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Article

Pterocarpans and a Novel Flavanone from *Harpalyce brasiliana* Roots

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A nova "(-)-7,8,3',4'-tri-hidróxi-8-(3'',7''-dimetil-octa-2'',6''-dienoil)-flavanona foi isolada a partir do extrato etanólico das raízes de *Harpalyce brasiliana*, juntamente com os pterocarpanos conhecidos (-)-2-geranil-3-hidróxi-8,9-metilenodioxipterocarpano, harpalicina, medicarpina, maackiaina e as cabenegrinas A-I e A-II. Os metabólitos isolados foram caracterizados com base nos seus dados espectroscópicos, principalmente RMN uni e bidimensional.

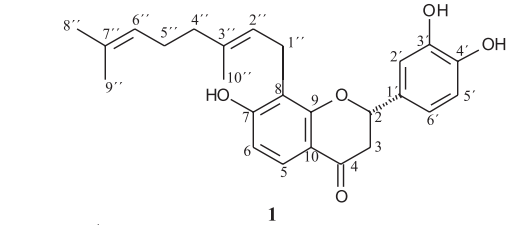
The novel (-)-7,8,3',4'-trihydroxy-8-(3'',7''-dimethyl-octa-2'',6''-dienoyl)-flavanone was isolated from the EtOH extract from the roots of *Harpalyce brasiliana* (Leguminosae) together with the known pterocarpans (-)-2-geranyl-3-hydroxy-8,9-methylenedioxypterocarpan, harpalicin, medicarpin, maackiain and cabenegrins A-I and A-II. The isolated metabolites were characterized on the basis of spectroscopic data, mainly 1D and 2D NMR.

Keywords: *Harpalyce brasiliana*, flavanone, pterocarpans, NMR assignments

Introduction

Medicinal plants have often been used by humans in folk medicine to treat snakebites envenomation as an alternative and supplementary therapy. Previous studies on the anti-ophidian activity of extracts and fractions of several Brazilian plant species used popularly in some communities have been investigated scientifically.[1-7] Among the class of the pharmacologically active secondary metabolites isolated from plants, the flavonoids are the most frequently cited. The coumestan wedelolactone from *Eclipta prostata* and some of its synthetic derivatives are the most active PLA2 inhibitors.[8] The prenylated pterocarpan edunol and its synthetic derivatives showed antimyotoxic, antiproteolytic and PLA2 inhibitor properties.[9] In addition, Nakagawa *et al.*[10] indicated the cabenegrines A-I and A-II as the responsible for anti-ophidian activity of the "Específico Pessoa", a phytotherapic tincture of the Brazilian folk medicine used against snakebites.

Harpalyce brasiliana Benth (Leguminoseae-Papilionoideae), popularly known in Northeast of Brazil as "raiz-de-cobra" (Port. Lit.: snake's root), is reported as a medicinal antidote to snake bites through the use of a hydroalcohol solution from its roots. We have previously reported the structure of several prenylated pterocarpans,[11-13] reputed as possessing venoms antidote properties.[9,10] In continuation to the search for bioactive flavonoids from the roots of *H. brasiliana*, we are reporting now the structure elucidation of the novel geranyl-flavanone (-)-7,8,3',4'-trihydroxy-8-(3'',7''-dimethyl-octa-2'',6''-dienoyl)-flavanone (**1**), along with the known pterocarpans (-)-2-geranyl- 3-hydroxy-8,9-methylenedioxypterocarpan (**2**),[14] harpalicin (**3**),[12] medicarpin (**4**),[15] maackiain (**5**)[16] and cabenegrins A-I (**6**) and A-II (**7**).[10]



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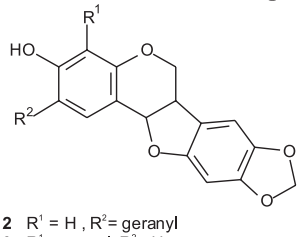

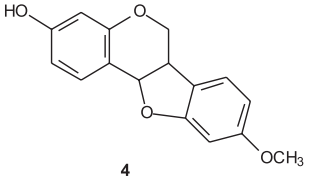

4

2 R^1 = H , R^2= geranyl
3 R^1 = prenyl, R^2= H
5 R^1 = H, R^2= H
6 R^1 = -CH_2-CH=CH(CH_3)CH_2OH, R^2 = H
7 R^1 = H, R^2 = -CH_2-CH_2-CH(CH_3)CH_2OH

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Results and Discussion

Compound **1** was isolated as a yellow solid with mp 96.3-97.1 °C and optical rotation −108° ($CHCl_3$; *c* 0.1). Its molecular formula of $C_{22}H_{24}O_{11}$ was established by the quasi-molecular ion at *m/z* 409.20157 $[M + H]^+$ in the HRESIMS spectrum. The absorption bands at 3366 cm^{-1} and 1748 in the IR spectrum, were consistent with hydroxyl and carbonyl groups, respectively. The analysis of ^1H NMR spectrum in addition to the COSY experiment data revealed the presence of two sets of ABX coupling system protons, through the correlations of the aromatic hydrogens at δ 6.93 (dd, *J* 1.6, 8.2 Hz, H-2´), 6.81 (dd, *J* 1.6,8.2 Hz, H-6´) and 6.78 (d, *J* 8.2 Hz, H-5´), and of the hydrogens of a typical flavanone skeleton at δ 5.26 (dd, *J* 2.8, 12.9 Hz, H-2), 2.96 (dd, *J* 12.9, 16.9 Hz, H-3) and 2.68 (dd, *J* 2.8, 16.9, H-3´).[17] The presence of a geranyl group was suggested from the characteristic signals of the three methyl groups at δ 1.59 (s, H-10´´), 1.57 (s, H-8´´) and 1.52 (s, H-9´´), two olefinic hydrogens at δ 5.02 (t, *J* 6.0 Hz, H-6´´) and 5.17 (t, *J* 7.8 Hz, H-2´´), and three methylenes at δ 2.01 (d, *J* 7.8 Hz, H-4´´), 2.09 (m, H-5´´) and 3.44 (d, *J* 7.0 Hz, H-1´´). In the aromatic region of the spectrum, the two remaining aromatic protons occurred as a set of *orto* coupled doublets at δ 6.50 (d, *J* 8.7 Hz, H-6) and 7.57 (d, *J* 8.7 Hz, H-5).

The support for a geranyl-flavanone structure came from the comparative analysis of the ^{13}C NMR and DEPT spectra that indicated the presence of signals for 25 carbon atoms, revealing 16 sp^2 carbons, from which 10 non-hydrogenated and 8 methines. The remaining signals comprised one carbonyl (δ 194.3), four methylenes, one oxygenated methine and three methyl groups (Table 1).

The assignment of the relative position of the geranyl moiety was defined in the HMBC spectrum by the cross-peaks of the allylic methylene protons at δ 3.44 (H-1´´) with the carbons at δ 163.2 (C-7) and 164.1 (C-9), in addition to the correlation of the signal at δ 7.57 (H-5) with the carbonyl at δ 194.3 (C-4) and the carbon at δ 163.2 (C-7), and of the hydrogen at δ 7.57 (H-5) with the carbons at δ 115.2 (C-8) and 163.2 (C-7). These correlation data of the A-ring protons definitively established that the OH and geranyl groups were located at the C-7 and C-8, respectively. Moreover, the concomitant correlations of the hydrogens of the B-ring at δ 6.81 (H-6´) and 6.93 (H-2´) with the carbons at δ 81.0 (C-2) and 81.0 (C-4´) confirmed the respective relative locations at C-3´ and C-4´ of the two hydroxyl groups in the trisubstituted aromatic, and gave the clear evidence of a 7,8, 3',4'-substitution pattern for the flavanone moiety.

Table 1. ^1H and ^{13}C NMR data for compound **1**[a]

Carbon	^1H NMR (δ_H)	^{13}C NMR (δ_C)
2	5.26 dd (2.8; 12.9)	81.0
3	2.68 dd (2.8; 16.9) 2.96 dd (12.9; 16.9)	45.1
4		194.3
5	7.57 d (8.7)	126.8
6	6.50 d (8.7)	111.0
7		163.2
8		115.2
9		164.1
10		114.9
1´		132.1
2´	6.93 dd (1.6; 8.2)	114.9
3´		146.8
4´		146.6
5´	6.78 d (8.2)	115.2
6´	6.81 dd (1.6; 8.2)	119.3
1´´	3.44 d (7.8)	23.0
2´´	5.17 t (7.8)	123.5
3´´		136.0
4´´	2.01 d (7.8)	41.0
5´´	2.09 m	26.6
6´´	5.02 t (6.0)	125.6
7´´		132.5
8´´	1.57 s	26.0
9´´	1.52 s	17.9
10´´	1.59 s	16.5

[a] ^1H and ^{13}C NMR spectra were acquired in CD_3OD at 300 and 75 MHz, respectively. Chemical shifts are shown in the δ scale and the coupling constants (*J* values in Hz) are given in parentheses.

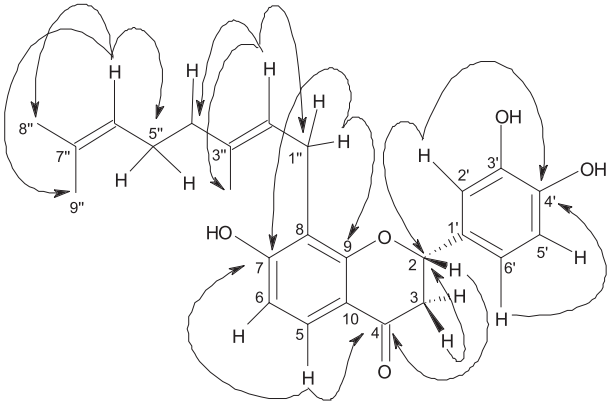

Figure 1. Key HMBC correlations observed for compound **1.**

Based on these findings, the structure of **1** was proposed to be the new (-)-7, 8, 3', 4'-trihydroxy-8-(3'',7''-dimethyl-octa-2'',6''-dienoyl)-flavanone.

The isolated compounds were tested for their cytotoxicity using five tumor cell lines: two human leukemias (HL-60

and CEM), human breast adenocarcinoma (MCF-7), human colon adenocarcinoma (HCT-8) and murine melanoma (B16), but none showed any cytotoxic activity.

Experimental

General procedures

IR spectra were recorded using a Perkin Elmer 1000 FT-IR spectrophotometer. Optical rotations were measured on a Perkin Elmer 341 polarimeter. The mass spectra were obtained on a Hewlett-Packard 5971 mass spectrometer by electron impact ionization (70 eV). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance DRX-500 (500 MHz for ^{1}H and 125 MHz for ^{13}C); chemical shifts were expressed in δ scale and were referenced to residual CD_3OD (3.31 and 49.15 ppm). Silica Gel 60 (Merck, 230-400 mesh) was used for analytical TLC. Column chromatographies were performed over Sephadex LH-20 (Amersham Pharmacia Biotech AB, Sweden) and silica gel (Merck, 60 F_{254} 230-240 mesh). HPLC separations were conducted on a Waters-1525 pumping system equipped with a PDA detector Waters-2996 (265 nm) and a XTerra RP-18 column (4.6 × 250 mm, 5 μmol L^{-1}). All compounds were visualized on TLC by spraying with vanillin/perchloric acid/EtOH followed by heating.

Plant material

Roots of *Harpalyce brasiliana* Benth were collected at Chapada do Araripe, Crato, Ceará State, and authenticated by Prof. Edson P. Nunes of the Departamento de Biologia, Universidade Federal do Ceará, CE, Brasil. Voucher specimen (32525) has been deposited at the Herbário Prisco Bezerra (EAC), Departamento de Biologia, Universidade Federal do Ceará, Fortaleza, Ceará, Brazil.

Extraction and isolation

Roots of *Harpalyce brasiliana* (3.5 kg) were pulverized and extracted with EtOH at room temperature. The solvent was removed under reduced pressure to give a dark viscous extract (260.0 g). Liquid-liquid partition of an aliquot of the EtOH extract (100.0 g) using petrol ether, $CHCl_3$, EtOAc and n-BuOH as solvents yielded four fractions. The $CHCl_3$ fraction (30.0 g) was further purified over Sephadex LH-20 by elution with MeOH to give five fractions (F1-to F-5). Flash chromatography of F-3 (8.9 g) using $CHCl_3$, EtOAc and MeOH as binary mixtures of increasing polarity afforded 73 fractions, which were pooled to 17 fractions after TLC analysis. The sub-fraction F-3 (1-3) was further purified by HPLC using a mixture of CH_3CN/H_2O (80/20, v/v) (flow rate 4.72 mL min^{-1}), to yield the (-)-2-geranyl- 3-hydroxy-8,9-methylenedioxypterocarpan (**2**) (10.0 mg) and harpalicin (**3**) (3.0 mg). Using the same method, sub-fraction F-3 (9-11) (50.0 mg) was purified, using a mixture of acetonitrile/H_2O (60/40, v/v) (flow rate 4.7 mL min^{-1}) as eluent, to yield medicarpin (**4**) (4.0 mg) and maackiain (**5**) (12.0 mg). Purification of sub-fraction F-3 (23-29) (100.0 mg) using a mixture of acetonitrile/H_2O (50/50, v/v) (flow rate 4.72 mL min^{-1}) afforded cabenegrins A-I (**6**) (29.0 mg) and A-II (**7**) (35.0 mg). The compound (-)-7,8,3',4'-Trihydroxy-8-(octa-2'',6''-dienoyl-3'',7''-dimethyl)-flavanone **1** (28.0 mg) was obtained from sub-fraction F-3 (37-44) (40.0 mg) by elution with a mixture of acetonitrile/H_2O (60/40 v/v) (flow rate 1.0 mL min^{-1}).

Cytotoxic assay

All compounds were tested for cytotoxic activity against five tumor cells lines (National Cancer Institute, Bethesda, MD); B16 (murine melanoma), HCT-8 (human colon), MCF-7 (human breast), CEM and HL-60 (leukemia) after 72 h of incubation. Doxorubicin (0.01 to 0.58 μg mL^{-1}) was used as positive control. The general viability of culture cells was determined by reduction of yellow dye 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-*2H*-tetrazolium bromide (MTT) to a blue formazan product as described by Mosmann.[18]

*(-)-7,8,3',4'-Trihydroxy-8-(3'',7''-dimethyl-octa-2'',6''-dienoyl)-flavanone (**1**)*

Yellow solid. mp 96.3-97.1 °C. $[\alpha]_D^{20}$ -108° ($CHCl_3$; *c* 0.1). IR (film) ν_{max}/cm^{-1}: 3366, 2925, 2852, 1748, 1493, 1380, 1347, 1131, 1092, 1073, 1002, 944, 823, 702. HRESIMS [M + H] 409.20157 (calculated for $C_{25}H_{29}O_5$ 409.2016). EIMS *m/z* 408 ([M] 10%), 393, 365, 339, 285, 272, 229, 203, 161, 149, 136, 123, 69, 41. ^{1}H and ^{13}C NMR data are given in Table 1.

*Cabenegrin A-II (**7**)*

White solid. mp 162.8-169.6 °C. $[\alpha]_D^{20}$ -190° (MeOH; *c* 0.02). IR (film) ν_{max}/cm^{-1}: 3399, 2915, 1622, 1509, 1472, 1376, 1326, 1252, 1139, 1120, 1034, 933, 847, 766. EIMS *m/z* 370 ([M] 10%), 297, 267, 175, 162, 148, 115, 91, 77, 69, 53. ^{1}H NMR (identical to literature)[10] .^{13}C NMR: 17.3 (C-5'), 28.2 (C-1'), 35.0 (C-2'), 36.8 (C-3'), 41.8 (C-6a), 67.6 (C-6), 68.7 (C-4'), 80.4 (C-11a), 94.4 (C-10), 102.6 (C-6'), 103.8 (C-4), 106.1 (C-7), 112.6 (C-1a), 120.1 (C-7a), 124.8 (C-2), 133.1(C-1), 143.2 (C-8), 149.5 (C-9), 156.0 (C-4a), 155.7 (C-10a), 157.8 (C-3).

Acknowledgments

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Supplementary Information

^{1}H NMR, ^{13}C NMR, COSY, HMQC, HMBC, IR and EIMS spectra of compound **1** are available free of charge at http:jbcs.sbq.org.br, as PDF file.

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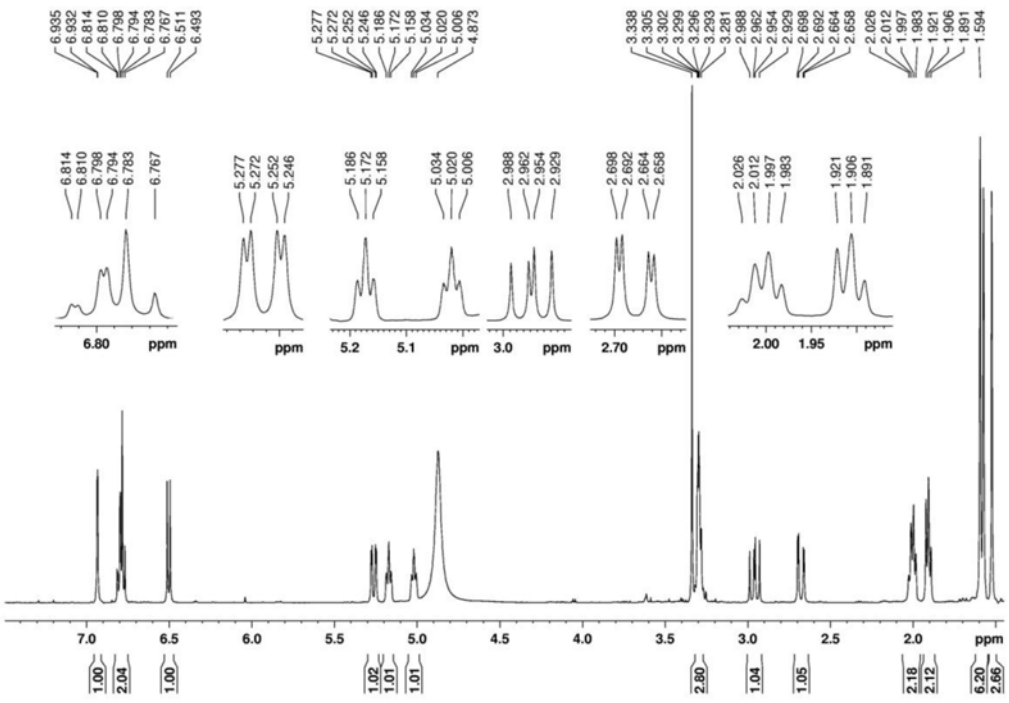

Figure S1. ^{1}H NMR spectrum (500 MHz, CD_3OD) of compound **1**.

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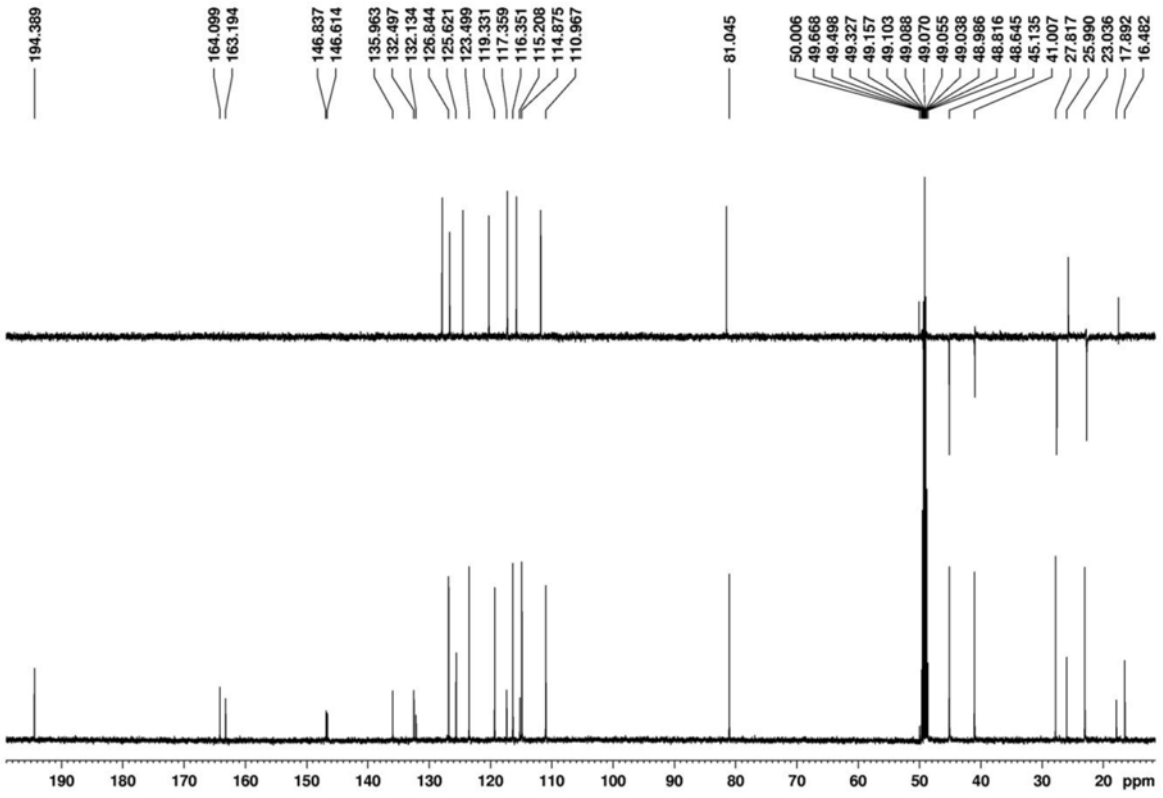

Figure S2. ^{13}C-BB and DEPT 135 NMR spectrum (125 MHz, CD_3OD) of compound **1**.

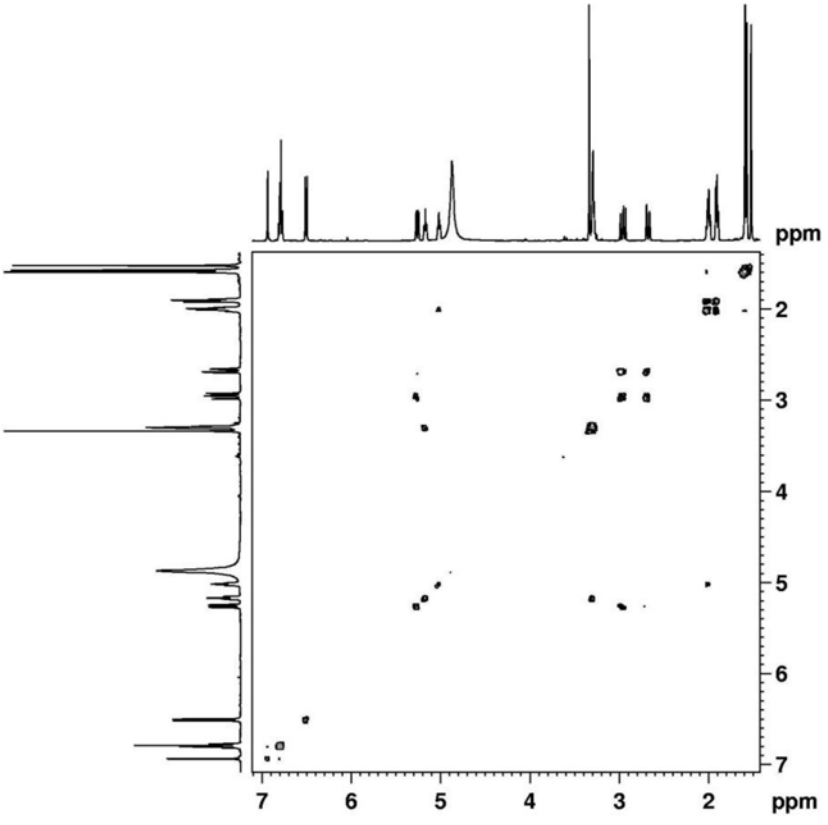

Figure S3. COSY spectrum (CD_3OD) of compound **1**.

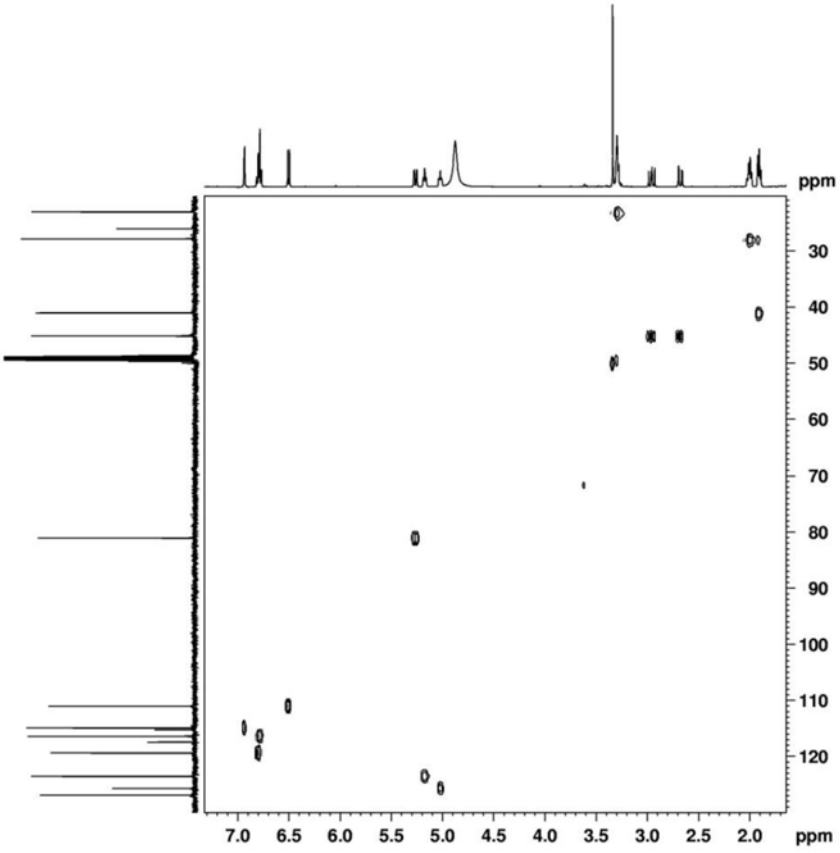

Figure S4. HSQC spectrum (CD_3OD) of compound **1**.

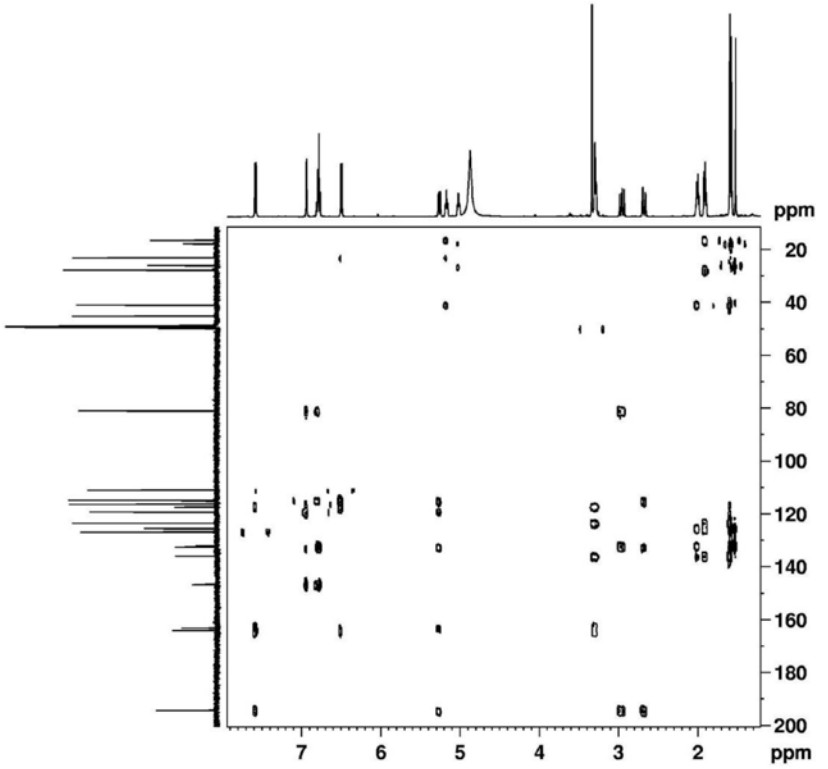

Figure S5. HMBC spectrum (CD_3OD) of compound **1**.

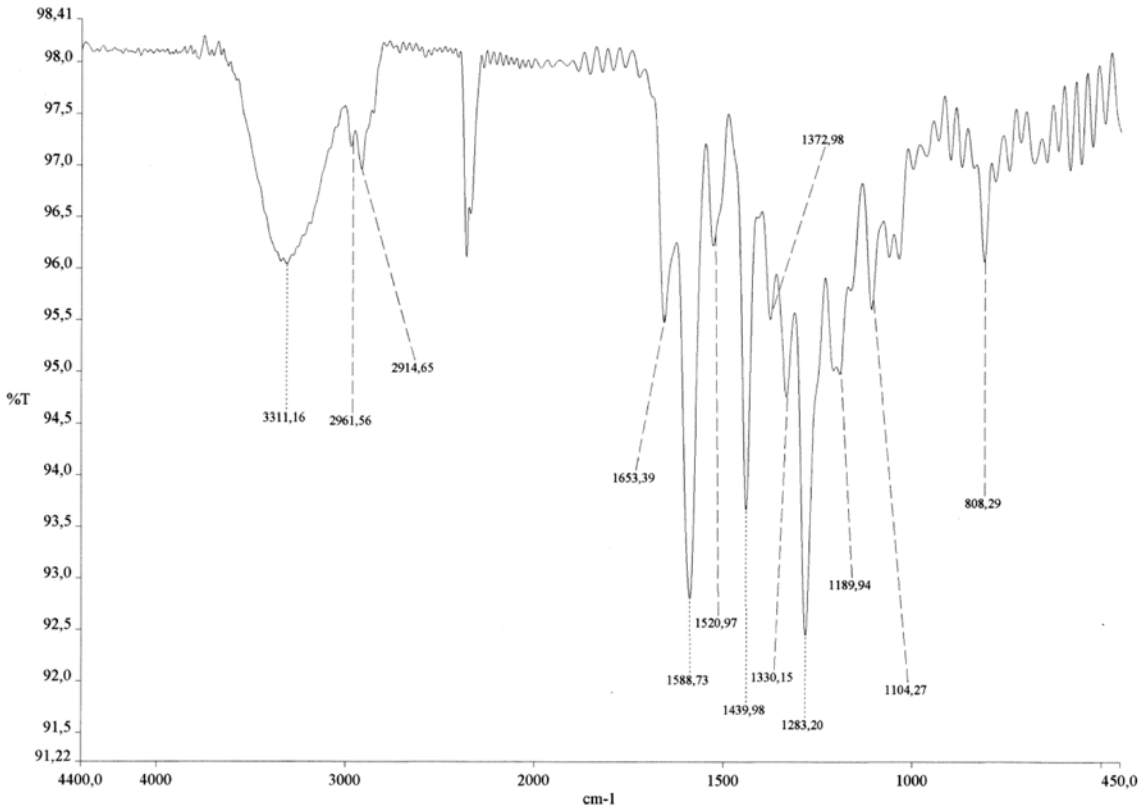

Figure S6. IR spectrum of compound **1**.

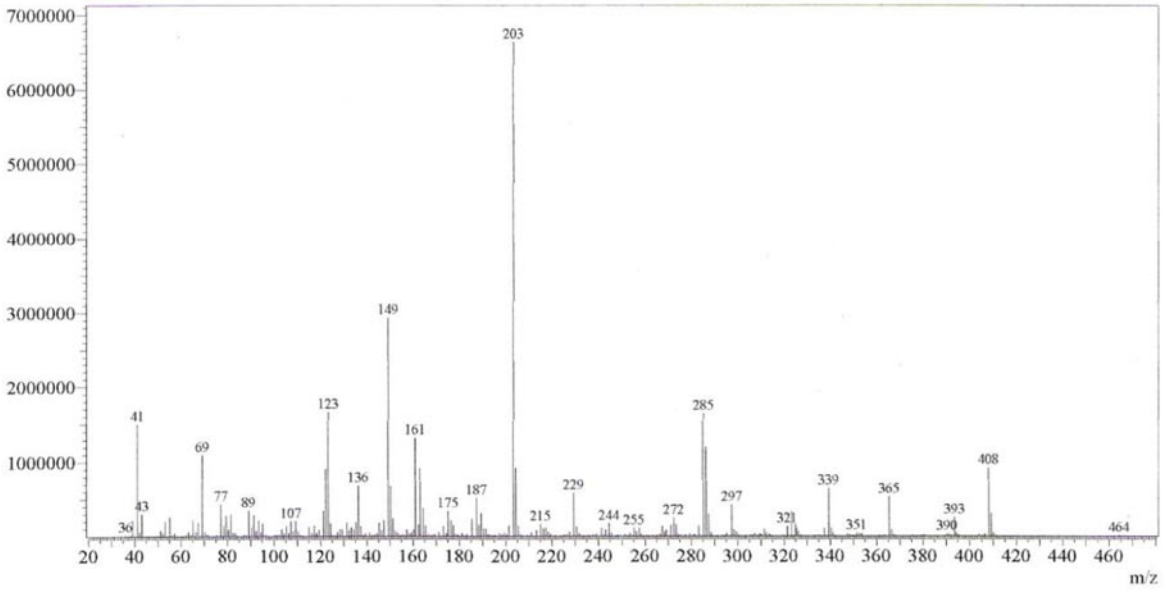

Figure S7. EIMS spectrum of compound **1**.