors, and using a Jasco LC-NetII/ADC, equipped with photodiode array (MD-2018 Plus) and CD (2095 Plus) detectors. The columns were RP-18 (Varian, C18, with a particle size of 5 μm, 250 × 4.6 mm for analytical analysis, and 250 × 20 mm for semi-preparative analysis), and chromatograms were acquired at 336 and 254 nm. TLC: silica gel 60 PF$_{254}$.

3.2. Plant Materials

The plant materials were collected in Ituiutaba, MG, Brazil, in February 2008, and identified as *Holostylis reniformis* Duch. by Dr. Vinícius C. Souza and Dr. Lindolpho Cappellari Jr. A voucher specimen (ESA 88282/2008) was deposited at the herbarium of the Escola Superior de Agricultura, Luiz de Queiroz (ESALQ), Piracicaba, SP, Brazil. The material was separated according to the plant parts and dried (~45 °C).

3.3. Extraction and Isolation of the Chemical Constituents

The roots (107.9 g) were ground and exhaustively extracted successively at room temperature with hexanes, acetone, and ethanol [4 × (~200 mL, 2 days) each solvent]. The residues were extracted with ethanol in a Soxhlet apparatus and the extracts were individually concentrated. The crude hexane extract (6.17 g) was subjected to CC (6.0 by 40.0 cm, silica gel 60H, 127.3 g, hexanes/EtOAc gradient, 19:1 to 100% EtOAc) to give 28 fractions (*ca.* 125 mL each) as previously described [3]. Fraction 5 (50.0 mg) gave bulnesol. Fraction 20 (280.0 mg) gave **1** (19.3 mg) after semi-preparative HPLC (MeOH–H_2O, 3:2).

The acetone extract from roots (3.73 g) was also subjected to CC to give 26 fractions, as previously described [4]. Fraction 17 (15.5 mg) was partially dissolved with CH_3CN and the soluble-portion was subjected to HPLC (MeOH-H_2O 7:3) to give **3** (11.6 mg). Fraction 23 (9.0 mg) was washed with MeOH and the resulting soluble-portion gave sitosterol-3-*O*-β-D-glucopyranoside (3.0 mg).

rel.-(2S,3S,6R)-6-(2-Hydroxypropan-2-yl)-3-methyl-2-(3-oxobutyl)cycloheptanone (7-*epi*-11-*hydroxy-chabrolidione A*, **1**): Colorless oil; $[\alpha]_D^{25}$ +50.7 (*c* 0.9, CHCl$_3$); IR (KBr) ν_{max} 3440, 2972, 2925, 2875, 1707, 1376 cm^{-1}; ^{1}H and ^{13}C-NMR (CDCl$_3$) see Table 1; HRESIMS (probe) 4.5 eV, positive mode, *m/z* (rel. int.): 277.1774 [M+Na]$^+$ ($C_{15}H_{26}O_3$+Na) (94) (calculated for $C_{15}H_{26}O_3$+Na = 277.1774), 237.18 [M+H−H_2O]$^+$ (100); ESIMS 20 eV, positive mode, *m/z* (rel. int.): 237 (100), 235 (35).

rel.-(5S,8R)-5,9,9-Trimethyl-8-(3-oxobutyl)oxonane-2,6-dione (holostylactone, **2**): Colorless oil; $[\alpha]_D^{25}$ −20.0 (*c* 1.0, CHCl$_3$); IR (KBr) ν_{max} 2912, 2862, 1732, 1707, 1389, 1100 cm^{-1}; ^{1}H and ^{13}C-NMR (CDCl$_3$) see Table 2; HRESIMS (probe) 4.5 eV, positive mode, *m/z* (rel. int.): 291.1567 (calc for $C_{15}H_{24}O_4$+Na = 291.1567) (100) and *m/z* 269.1745 [M+H]$^+$ (calc for $C_{15}H_{25}O_4$ 269.1747) (60); ESIMS 20 eV, positive mode, *m/z* (rel. int.): 269 (100), 251 (55), 233, [M+Na−H_3CCOCH_3] (18).

(6S,7E,9S)-6,9-Dihydroxy-10-(2'-hydroxyethoxy)-4,7-megastigmadien-3-one (holostymegastigmadienone, **3**): Yellow oil; $[\alpha]_D^{25}$ +68.6 (*c* 0.9, MeOH); IR (KBr) ν_{max} 3452, 1666, 1643, 1384 cm^{-1}; ^{1}H and ^{13}C-NMR (DMSO-d_6) see Table 3; HRESIMS (probe) 4.5 eV, positive mode, *m/z* (rel. int.): 307.1525 [M+Na]$^+$ (100) (calculated for $C_{15}H_{24}O_5$+Na = 307.1522); ESIMS 20 eV, positive mode, *m/z* (rel. int.): 307 (38), 277 (87), 275 (100), 237 (43), 205 (47), 130 (24). CD (MeOH, *c* 0.1): $[\theta]_{221}$ 0, $[\theta]_{243}$ + 7590, $[\theta]_{271}$ 0, $[\theta]_{312}$ −1815.

4. Conclusions

H. reniformis can synthesize a variety of seco compounds, including lignans and sesquiterpenes. Among them, bulnesol and two unusual 4,5-seco-guainanes, which may be biogenetic derivatives of hedycaryol, were isolated from the roots of this species. In addition, a new megastigmadienone was isolated.

Supplementary Materials

Supplementary materials can be accessed at: http://www.mdpi.com/1420-3049/17/12/14046/s1.

Acknowledgments

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Sample Availability: Samples of the compounds **1**–**3** are available from the authors.