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Article

New *neo*-Clerodanes from *Tinnea antiscorbutica* Welv.

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Três novos *neo*-clerodanos, antiscorbuticano A, antiscorbuticano B, e antiscorbuticano C e os compostos conhecidos glutinol, friedelina, 5,7-di-hidroxiflavanona (pinocembrina), 5-hidroxi-3,6,7,4'-tetrametoxiflavona, 5-hidroxi-3,6,7,3',4'-pentametoxiflavona (artemetina), 5,4'-di-hidroxi-3,6,7,3'-tetrametoxiflavona (penduletina) e 5,3',4'-tri-hidroxi-3,6,7-trimetoxiflavona (crisosplenol D) foram isolados do extrato de metanol de *Tinnea antiscorbutica*. O composto antiscorbuticano B não apresentou atividade mutagênica para doses até 250 μg por caixa (teste de Ames) e não induziu micronúcleos na linha celular V79 em doses até 100 μg mL^{-1}.

Three new *neo*-clerodanes, antiscorbuticane A, antiscorbuticane B and antiscorbuticane C, and known compounds glutinol, friedelin, 5,7-dihydroxyflavanone (pinocembrin), 5-hydroxy-3,6,7,4'-tetramethoxyflavone, 5-hydroxy-3,6,7,3',4'-pentamethoxyflavone (artemetin), 5,4'-dihydroxy-3,6,7,3'-tetramethoxyflavone (penduletin) and 5,3',4'-trihydroxy-3,6,7-trimethoxyflavone (chrysosplenol D), were isolated from the methanol extract of *Tinnea antiscorbutica*. Antiscorbuticane B exhibited no mutagenic activity at doses of up to 250 μg *per* plate (Ames test) and did not induce micronucleus formation in the V79 cell line at doses of up to 100 μg mL^{-1}.

Keywords: *Tinnea antiscorbutica, neo*-clerodanes, mutagenic activity, cytotoxic activity, genotoxicity

Introduction

The *Tinnea* genera belong to the Labiatea Juss. family[1] and comprise 19 species restricted to Africa. Originally from the north of Angola, in the province of Kuanza Norte (Dembos region), *T. antiscorbutica* Welv., which is traditionally named "Tete-Mbula", is a small shrub that can be collected in several regions of Angola and is used in folk medicine to treat scurvy.[1] Despite the use of *T. barbata* as a flowering shrub,[2] to the best of our knowledge there have been no chemical studies of the *Tinnea* genera.

Following our research on Angolan plants,[3-6] we report the isolation of the new *neo*-clerodanes antiscorbuticane A (**3**), antiscorbuticane B (**8**) and antiscorbuticane C (**10**) and the known compounds glutinol (**1**),[7,8] friedelin (**2**),[9] 5,7-dihydroxyflavanone (pinocembrin) (**4**),[10] 5-hydroxy-3,6,7,4'-tetramethoxyflavone (**5**),[11,12] 5-hydroxy-3,6,7,3',4'-pentamethoxyflavone (artemetin) (**6**),[13] 5,4'-dihydroxy-3,6,7,3'-tetramethoxyflavone (**7**),[14] 5,3',4'-trihydroxy-3,6,7-trimethoxyflavone (**9**) (chrysosplenol D)[15] from the methanol extract of the aerial parts of *T. antiscorbutica* (Figure 1). Their structures were characterized by spectroscopic methods and comparison with literature data.

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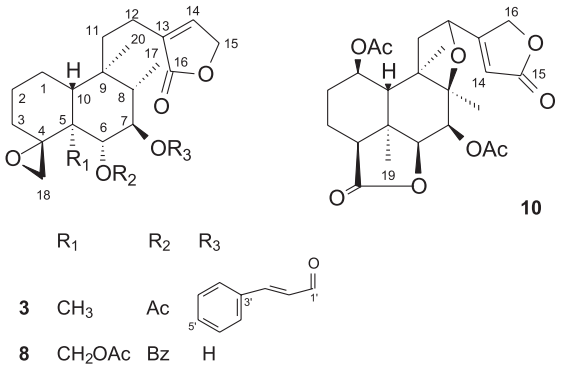

Figure 1. New *neo*-clerodanes from *Tinnea antiscorbutica*.

Experimental

General experimental procedures

The optical rotations were obtained with a Bellingham+Stanley Ltd. ADP 220 polarimeter. The high resolution electron ionization mass spectrometry (HREIMS) measurements were performed on a VG Autospec M and were recorded at 70 eV. The infrared (IR) spectra were measured with a Unicam Mattson 5000 FTIR. The nuclear magnetic resonance (NMR) spectra were recorded with a Bruker Avance II at 600 MHz (^{1}H NMR) and 150.9 MHz (^{13}C NMR) in $CDCl_3$. The chemical shifts are given in δ ppm and are referenced to the residual $CHCl_3$ at 7.26 ppm for the ^{1}H spectrum and 77.0 ppm for the ^{13}C spectrum. Two-dimensional experiments were performed with standard Bruker software. Column chromatography was performed on silica gel 60 (70-230 mesh, Merck, Darmstadt, Germany).

Plant material

The aerial parts of *Tinnea antiscorbutica* were collected in the Chibia road at the Comuna da Huíla, Huíla province (Angola), in July 2001 and were identified by Professor Esperança da Costa, Agostinho Neto University. A voucher specimen (No. 3742) has been deposit at the Lubango Herbarium, Angola.

Extraction and isolation

The dried aerial parts (1.5 kg) were macerated in methanol for a week at room temperature; the procedure was performed three times. After being concentrated, the methanol extract (42.7 g) was partitioned between MeOH-H_2O (5:1) and hexane to yield 19.0 g of the hexane fraction. The aqueous methanolic fraction was concentrated under vacuum, H_2O was added, and the fraction was extracted with chloroform to give the chloroform fraction (2.8 g). Finally, the aqueous fraction was extracted with EtOAc to yield 9.3 g of the EtOAc fraction, and the remaining material was considered to be the aqueous fraction (10.4 g).

A sample of the hexane fraction (2 g) was fractionated on a silica gel column with a hexane/EtOAc, EtOAc and EtOAc/MeOH gradient. The fraction eluted with hexane/EtOAc (9:1) was separated on a silica gel column with a hexane/EtOAc gradient (99:1; 49:1; 9:1; 4:1; 7:3; 1:1) to yield glutinol (**1**) (7.9 mg) and friedelin (**2**) (5.4 mg). The fraction eluted with hexane/EtOAc 3:2 was separated on a silica gel column with a hexane/EtOAc gradient (4:1; 7:3; 3:2; 1:1) and EtOAc to yield antiscorbuticane A (**3**) (5.5 mg).

The chloroform fraction (2.8 g) was fractionated on a silica gel column with a hexane/EtOAc, EtOAc/$CHCl_3$ and EtOAc/CH_3OH gradient. The fraction eluted with hexane/EtOAc 9:1 was separated on a silica gel column with a hexane/EtOAc gradient (9:1; 4:1; 7:3; 1:1) to yield 5,7-dihydroxyflavanone (**4**) (2.5 mg) and 5-hydroxy-3,6,7,4'-tetramethoxyflavone (**5**) (3.5 mg). The fractions eluted with the EtOAc/CH_3OH gradient were combined and subjected to successive purification on a silica gel column to yield 5-hydroxy-3,6,7,3',4'-pentamethoxyflavone (**6**) (5.2 mg), 5,4'-dihydroxy-3,6,7,3'-tetramethoxyflavone (**7**) (3.5 mg), antiscorbuticane B **8** (25.1 mg) and 5,3',4'-trihydroxy-3,6,7-trimethoxyflavone (**9**) (11.6 mg).

The ethyl acetate fraction (9.3 g) was fractionated on a silica gel column with a hexane/EtOAc, EtOAc and EtOAc/MeOH gradient. The fraction eluted with hexane/EtOAc (3:2) was separated on a silica gel column with a hexane/$CHCl_3$ gradient to yield antiscorbuticane C (**10**) (9.3 mg).

Antiscorbuticane A (**3**)

Colorless oil; $[\alpha]_D^{21}$ = + 22.2 (*c* 0.045, $CHCl_3$); IR υ_{max}/cm^{-1} 2929, 1750-1715, 1636, 1451, 1251, 1203, 1168, 754; ^{1}H NMR ($CDCl_3$, 600 MHz) and ^{13}C NMR ($CDCl_3$, 150.9 MHz): see Table 1; HR-FAB-MS (pos.) *m/z* 523.2686 $[M+H]^+$ (calcd. for $C_{31}H_{39}O_7$, 523.2696).

Antiscorbuticane B (**8**)

White amorphous solid; $[\alpha]_D^{18}$ = + 19.2 (*c* 0.26, $CHCl_3$); IR υ_{max}/cm^{-1} 2982, 1762-1717, 1638, 1240, 1165, 750; ^{1}H NMR ($CDCl_3$, 600 MHz) and ^{13}C NMR ($CDCl_3$, 150.9 MHz): see Table 1; HR-FAB-MS (pos.) *m/z* 535.2296 $[M+Na]^+$ (calcd. for $C_{29}H_{36}O_8Na$, 535.2308).

Antiscorbuticane C (**10**)

Colorless oil; $[\alpha]_D^{16}$ = + 57.1 (*c* 0.07, $CHCl_3$); IR υ_{max}/cm^{-1} 2986, 1778, 1747, 1639, 1619, 1444, 1372, 1228, 1170, 1032, 756. ^{1}H NMR ($CDCl_3$, 600 MHz) and ^{13}C NMR

($CDCl_3$, 150.9 MHz): see Table 2; HR-TOF-MS-EI (pos.) *m/z* 462.1885 $[M]^+$ (calcd. $C_{24}H_{30}O_9$, 462.1890).

MTT cytotoxicity assay

The MTT assay was conducted in V79 Chinese hamster cells as described elsewhere.[6] Three independent experiments were performed.

Ames assay

Mutagenicity testing was conducted through the plate incorporation assay described by Maron and Ames[16] with the *Salmonella typhimurium* strains TA 98, TA 100 and TA 102 in the presence or absence of S9 mix.[16] At least two independent experiments were performed for each assay.

Cytokinesis-block micronucleus assay (CBMN)

Cytokinesis-block micronucleus assay was conducted as described elsewhere.[6] At least two independent experiments were performed for each assay.

Results and Discussion

Previous phytochemical studies demonstrated that certain of the known compounds isolated from *Tinnea antiscorbutica* present different biological activities. Glutinol presents analgesic and anti-inflammatory properties;[17-19] 5,7-dihydroxyflavanone (pinocembrin) is well known for its vasorelaxing effects,[20,21] antimutagenic activity,[22] induction of apoptosis,[23] bacteriostactic activity[24] and fasciolicide, ovicide and larvicide activities.[15] 5-Hydroxy-3,6,7,4'-tetramethoxyflavone presents antifungal activity[11] and inhibitory activity against prolylendopeptidase and thrombin.[25] 5-Hydroxy-3,6,7,3',4'-pentamethoxyflavone (artemetin) induces apoptosis in different target cells[26,27] and has cytotoxic and antioxidant activity.[28,29] 5,4'-Dihydroxy-3,6,7,3'-tetramethoxyflavone presents cytotoxic activity.[30] 5,3',4'-Trihydroxy-3,6,7-trimethoxyflavone (chrysosplenol D) induces apoptosis in mammalian cancer cells.[26]

Compound **3** was obtained as a colorless oil with an $[\alpha]_D^{21}$ value of + 22.2° (*c* 0.045, $CHCl_3$). The molecular formula $C_{31}H_{38}O_7$ was established by HR-FAB-MS, which showed a quasi-molecular ion peak at *m/z* 523.2686 $[M+H]^+$ (calculated at 523.2696) and implied 13 degrees of unsaturation.

The ^{1}H-NMR spectrum of compound **3** (Table 1) displayed signals for four methyl groups: one acetate at δ_H 1.90, two Me singlets at δ_H 1.38 and 0.92, and a secondary Me at δ_H 0.88 (d, 3H, *J* 6.7 Hz); one diastereotopic oxymethylene, which presented HMBC correlations with C-3, C-4 (Figure 2), at δ_H 3.31 (d, 1H, *J* 3.7 Hz) and 2.37 (d, 1H, *J* 3.7 Hz); an *E*-cinnamoyloxy moiety (δ_H 6.38, d, 1H, *J* 16.0 Hz, H-2'; 7.67, d, 1H, *J* 16.0 Hz, H-3'; 7.40, 7.53, 7.65, m, 5H, H-5', 6', 7', 8', 9'); an α-substituted butenolide ring[31] with H-14 at δ_H 7.10 (quint, 1H, *J* 1.6 Hz) that presented a vicinal coupling to H-15 at δ_H 4.78 (t, 2H, *J* 1.6 Hz); and an allylic coupling to H-12 characteristic of some *neo*-clerodane diterpenoides.[31,32] The ^{13}C NMR spectrum (Table 1) showed 29 signals corresponding to 31 carbons, which were determined to be four methyls, seven methylenes, twelve methines and eight quaternary carbons from the DEPT spectrum of **3**. The ^{13}C-NMR chemical shifts of the three methyls (δ_C 18.8, 15.7, and 10.6), the oxymethylene (δ_C 52.3), the four methines (δ_C 75.4, 73.7, 46.8, and 40.3) and the three quaternary carbons (δ_C 66.8, 42.5, and 39.8) were found to be consistent with a *trans*-fused A/B ring clerodane structure[31,33] in which Me-18 was transformed into a 4,18-epoxy ring and ring *B* contained an acetate and an *E*-cinnamate group. The α-substituted butenolide ring (δ_C 134.3, 143.9, and 70.2 as C-13, C-14, and C-15 and 174.1 as C-16) can unambiguously be assigned to the H-14 and CH_2-15 groups with the aid of the ^{1}H–^{1}H COSY, HSQC and HMBC data (Table 1, Figure 2*)*. The NOESY correlations (Figure 3) between Me-17/Me-19, H_α-7, and Me-19/Me-20 indicated that H-7, Me-17, Me-19, and Me-20 were on the α-face of the molecule. Additionally, the NOESY correlation between H_β-10/H_β-6 suggested that these hydrogens were on the β-face of the molecule. Thus, the structure of compound **3** was established as 6α-acetoxy-(*E*),7β-cinnamoyloxy-4α,18-epoxy-*neo*-clerodan-15,16-olide and was called antiscorbuticane A (Figure 1).

Compound **8** was obtained as a colorless oil with an $[\alpha]_D^{18}$ value of + 19.2° (*c* 0.26, $CHCl_3$). The molecular

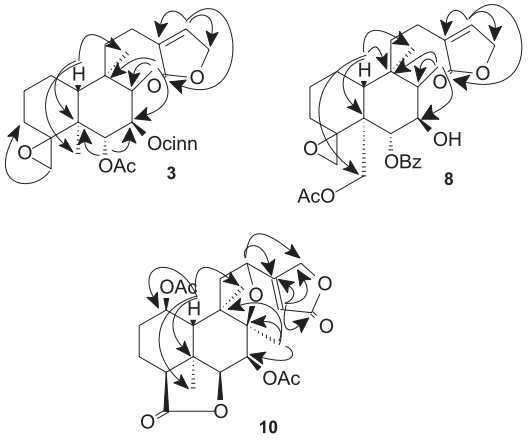

Figure 2. Key ^{1}H-^{13}C long-range correlations (H → C) of **3**, **8** and **10**.

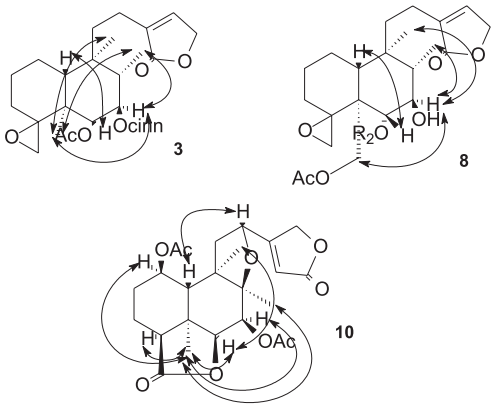

Figure 3. NOESY correlations of **3**, **8** and **10**.

formula $C_{29}H_{36}O_8$ was established by HR-FAB-MS by observing the ion of the Na^+-adduct at *m/z* 535.2296 (calculated for 535.2308), which implied 11 degrees of unsaturation.

Compound **8** had a ^{1}H-NMR profile similar to that of **3** except for the moieties at C-6 and C-7 and the absence of Me-19. The ^{1}H and ^{13}C-NMR spectra (Table 1) indicated the presence of two methyls (δ_H 1.02, d, 3H, *J* 6.6 Hz; and 0.81, s, 3H; δ_C 10.6, 18.5), an acetate group (δ_H 1.98, s, 3H; δ_C 171.3, 21.2) attached to C-19, a benzoyloxy moiety (δ_H 8.00, dd, 2H *J* 7.2 Hz, 1.2 Hz, H-2' and H-6'; 7.50, tt, 1H, *J* 7.8 Hz, 1.2 Hz, H-4'; and 7.38, td, 2H, *J* 7.8 Hz, 1.8 Hz, H-3' and H-5'; δ_C 166.5, 6-OOCPh; 130.5 C-1';

Table 1. ^{1}H NMR and ^{13}C NMR data and HMBC correlations of compounds **3** and **8**[a]

Position	**3**			Position	**8**		
	δ ^{13}C	δ ^{1}H (mult, nH, *J* / Hz)	HMBC		δ ^{13}C	δ ^{1}H (mult, nH, J / Hz)	HMBC
1	21.0	1.67 (m, 1H) 2.03 (m, 1H)	9, 10	1	21.0	1.95 (m, 1H) 2.01 (m, 1H)	2, 9
2	24.7	1.64 (m, 1H) 1.93 (m, 1H)	4, 5, 10	2	24.8	1.95 (m, 2H)	1, 4, 10
3	31.7	2.15 (m, 2H)	4, 5, 18	3	32.5	1.01 (m, 1H) 1.99 (m, 1H)	1, 2, 4, 5, 18
4	66.8			4	65.0		
5	42.5			5	45.9		
6	75.4	4.81 (d, 1H, 10.0) β	4, 5, 7, 19, 6-OAc	6	77.7	4.84 (d, 1H, 9.6) β	4, 5, 7, 8, 19, 6-OOCPh
7	73.7	5.26 (m, 1H) α	6, 8, 9, 17, 7-Ocinn	7	72.0	3.67 (dd, 1H, 10.5, 9.6) α	5, 6, 8, 9, 17
8	40.3	1.83 (m, 1H) β	6, 7, 9, 17, 20	8	42.2	1.64 (m, 1H) β	6, 7, 9, 10, 17, 20
9	39.8			9	39.3		
10	46.8	1.53 (m, 1H) β	5, 19, 20	10	47.6	1.68 (m, 1H) β	1, 5, 9, 19, 20
11	35.8	1.67 (m, 2H)	8, 9, 12	11	35.7	1.55 (m, 1H) 1.63 (m, 1H)	8, 9, 10,12, 13
12	18.9	2.20 (m, 2H)		12	18.9	2.06 (m, 1H) 2.19 (m, 1H)	9, 11, 13, 14, 16
13	134.3			13	134.0		
14	143.9	7.10 (quint, 1H, 1.6)	13, 15, 16	14	144.1	7.10 (t, 1H, 1.7)	13, 15, 16
15	70.2	4.78 (t, 2H, 1.6)	13, 14, 16	15	70.2	4.76 (t, 2H, 1.7)	13, 14,16
16	174.1		–	16	174.2		
17	10.6	0.88 (d, 3H, 6.7) α	7, 8, 9, 11	17	10.6	1.02 (d, 3H, 6.6) α	7, 8, 9
18	52.3	2.37 (d, 1H, 3.7) 3.31 (d, 1H, 3.7)	3, 4, 5	18	48.7	2.22 (d, 1H, 3.8) 3.18 (dd, 1H, 3.6, 2.3)	3, 4, 5
19	15.7	1.38 (s, 3H) α	4, 5, 6, 10	19	63.2	4.62 (d, 1H, 12.0) 4.68 (d, 1H, 12.0)	4, 5, 6, 10, 19-OAc
20	18.8	0.92 (s, 3H) α	5, 8, 9, 10, 11	20	18.5	0.81 (s, 3H) α	8, 9, 10, 11
6-H_3C-C=O	170.3			19-H_3C-C=O	171.3		
6-H_3C-C=O	20.8	1.90 (s, 3H)	6, 6-H_3C-C=O	19-H_3C-C=O	21.2	1.98 (s, 3H)	19, 19-H_3C-C=O
1'	166.7			6-OOCPh	166.5		
2'	117.4	6.38 (d, 1H, 16.0)	1', 3', 4'	1'	130.5		
3'	145.6	7.67 (d, 1H, 16.0)	1', 2', 4', 5', 9'	2', 6'	129.8	8.00 (dd, 2H, 7.2, 1.2)	
4'	134.2			3', 5'	128.1	7.38 (td, 2H, 7.8, 1.8)	
5', 9'	128.9	7.40 (m, 2H)	4',5', 6', 7', 8', 9'	4'	132.7	7.50 (tt, 1H, 7.8, 1.2)	
6', 8'	128.2	7.53 (m, 2H)					
7'	130.4	7.65 (m, 1H)					

[a]Spectra were recorded at 600 MHz for ^{1}H NMR and 150.9 MHz for ^{13}C NMR; multiplicity and coupling constants (*J* / Hz) are in parenthesis.

129.8, C-2' and C-6'; 128.1 C-3' and C-5', and 132.7, C-4') attached to C-6; an α-substituted butenolide ring (δ_H 7.10, 1H, t, *J* 1.7 Hz, H-14; and 4.76, 2H, q, *J* 1.7 Hz, H-15; δ_C 134.0, 144.1, and 70.2, C-13, C-14, and C-15, respectively; and 174.2, C-16),[31] and two diastereotopic oxymethylenes (δ_H 3.18, dd, 1H, *J* 3.8 Hz, 2.3 Hz and 2.37, d, 1H, *J* 3.6 Hz, H-18; and 4.68, 4.62, 1H, d, *J* = 12.0 Hz each, H-19; δ_C 48.7, C-18; 63.2, C-19). The ^{1}H and ^{13}C-NMR data were found to be consistent with a *trans*-fused *A*/*B* ring clerodane structure[31,33] in which Me-18 was transformed into a 4,18-epoxy ring, Me-19 transformed into an oxymethylene bearing an acetate moiety, and ring *B* bears an hydroxyl and a benzoyl group (Figure 1).

The NOESY correlations (Figure 3) between H_α-7/Me-17, Me-20, and CH_2-19 indicated that H-7, Me-17, CH_2-19 and Me-20 were on the α-face of the molecule. Additionally, the NOESY correlation between H_β-10 and H_β-6 suggested that these hydrogens were on the β-face of the molecule. Thus, the structure of compound **8** was established as 19-acetoxy-6α-benzoyloxy-4α,18-epoxy-7β-hydroxy-*neo*-clerodan-15,16-olide and was named antiscorbuticane B (Figure 1).

Compound **10** was obtained as a colorless oil with an $[\alpha]_D^{16}$ value of + 57.1° (*c* 0.07, $CHCl_3$). The molecular formula $C_{24}H_{30}O_9$ was established by HR-TOF-MS-EI, which showed a molecular ion peak at *m/z* 462.1885 (calculated for 462.1890) and implied 10 degrees of unsaturation.

The ^{1}H and ^{13}C-NMR spectra of **10** (Table 2) showed signals for two acetate groups (δ_H 2.08, s 3H, and 2.17, s, 3H, ; δ_C 21.4, 169.9 and 21.0, 170.5), three methyl groups (δ_H 1.18, s, 3H, Me-20; 1.15, s, 3H, Me-17 and 1.07, s, 3H, Me-19), a β-substituted butenolide ring (δ_H 5.99, br s, 1H and 4.83, br s, 2H; δ_C 115.1 and 70.9),[34] which can be assigned to the H-14 vinylic proton and to the C-16 methylene, respectively, with the aid of ^{1}H–^{1}H COSY, HSQC and HMBC data (Table 2, Figure 2); and four oxymethines (δ_H 5.47, d, 1H, *J* 11.0 Hz, H-7; 5.23, td, 1H, *J* 10.8, 4.9 Hz, H-1; 5.03, dd, 1H, *J* 9.9 Hz, 7.2 Hz, H-12 and 4.28, d, 1H, *J* 11.0 Hz, H-6; δ_C 71.9, 70.3, 72.5 and 82.8). The δ_H 5.23 and 5.47 methines showed HMBC correlations with the δ_C 169.9 and 170.5 carbonyl acetates, respectively, and were located on C-1 and C-7. The ^{1}H–^{1}H COSY correlations showed the connectivity between the protons at δ_H 4.28 and H-7 and suggested the placement of this methine at C-6. The ^{1}H and ^{13}C NMR data were found to be consistent with a *trans*-fused A/B ring clerodane structure,[31,33] in which Me-18 was transformed into a lactone carbonyl (δ_C 174.50). The low field shifts of Me-17 and Me-20 and the H-12/C-8 HMBC correlation clearly indicated the presence of an 8β,12 cyclic ether.[35] Because the α position of Me-19 was already established (*trans*-fused A/B ring), the NOESY correlations (Figure 3) between Me-19/H_α-1, H_α-4, H_α-7 and H_α-7/H_α-4, H_α-6, Me-17 indicated that H-1, H-4, H-6, H-7, Me-17 and Me-19 were on the α-face of the molecule. Additionally, the NOESY correlation between H_β-10 and H_β-12 suggested that these hydrogens were on the β-face of the molecule. Thus, the structure of compound **10** was established as 1β,7β-diacetoxy-8β,12-epoxy-*neo*-clerodan-16,15:18β,6β-diolide and was named antiscorbuticane C (Figure 1).

Table 2. ^{1}H NMR and ^{13}C NMR data and HMBC correlations of compound 10[a]

Position	10		
	δ ^{13}C	δ ^{1}H (mult, nH, *J* / Hz)	HMBC
1	70.3	5.23 (td, 1H, 10.8, 4.9) α	2, 3, 9, 10, 1-OAc
2	18.9	2.02 (m, 1H) α 2.11 (m, 1H) β	1, 4, 10
3	32.4	1.39 (m, 1H) α 2.36 (m, 1H) β	1, 2, 4, 5, 10
4	53.8	2.29 (m, 1H) α	2, 3, 5, 6, 10, 18, 19
5	44.6		
6	82.8	4.28 (d, 1H, 11.0) α	4, 5, 7, 8, 10, 19
7	71.9	5.47 (d, 1H, 11.0) α	5, 6, 17, 7-COO
8	90.4		
9	51.2		
10	48.5	1.88 (d, 1H, 10.8) β	1, 4, 5, 6, 9, 11, 19, 20
11	45.9	1.81 (m, 1H) α 2.73 (dd, 1H, 12.8, 7.2) β	8, 9, 10,12, 13, 20
12	72.5	5.03 (dd, 1H, 9.6, 7.2) β	8, 11, 13, 14, 16
13	170.8		
14	115.1	5.99 (brs, 1H)	12, 13, 15, 16
15	173.1		
16	70.9	4.83 (brs, 2H)	13, 14, 15
17	22.5	1.15 (s, 3H) α	7, 8, 9
18	174.5		
19	12.8	1.07 (s, 3H) α	4, 5, 6, 10
20	18.8	1.18 (s, 3H) α	8, 9, 10, 11
1-H_3C-C=O	21.4	2.08 (s, 3H)	1, 1-H_3C-C=O
1-H_3C-C=O	169.9		
7-H_3C-C=O	21.0	2.17 (s, 3H)	7, 7-H_3C-C=O
7-H_3C-C=O	170.5		

[a]Spectra were recorded at 600 MHz for ^{1}H NMR and 150.9 MHz for ^{13}C NMR; multiplicity and coupling constants (*J* / Hz) are in parenthesis.

Regarding the potential genetic damage induced by compound **8,** there is no evidence of mutagenic activity at doses of up to 250 μg *per* plate (Ames test, Table 3), and compound **8** does not induce micronuclei in the V79 cell line at doses of up to 100 μg mL^{-1} (Table 4). Furthermore compound **8** don't present cytotoxic activity (Table 5). Compounds **3** and **10** were not tested due the lack of available sample amount.

Table 3. Mutagenic activity of compound **8** in the Ames assay in the presence and in the absence of metabolic activation (S9) (*Salmonella typhimurium* strains TA 98, 100, 102)

Dose (μg *per* plate)	Revertants *per* plate					
	TA 98		TA 100		TA 102	
	–S9	+S9	–S9	+S9	–S9	+S9
0	17.5 ± 3.5	27.5 ± 9.2	130.5 ± 29.0	123.5 ± 20.5	268.0 ± 56.6	342.0 ± 31.1
5	20.5 ± 0.7	29.0 ± 4.2	121.5 ± 23.3	99.5 ± 16.3	298.5 ± 13.4	343.5 ± 24.7
25	14.5 ± 0.7	21.5 ± 3.5	124.0 ± 22.6	100.0 ± 1.4	258.5 ± 2.1	348.0 ± 50.9
50	15.5 ± 6.4	27.0 ± 1.4	108.0 ± 4.2	123.0 ± 4.2	233.5 ± 7.8	410.0 ± 28.3
250	16.5 ± 0.7	26.0 ± 5.7	117.0 ± 4.2	85.0 ± 9.9	167.5 ± 17.7	134.0 ± 32.5
Quercetin						
10	284.0 ± 77.7	1314.5 ± 102.5				
4-NQO[a]						
10			1432		2842	

[a]4-NQO: 4-nitroquinoline-1-oxide. Values are presented as the mean ± standard error (n = 2). Quercetin and 4-NQO were used as positive controls. Values are not significant (p>0.05).

Table 4. Effect of compound **8** on the frequency of micronucleated binucleated cells (% MNBN) in V79 Chinese hamster cells in the presence (+S9) and absence (–S9) of metabolic activation

Dose / (μg mL^{-1})	MN/BN		%MNBN		%BN	
	–S9	+S9	–S9	+S9	–S9	+S9
0	0.003 ± 0.001	0.002 ± 0.001	0.300 ± 0.071	0.167 ± 0.058	40.950 ± 14.213	37.400 ± 2.263
20	0.005 ± 0.004	0.002 ± 0.000	0.350 ± 0.212	0.200 ± 0.000	32.500 ± 5.091	36.100 ± 0.990
100	0.004 ± 0.001	0.001 ± 0.001	0.300 ± 0.000	0.100 ± 0.141	44.200 ± 1.556	35.200 ± 1.838
Mytomicin C						
2.5	0.154 ± 0.022	–	10.475 ± 1.790	–	25.000 ± 4.243	–
Cyclophosphamide						
2.0	–	0.036 ± 0.022	–	2.100 ± 0.265	–	38.600 ± 0.141

Results are expressed as mean values ± standard deviations (SD) (n = 2 and n = 5 for negative control). In each experiment 1000 binuleated cells were analyzed for the presence of micronuclei. % of binucleated cells (%BN) was use as index of cell proliferation. Mytomicin C and cyclophosphamide as positive controls, dose 0 as negative control. Values are not significant (p>0.05).

Table 5. Effect of compound **8** on cell viability of V79 Chinese hamster cells using the MTT assay

Dose / (μg *per* well)	Viability[a] / %
25	132.5 ± 15.7
50	162.7 ± 23.0
250	184.5 ± 101.6

[a]Viability is expressed as percentage values relative to control cells. Results are expressed as mean value %Viability ± standard deviations (SD) (n = 3). In each independent experiment four replicate cultures were used.

Conclusions

The present phytochemical investigation of aerial parts of *T. antiscorbutica* Welv., afforded three new *neo*-clerodanes named as antiscorbuticane A, antiscorbuticane B and antiscorbuticane C, and seven known compounds, glutinol, friedelin, 5,7-dihydroxyflavanone (pinocembrin), 5-hydroxy-3,6,7,4'-tetramethoxyflavone, 5-hydroxy-3,6,7,3',4'-pentamethoxyflavone (artemetin), 5,4'-dihydroxy-3,6,7,3'-tetramethoxyflavone (penduletin) and 5,3',4'-trihydroxy-3,6,7-trimethoxyflavone (chrysosplenol D).

Genotoxicity, mutagenicity and cytotoxicity were tested for antiscorbuticane B but all assays were negative concluding that this particular compound has no potential risk regarding their future use as bioactive compound.

Supplementary Information

1D and 2D NMR spectra data associated with this article are available free of charge at http://jbcs.sbq.org.br as a PDF file.

Acknowledgments

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Supplementary Information

New *neo*-Clerodanes from *Tinnea antiscorbutica* Welv.

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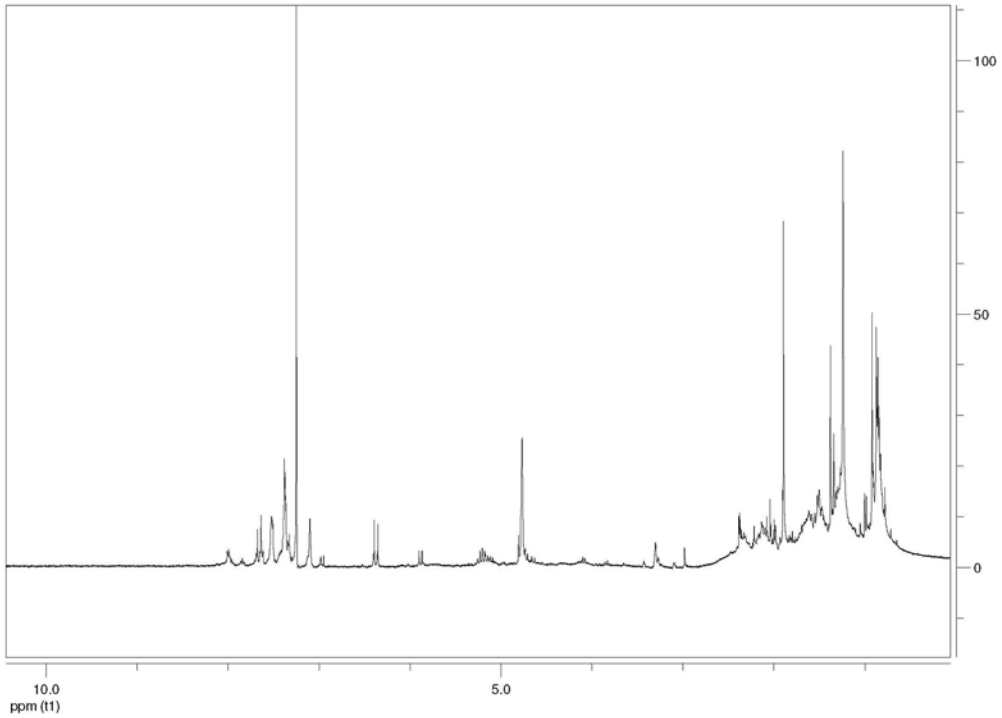

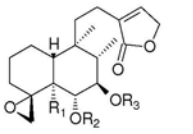

	R_1	R_2	R_3
3	CH_3	Ac	*E*-Cinn

Figure S1. ^{1}H NMR (600 MHz, $CDCl_3$) of compound **3**.

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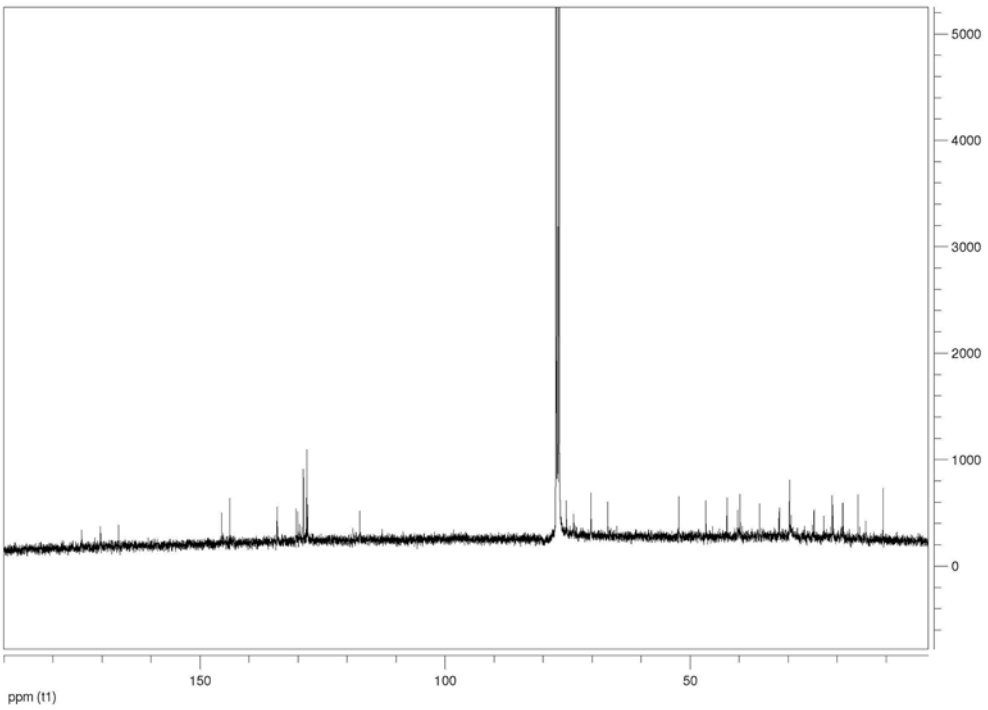
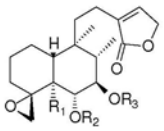
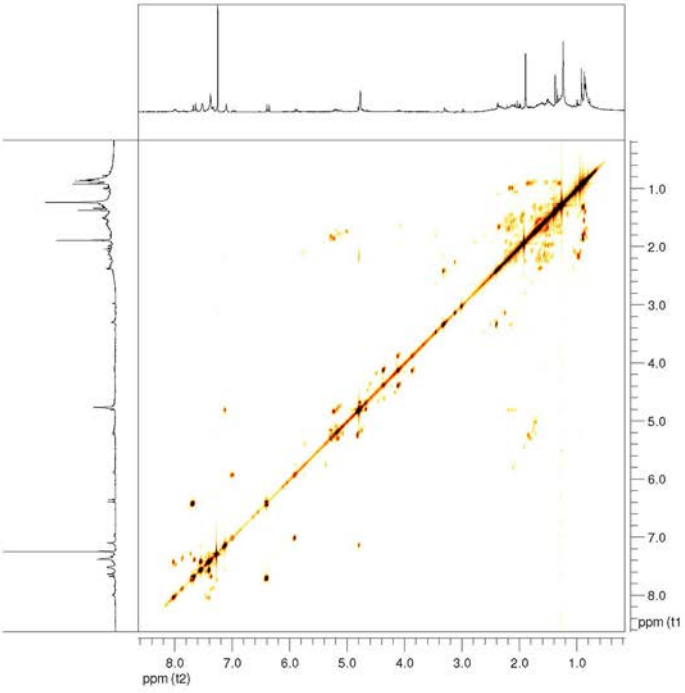
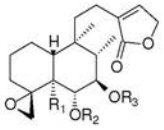




	R_1	R_2	R_3
3	CH_3	Ac	*E*-Cinn

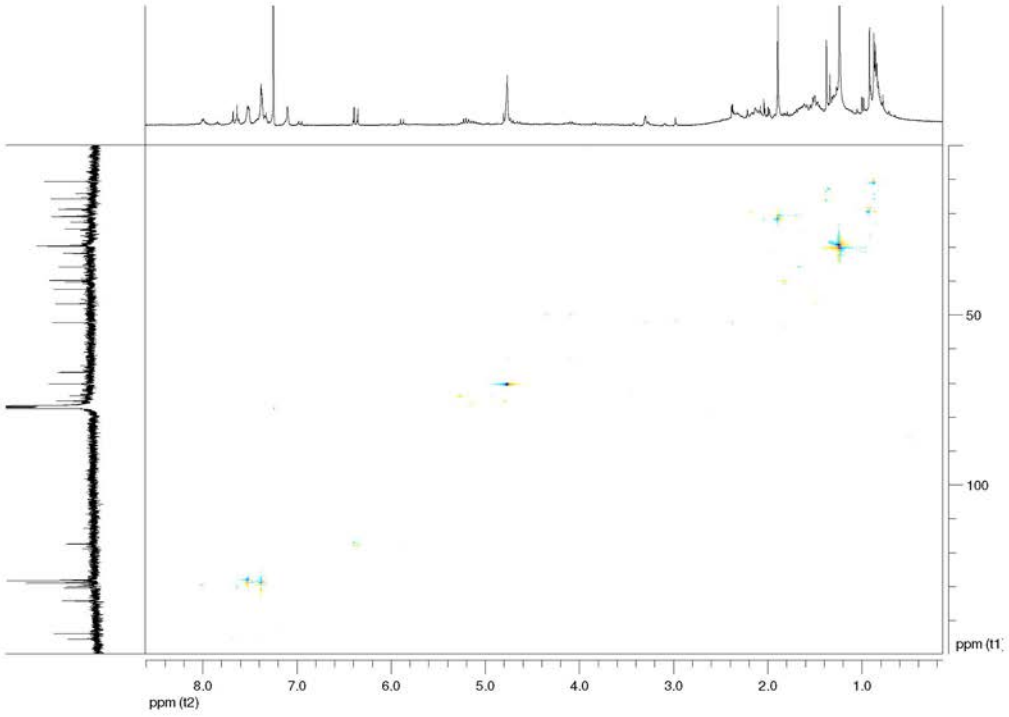
Figure S2. ^{13}C NMR (150.9 MHz, $CDCl_3$) of compound **3**.

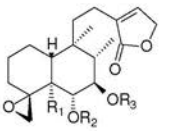




	R_1	R_2	R_3
3	CH_3	Ac	*E*-Cinn

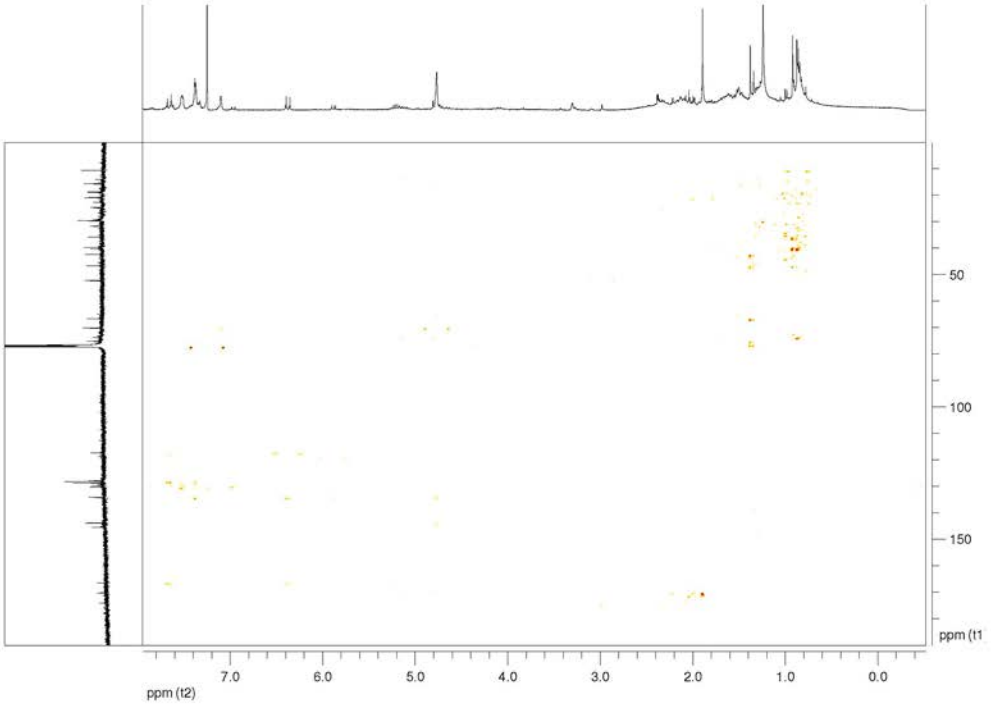
Figure S3. ^{1}H-^{1}H COSY (600 MHz, $CDCl_3$) of compound **3**.

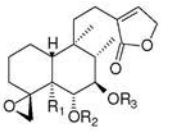




	R_1	R_2	R_3
3	CH_3	Ac	*E*-Cinn

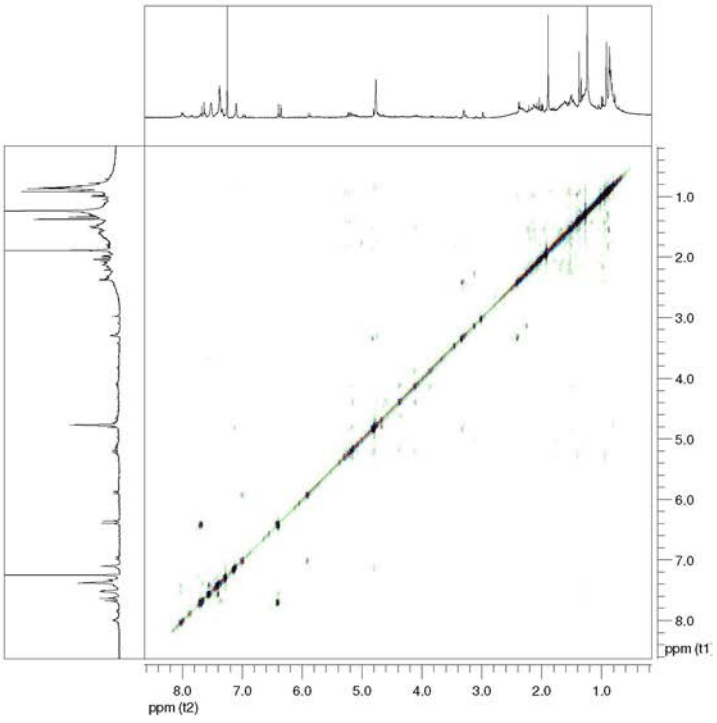
Figure S4. HSQC (600 MHz, $CDCl_3$) of compound **3**.

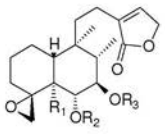




	R_1	R_2	R_3
3	CH_3	Ac	*E*-Cinn

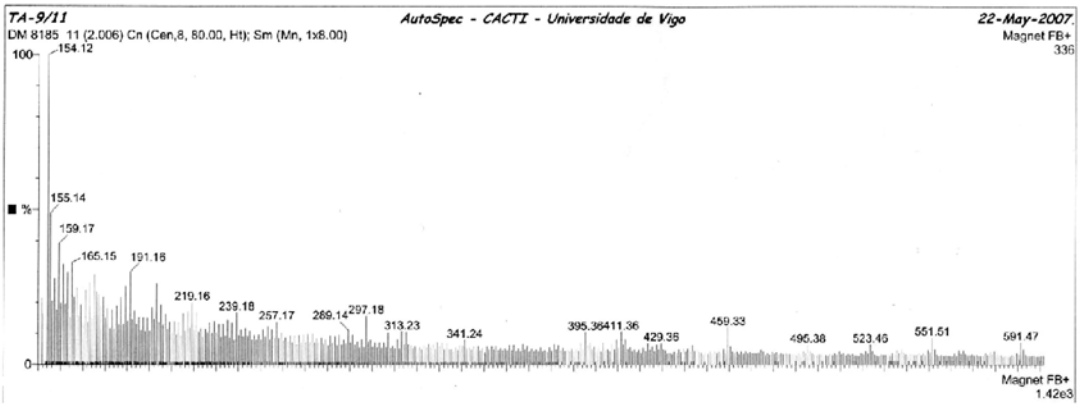
Figure S5. HMBC (600 MHz, $CDCl_3$) of compound **3**.

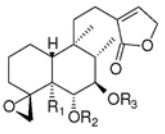




	R_1	R_2	R_3
3	CH_3	Ac	*E*-Cinn

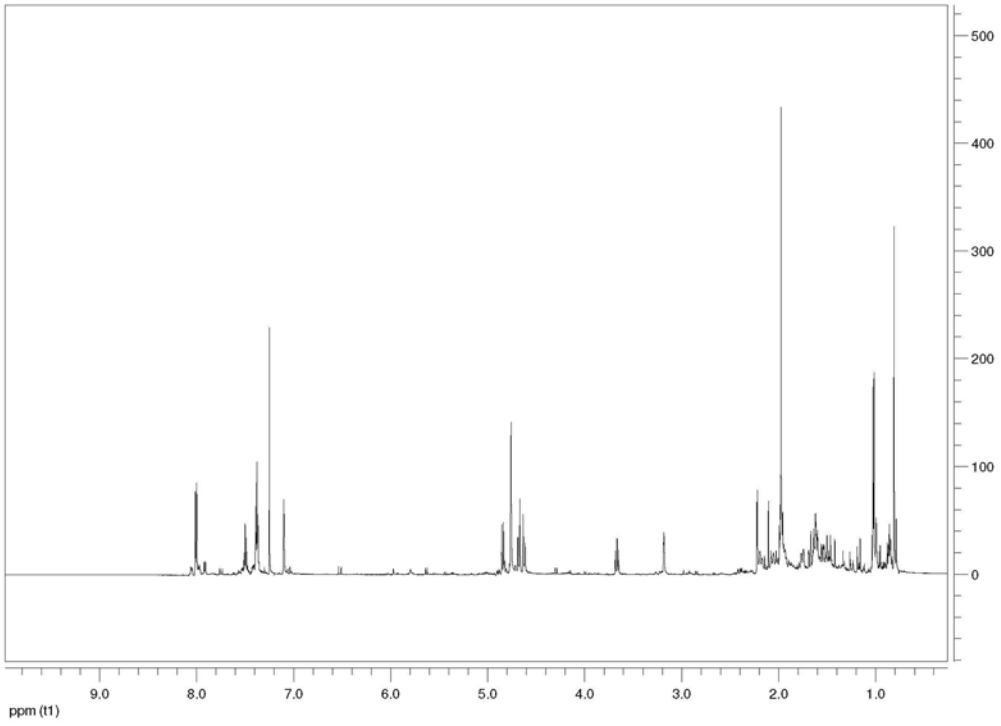
Figure S6. NOESY (600 MHz, $CDCl_3$) of compound **3**.

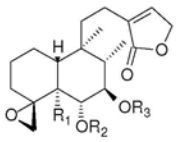




	R_1	R_2	R_3
3	CH_3	Ac	*E*-Cinn

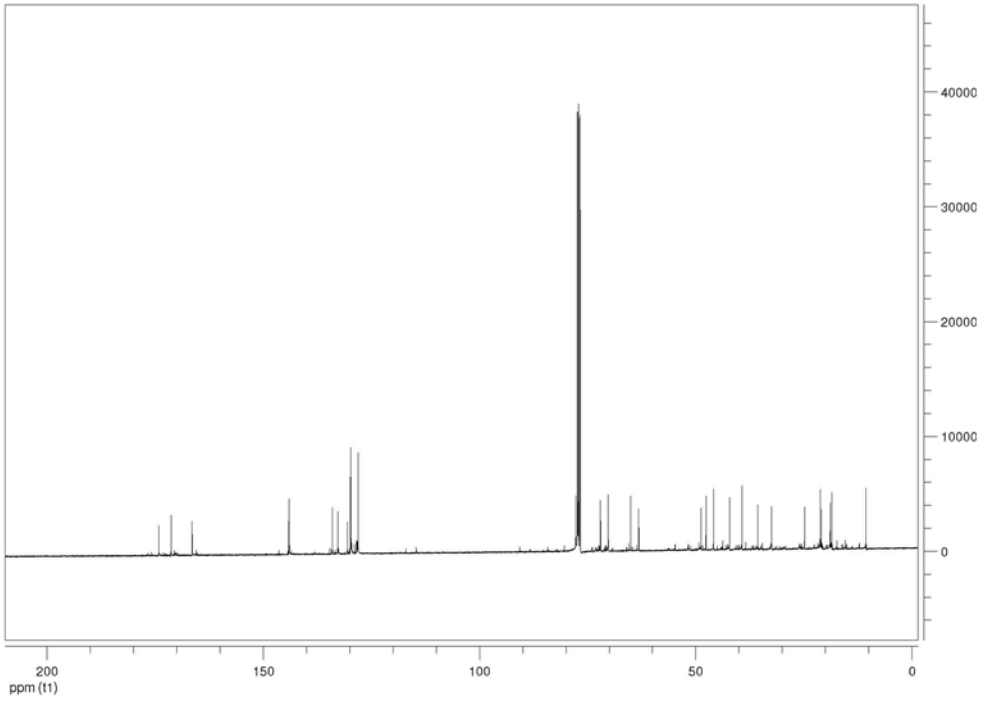
Figure S7 FAB-MS of compound **3**.

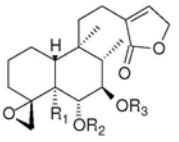




	R_1	R_2	R_3
8	CH_2OAc	Bz	H

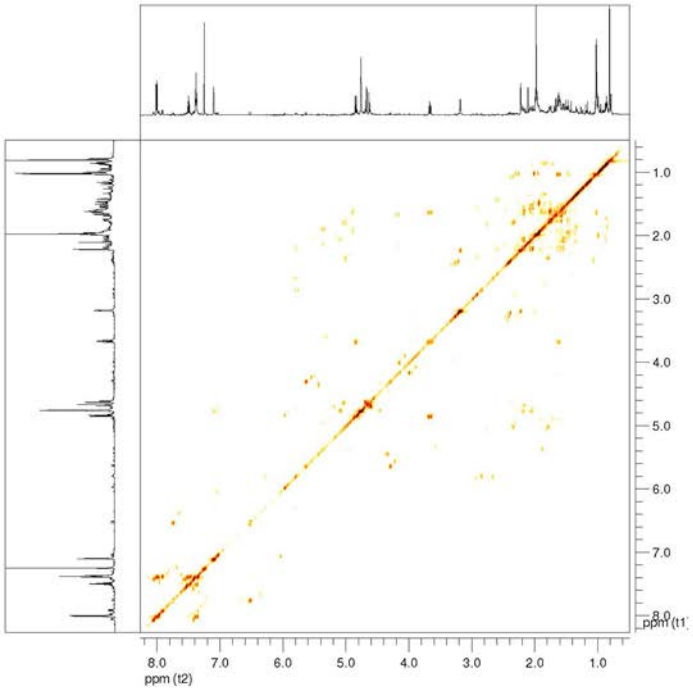
Figure S8. ^{1}H NMR (600 MHz, $CDCl_3$) of compound **8**.

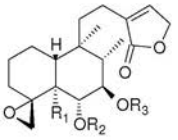




	R_1	R_2	R_3
8	CH_2OAc	Bz	H

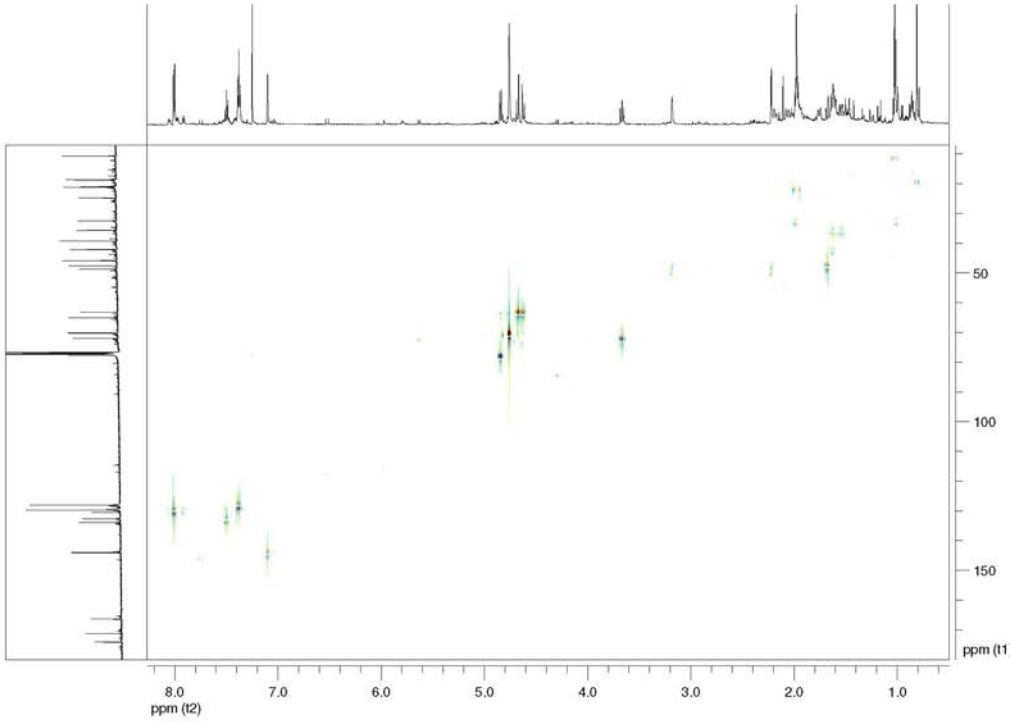
Figure S9. ^{13}C NMR (150.9 MHz, $CDCl_3$) of compound **8**.

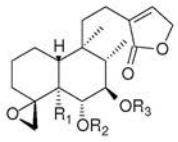




	R_1	R_2	R_3
8	CH_2OAc	Bz	H

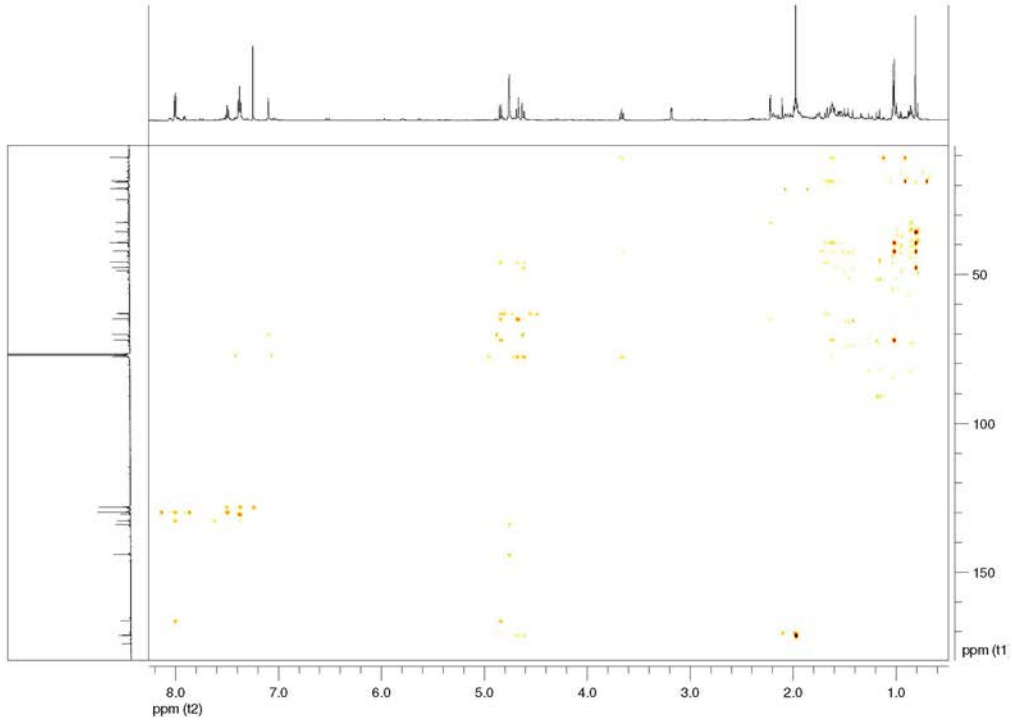
Figure S10. ^1H-^1H COSY (600 MHz, $CDCl_3$) of compound **8**.

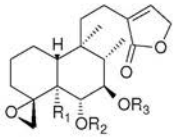




	R_1	R_2	R_3
8	CH_2OAc	Bz	H

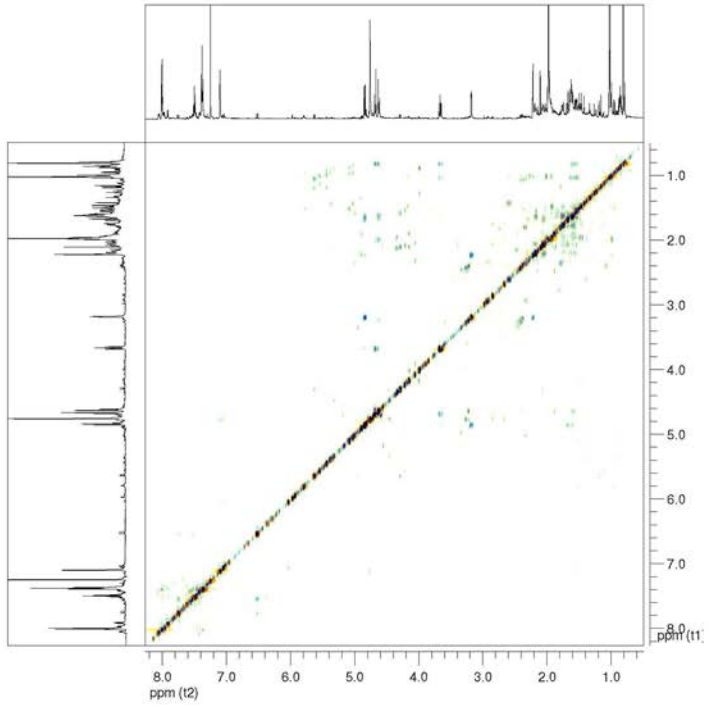
Figure S11. HSQC (600 MHz, $CDCl_3$) of compound **8**.

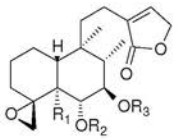




	R_1	R_2	R_3
8	CH_2OAc	Bz	H

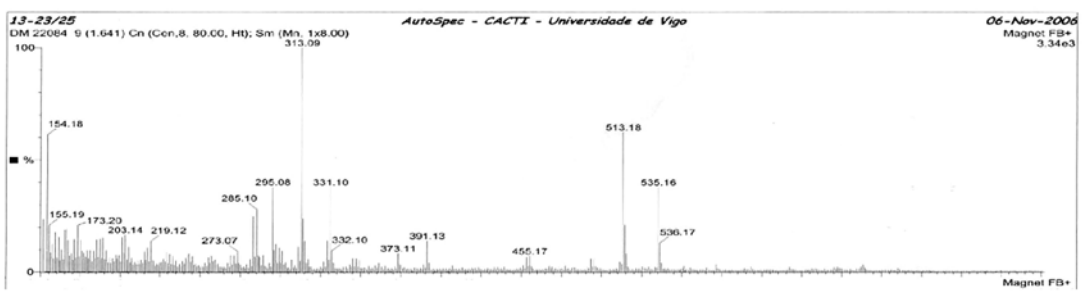
Figure 12. HMBC (600 MHz, $CDCl_3$) of compound **8**.

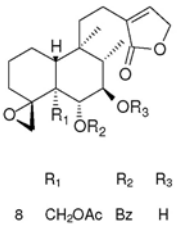




	R_1	R_2	R_3
8	CH_2OAc	Bz	H

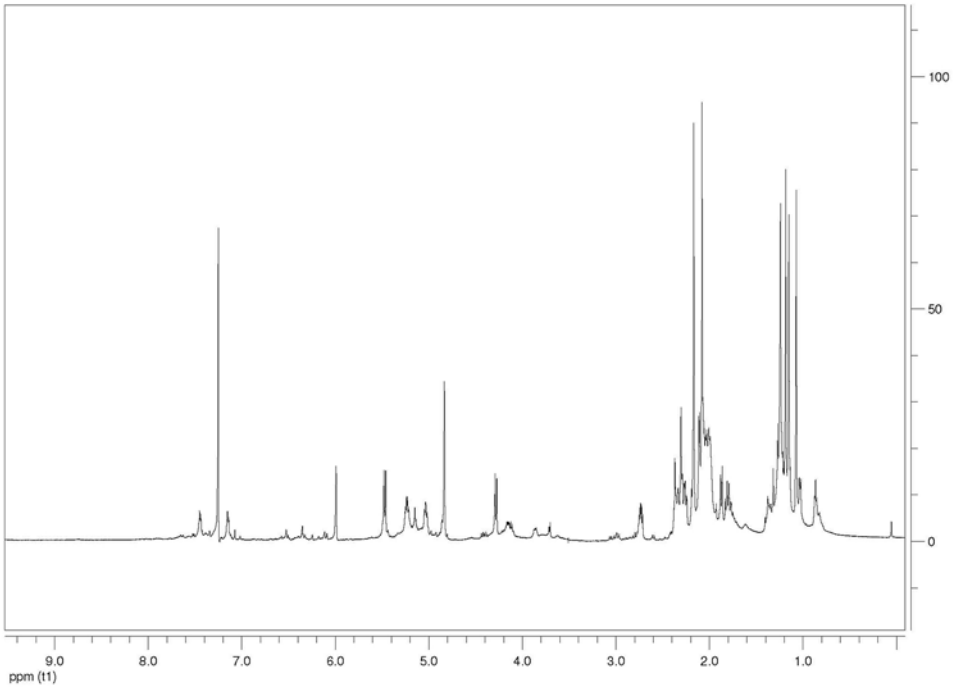
Figure S13. NOESY (600 MHz, $CDCl_3$) of compound **8**.





	R_1	R_2	R_3
8	CH_2OAc	Bz	H

Figure S14 FAB-MS of compound **8**.



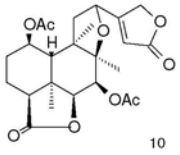


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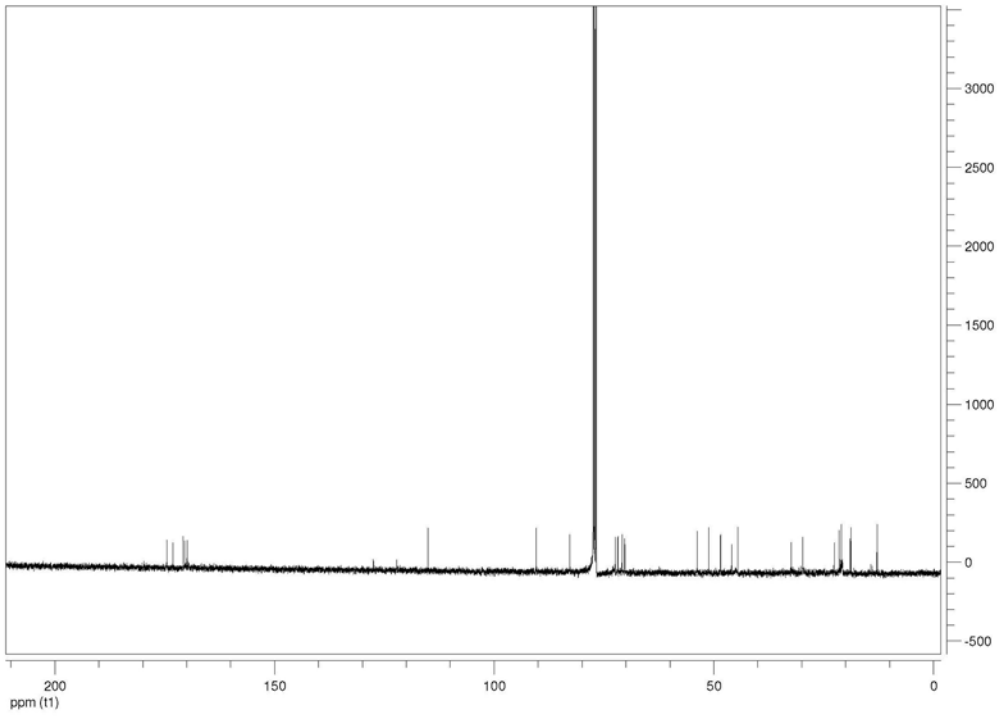
Figure S15. ^{1}H NMR (600 MHz, $CDCl_3$) of compound **10**.



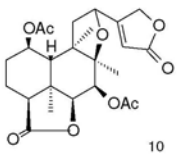
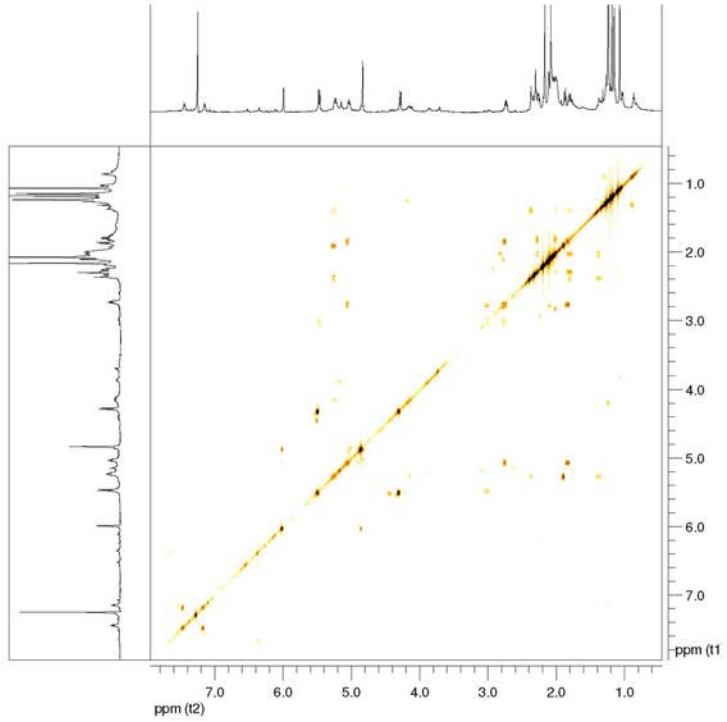


Figure S16. ^{13}C NMR (150.9 MHz, $CDCl_3$) of compound **10**.



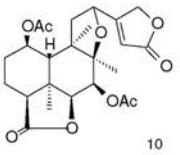
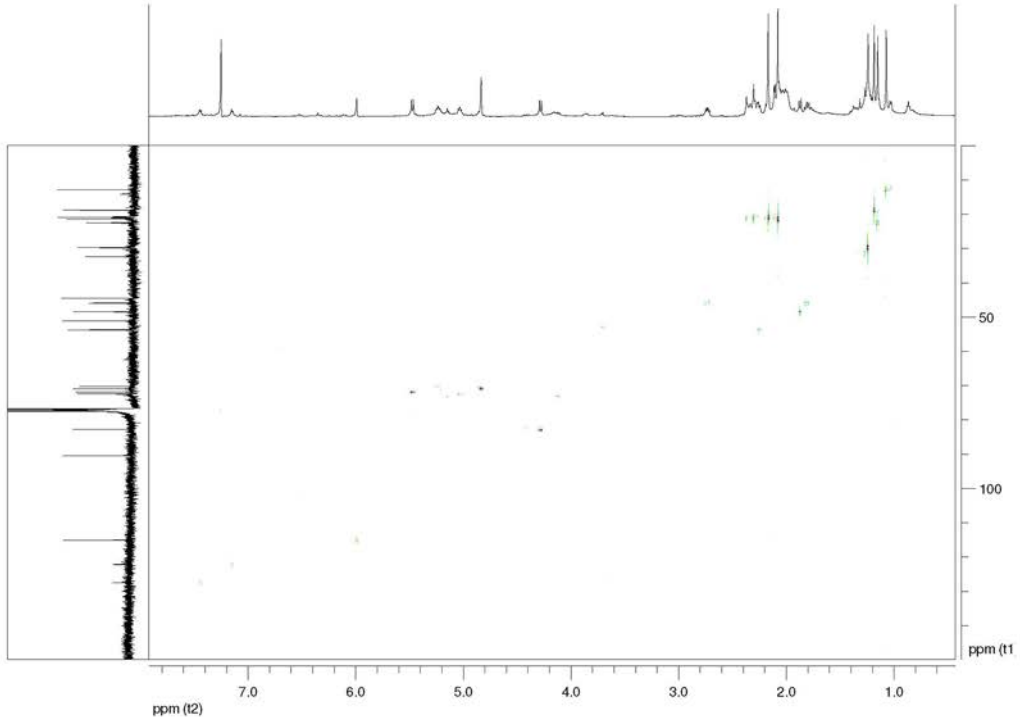


Figure S17. ^{1}H-^{1}H COSY (600 MHz, $CDCl_3$) of compound **10**.



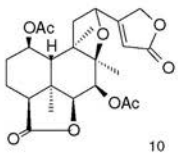


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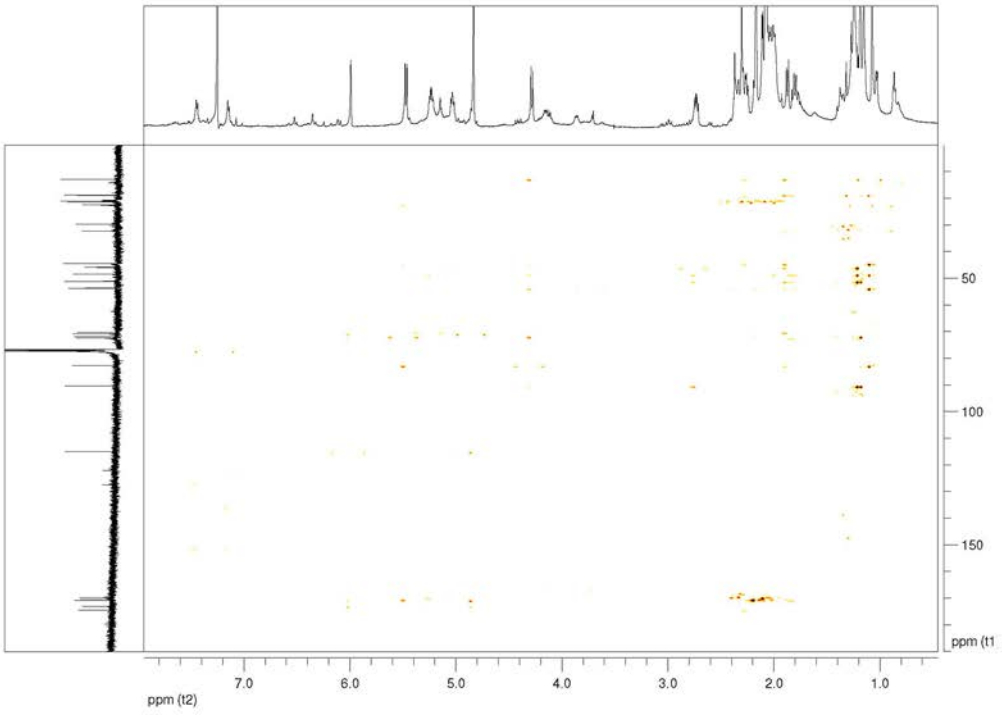
Figure S18. HSQC (600 MHz, $CDCl_3$) of compound **10**.



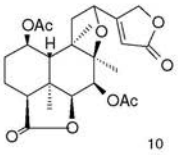


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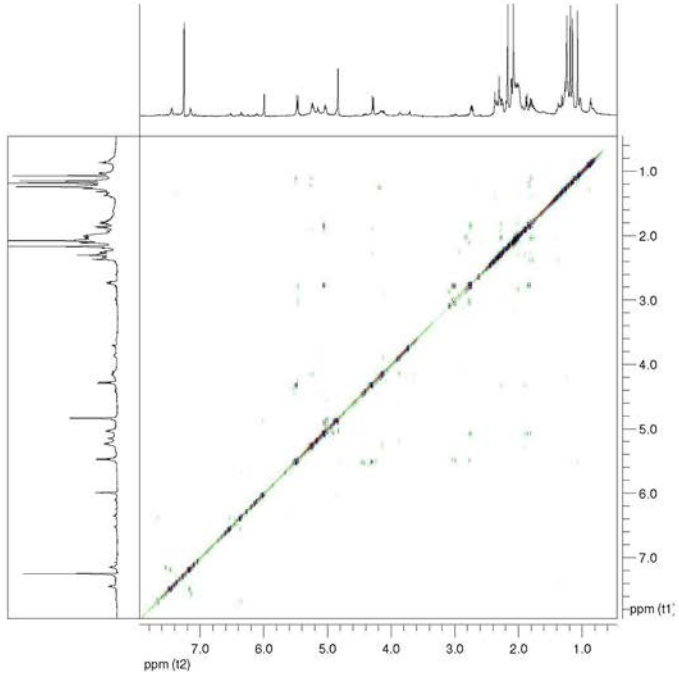
Figure 19. HMBC (600 MHz, $CDCl_3$) of compound **10**.



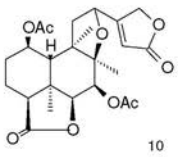
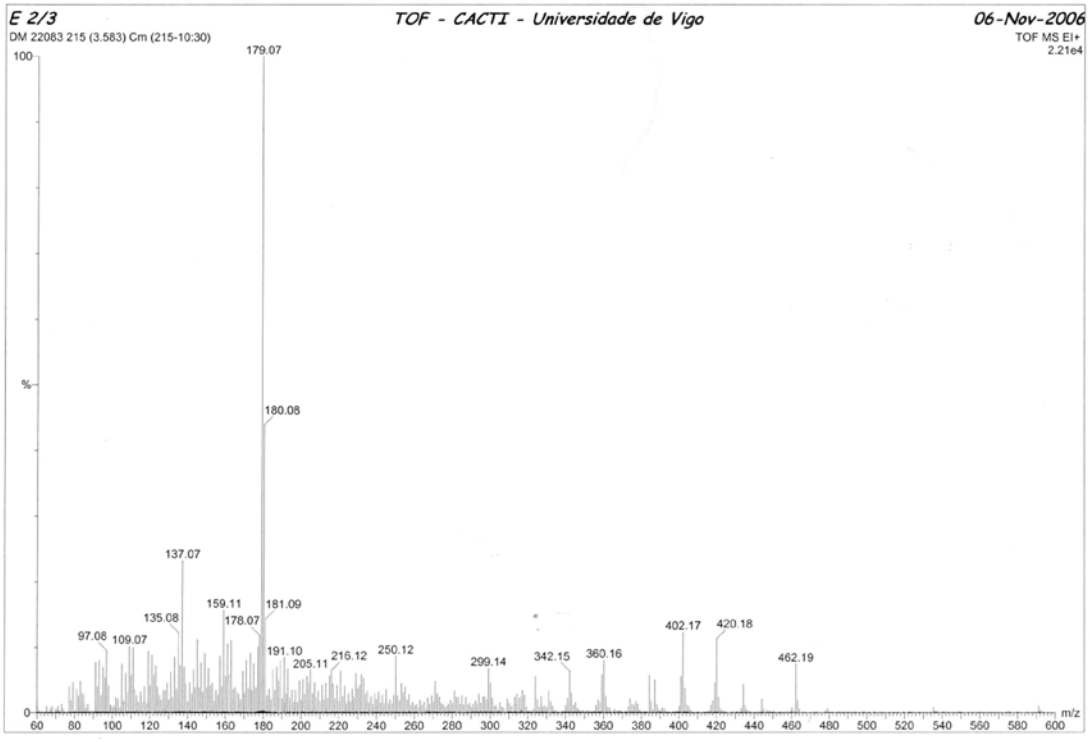


Figure S20. NOESY (600 MHz, $CDCl_3$) of compound **10**.



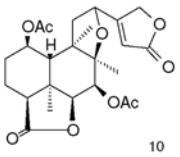


Figure S21 TOF-MS-EI of compound **10**.