

ANTIFUNGAL IRIDOIDS, TRITERPENES AND PHENOL COMPOUNDS FROM *Alibertia myrciifolia* SPRUNGE EX. SCHUM**João Henrique S. Luciano, Mary Anne S. Lima* e Edilberto Rocha Silveira**

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The new iridoid glucoside 10-*O*-vanilloyl-geniposidic acid has been isolated from the aerial parts of *Alibertia myrciifolia* along with hydroxyhopanone, 3 α ,22-dihydroxyhopane, ursolic acid, luteolin-3',4'-dimethyl ether, caffeic acid and geniposidic acid. The structures of the isolated compounds were determined by means of mass spectrometry and nuclear magnetic resonance spectral analyses. The antifungal activities of the iridoids 10-*O*-vanilloyl-geniposidic acid and geniposidic acid were evaluated against the phytopathogenic fungi strains *Colletotrichum gloeosporioides*, *Fusarium solani* and *Aspergillus niger*.

Keywords: *Alibertia myrciifolia*; triterpenes; iridoids.**INTRODUCTION**

Species of Rubiaceae have widespread occurrence in Brazil and are well known by their economic and therapeutic importance; however, plants belonging to the genus *Alibertia* have been limited to a few species. Previous reports reveal these species as rich sources of iridoids, flavonoids, caffeic acid and its esters, and pentacyclic triterpenes.^{1,2}

Alibertia myrciifolia Sprunge ex. Schum (Rubiaceae) is a small tree growing abundantly in the Brazilian Northeast region, specially in Araripe's Plateau, where is known as "café bravo" (port. lit.: wild coffee). In our previous work we have isolated D-mannitol, scopoletin, pomolic acid, methyl esters of ursolic and oleanolic acids and the cytotoxic flavonoids corymbosin, lethedocin, apometzgerin, acacetin and apigenin.²

In continuation of our chemical investigation of the aerial parts of this plant, the present study deals with the isolation and structural determination of the new iridoid 10-*O*-vanilloyl-geniposidic acid (**1**) along with the known geniposidic acid (**2**),³ hydroxyhopanone (**3**),⁴ 3 α ,22-dihydroxyhopane (**4**),⁴ ursolic acid (**5**),⁵ luteolin-3',4'-dimethyl ether (**6**)⁶ and caffeic acid (**7**).⁷ The structures of the known compounds were established by ¹H and ¹³C NMR spectral data and comparison with those reported in the literature. The antifungal activity of both iridoids **1** and **2** was evaluated against *Colletotrichum gloeosporioides*, *Fusarium solani* and *Aspergillus niger*.

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 Shimadzu QP 5000 DI-50 mass spectrometer by electron impact ionization (70 eV). ¹H and ¹³C NMR spectra were recorded on a Bruker Avance DRX-500 (500 MHz for ¹H and 125 MHz for ¹³C). Chromatography columns

were carried out on silica gel 60 (Merck, 230-400 mesh), Waters RP-18 and Sephadex LH-20, and silica gel 60 (Merck, 60 F₂₅₄, 0.2 mm) was used for analytical TLC. All compounds were visualized on TLC by spraying with vanillin/perchloric acid/EtOH followed by heating.

Plant material

Aerial parts of *Alibertia myrciifolia* Spruce ex K. Schum. were collected at Chapada do Araripe, Crato, Ceará State. A voucher specimen (31016) has been identified by Dr. E. B. de Souza of the Universidade Estadual do Vale do Acaraú, Sobral, Ceará State, and deposited at the Herbário Prisco Bezerra (EAC), Departamento de Biologia, Universidade Federal do Ceará, Fortaleza, Ceará, Brazil.

Extraction and isolation

The air dried aerial parts (790.0 g) of *A. myrciifolia* were pulverized and extracted with hexane at room temperature. The solvent was removed under reduced pressure yielding a greenish resinous extract (6.6 g). The marc obtained after hexane extraction was extracted with EtOH to yield a dark brown solid (67.0 g), after evaporation under vacuum. The hexane extract (6.6 g) was coarsely fractionated on a silica gel column by elution with hexane, CHCl₃, EtOAc and MeOH. Chromatography of the CHCl₃ fraction (1.4 g) on silica gel using hexane/EtOAc mixtures with increasing polarity yielded 31 fractions (10 mL). The sub-fraction F-22 yielded β -sistosterol (25.0 mg). Successive flash chromatography of the EtOAc fraction (3.2 g) using 0-100% hexane/EtOAc mixture yielded 23 fractions (20 mL), that were pooled together in 10 fractions after TLC analysis. Flash chromatography of the EtOAc sub-fraction F-2 (119.5 mg) using hexane/EtOAc 85:15 mixture yielded hydroxyhopanone (**3**) (41.0 mg). Chromatography of the EtOAc sub-fraction F(4-5) (331.4 mg) using hexane/EtOAc 9:1 mixture yielded 3 α ,22-dihydroxyhopane (**4**) (28.2 mg).

The EtOH extract (67.0 g) was redissolved in a mixture of MeOH:H₂O (1:1 v/v) and submitted to liquid-liquid partition with hexane, CHCl₃, EtOAc and n-BuOH to give four fractions. The CHCl₃ fraction (15.4 g) was re-chromatographed on Sephadex

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LH-20 to give 12 sub-fractions (50 mL), which were combined in 5 sub-fractions according to TLC analysis. From the CHCl_3 sub-fraction (8-9) luteolin-3',4'-dimethyl ether (**6**) (1.5 mg) was obtained. The EtOAc fraction (7.6 g) was further purified over Sephadex LH-20 by elution with MeOH to afford 20 sub-fractions (50 mL), which were combined in eight sub-fractions according to TLC. Flash chromatography of the EtOAc sub-fraction F(7-11) (4.7 g) using $\text{CHCl}_3/\text{EtOAc}$ mixtures with increasing polarity, afforded ursolic acid (**5**) (9.1 mg). The EtOAc F(12-13) sub-fraction (180.0 mg) was further purified by flash chromatography after elution with a mixture of $\text{CH}_2\text{Cl}_2:\text{MeOH}:\text{AcOH}$ (88:10:2) to yield caffeic acid (**7**) (16.4 mg). The n-BuOH fraction (9.4 g) was re-chromatographed on Sephadex LH-20, to give 11 sub-fractions (50 mL) which were combined in 5 according to TLC analysis. Chromatography of the n-BuOH sub-fraction F(7-8) (5.8 g) using Waters RP-18 column and the $\text{MeOH}:\text{H}_2\text{O}$ (1:1) as eluent yielded geniposidic acid (**2**) (10.5 mg). By the same procedure, the n-BuOH F(9-11) (544.5 mg) sub-fraction was rechromatographed on a Waters RP18 column to afford 10-*O*-vanilloyl geniposidic acid (**1**) (3.5 mg).

10-*O*-Vanilloyl geniposidic acid (1): yellow resin $[\alpha]_D^{20} + 23.7^\circ$ (CHCl_3 , c 0,1). IR (film) $\nu_{\text{max}}/\text{cm}^{-1}$: 3382, 2920, 2855, 1692, 1645, 1589, 1551, 1537, 1450, 1402, 1353, 1282, 1219, 1183, 1107, 1077, 1042, 908, 880. EI-MS, m/z 524 ($M^+ 10\%$) 503 (0,5%), 484 (0,5%), 471 (0,5%), 445 (0,5%), 430 (0,5%), 413 (0,5%), 385 (0,5%), 368 (0,5%), 342 (0,5%), 318 (1%), 290 (1%), 274 (3%), 260 (1%), 245 (2%), 230 (7%), 202 (10%), 168 (47%), 153 (33%), 124 (79%), 109 (100%), 97 (20%), 81 (53%), 60 (65%), 44 (98%). ^1H and ^{13}C NMR data are given in Table 1.

Antifungal assay

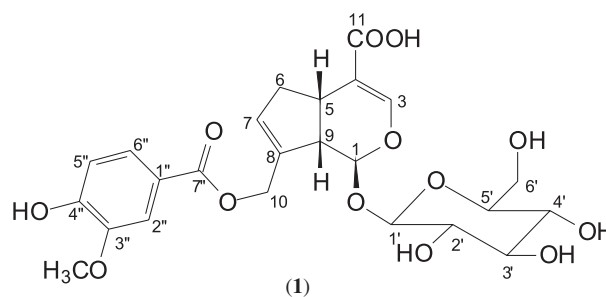
The filamentous fungus *Colletotrichum gloeosporioides* was obtained from the Departamento de Biologia, Universidade Federal do Ceará, Brazil, whereas *Fusarium solani* (URM 3708) and *Aspergillus niger* (URM 3292) were provided by the Departamento de Micologia, Universidade Federal Rural de Pernambuco, Brazil.

The assay for antifungal activity toward *Colletotrichum gloeosporioides*, *Fusarium solani* and *Aspergillus niger* was carried out in 100 x 15 mm Petri plates containing 25 mL of potato dextrose agar. After the mycelial colony had developed, sterile filter paper disks (0.6 cm in diameter, Whatman) were placed at a distance of 0.5 cm away from the rim of the mycelial colony. Aliquots (75 μL) of the iridoids **1** and **2** (solutions of 122.5 μg of **1** and 150 μg of **2** in 0.1% dimethyl sulfoxide) were added to the disks. Nistatin (2 μg) and DMSO were used as positive and negative control, respectively. The plates were incubated at 27 °C for 72 h until mycelial growth had enveloped the disks containing the negative control and had formed crescents halos of inhibition around disks containing samples with antifungal activity.⁸

RESULTS AND DISCUSSION

Compound **1** was obtained as a yellowish resin after isolation and purification. The IR spectrum showed absorption bands at 3382 cm^{-1} for hydroxyl; at 1645 and 1692 cm^{-1} for conjugated carbonyl groups, and at 1402 cm^{-1} for C-O-H, respectively. The low resolution EIMS of **1** showed a molecular ion at m/z 524, in agreement with the molecular formula $\text{C}_{24}\text{H}_{28}\text{O}_{13}$.

The ^1H NMR spectrum of **1** showed a very similar feature to those described for geniposidic acid (**2**), by the signals of two olefinic hydrogens at δ 7.23 (d, $J = 0.9$ Hz, H-3) and 5.86 (s, H-7), two diastereotopic methylene groups at δ 4.99 (d, $J = 14.0$ Hz, H-10eq)



and 4.87 (d, $J = 14.0$ Hz, H-10ax), and 2.89 (m, H-6) and 2.12 (m, H-6), and the β -D-glucopyranosyl moiety at δ 4.71 (d, $J = 7.7$ Hz, H-1'), 3.38 (m, H-5'), 3.33 (m, H-4'), 3.29 (m, H-3'), 3.25 (m, H-2'), 3.66 (dd, $J = 12.1, 5.3$ Hz, H-6'') and 3.85 (d, $J = 12.1$ Hz, H-6''). In addition, were also observed one methoxyl at δ 3.81 (s, OCH_3 -3'') and three aromatic hydrogens on an AMX spin system at δ 7.48 (dd, $J = 8.4, 2.1$ Hz, H-6''), 7.40 (d, $J = 2.1$ Hz) and 6.59 (d, $J = 8.4$ Hz) relative to the presence of an additional vanilloyl moiety.

The unequivocal NMR assignments of compound **1** shown in Table 1 was established by analysis from COSY, HMQC and NOESY experiments. The site of the vanilloyl ester linkage at C-10 was confirmed by the $^1\text{H}, ^1\text{H}$ scalar correlations observed from the COSY analysis, that revealed the correlations between the methylene group at δ 2.89 and 2.12 (2H-6) with the methine hydrogen at δ 3.37 (H-5) and the olefinic at δ 5.86 (H-7), which showed long-range coupling with the allylic methylene at δ 4.99 and 4.87 (2H-10). Although

Table 1. NMR assignments for compound **1**

Carbon	δ_{H}	δ_{C}	HMBC $^2J_{\text{CH}}$	$^3J_{\text{CH}}$
1	5.15 d (7.3)	98.0	H-9	H-3, H-1
3	7.23 d (0.9)	148.4		H-1
4		119.1	H-3, H-5	
5	3.37 m	37.7		H-1, H-3, H-7
6	2.12 m 2.89 m	40.4	H-7	
7	5.86 s	130.9		H-9
8		140.5	H-9, H-10	H-6
9	2.77 t (7.3)	48.4		H-7
10	4.87 d (14.0) 4.99 d (14.0)	63.8		
11		176.0		H-3
1'	4.71 d (7.7)	100.7		H-1
2'	3.25 m	75.1		
3'	3.29 m	78.4	H-2', H-4'	
4'	3.33 m	71.7	H-3'	
5'	3.38 m	78.1	H-6'	
6'	3.66 dd (2.2; 12.2) 3.85 d (2.2)	62.9		
1''		115.5		H-5''
2''	7.40 d (2.1)	113.4		H-6''
3''		151.5	H-2''	H-5'', - OCH_3
4''		163.4		H-2'', H-6''
5''	6.59 d (8.4)	118.4		
6''	7.48 dd (2.1; 8.4)	126.5		H-2''
7''		169.4		H-2'', H-6''
- OCH_3	3.81 s	56.3		

^1H and ^{13}C NMR spectra were acquired in CD_3OD at 500 and 125 MHz respectively. Chemical shifts are shown in the δ scale with J values (Hz) in parentheses.

no correlation of the olefinic hydrogen at δ 5.86 (H-7) with the carbon of the methylene group at C-10 was observed in the HMBC spectrum, cross-peak correlations of this hydrogen were found with the methine carbons at δ 48.4 (C-9) and 37.7 (C-5) and with the methylene at δ 40.4 (C-6), and the exact location of the double bond between the carbons atoms C-7 and C-8 was deduced. The relative stereochemistry of **1** was established by the NOESY experiment and revealed by the diagnostic NOE cross-peak between the hydrogen H-5 at δ 3.30 with the H-9 at δ 2.77, that in turn showed correlation with the H-1 at δ 5.15, in agreement with the relative stereochemistry related to the geniposidic acid.³ Therefore, the structure of compound **1** was elucidated to be the new iridoid 10-*O*-vanilloyl-geniposidic acid.

Geniposidic acid (**2**), but not 10-*O*-vanilloyl geniposidic acid (**1**), was inhibitory to the mycelial mass development of *C. gloeosporioides* at 150 μ g/mL (Figure 1S, supplementary material). In contrast, both compounds were inactive toward *A. niger* and *F. solani*.

SUPPLEMENTARY MATERIAL

The fungal growth inhibitory assay of compounds **1** and **2**, and ¹H NMR, ¹³C NMR, COSY, HMQC, HMBC, IR and EIMS spectra of compound **1** are available free of charge at <http://quimicanova.sbq.org.br>, as PDF file.

ACKNOWLEDGMENTS

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REFERENCES

1. Olea, R. S. G.; Roque, N. F.; Bolzani, V. S.; *J. Braz. Chem. Soc.* **1997**, *8*, 257; Silva, V. C.; Bolzani, V. S.; Young, M. C. M.; Lopes, M. N.; *J. Braz. Chem. Soc.* **2007**, *18*, 1405; Brochini, C. B.; Martins, D.; Roque, N. F.; Bolzani, V. S.; *Phytochemistry* **1994**, *36*, 1293; Young, M. C. M.; Braga, M. R.; Dietrich, S. M. C.; Gottlieb, H. E.; Trevisan, L. M. V.; *Phytochemistry* **1992**, *31*, 3433.
2. Luciano, J. H. S.; Lima, M. A. S.; Souza, E. B.; Silveira, E. R.; *Biochem. Syst. Ecol.* **2004**, *32*, 1227; Militão, G. C. G.; Pessoa, C.; Costa-Lotufo, L. V.; Moraes, M. E. A.; Moraes, M. O.; Luciano, J. H. S.; Lima, M. A. S.; Silveira, E. R.; *Pharm. Biol.* **2005**, *43*, 480.
3. Bitar, H. E.; Nguyen, V. H.; Gramain, A.; Sévenet, T.; Bodo, B.; *Tetrahedron Lett.* **2004**, *45*, 515.
4. Tanaka, R.; Matsunaga, S.; *Phytochemistry* **1992**, *31*, 3535.
5. Ahmad, V. U.; Rahman, A.; *Handbook of Natural Products Data. Pentacyclic Triterpenoids*, Elsevier: New York, 1994.
6. Stevens, J. F.; Wollenweber, E.; Ivancic, M.; Hsu, V. L.; Sundberg, S.; Deinzer, M. L.; *Phytochemistry* **1999**, *51*, 771.
7. Bolzani, V. S.; Trevisan, L. M.; Young, M. C. M.; *Phytochemistry* **1991**, *30*, 2089.
8. Chu, K. T.; Xia, L.; Ng, T. B.; *Peptides* **2005**, *26*, 2098.

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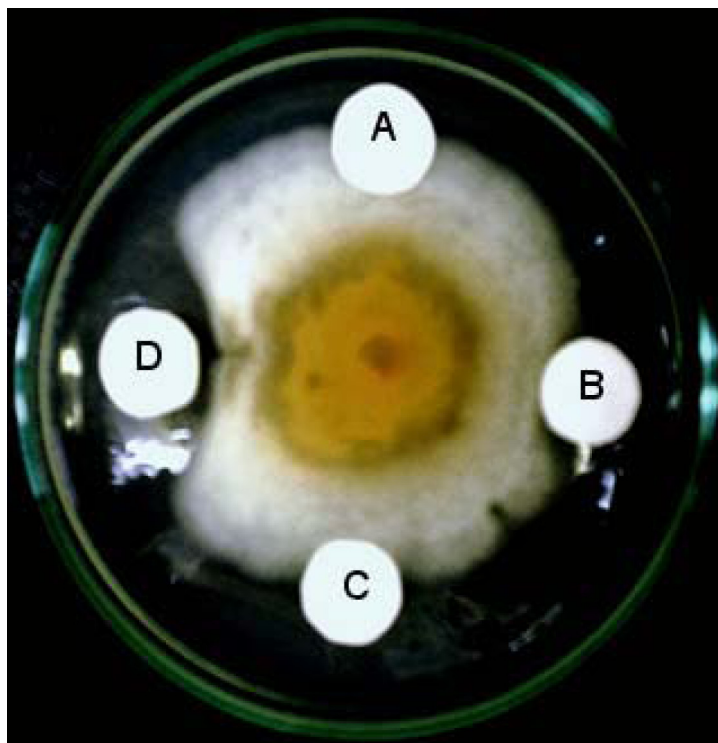


Figure 1S. Fungal growth inhibitory assay. (A) 0,1% DMSO as negative control; (B) 150 μg geniposidic acid (2); (C) 112,5 μg 10-O-vanniloyl geniposidic acid (1); (D) 2 μg nystatin as positive control

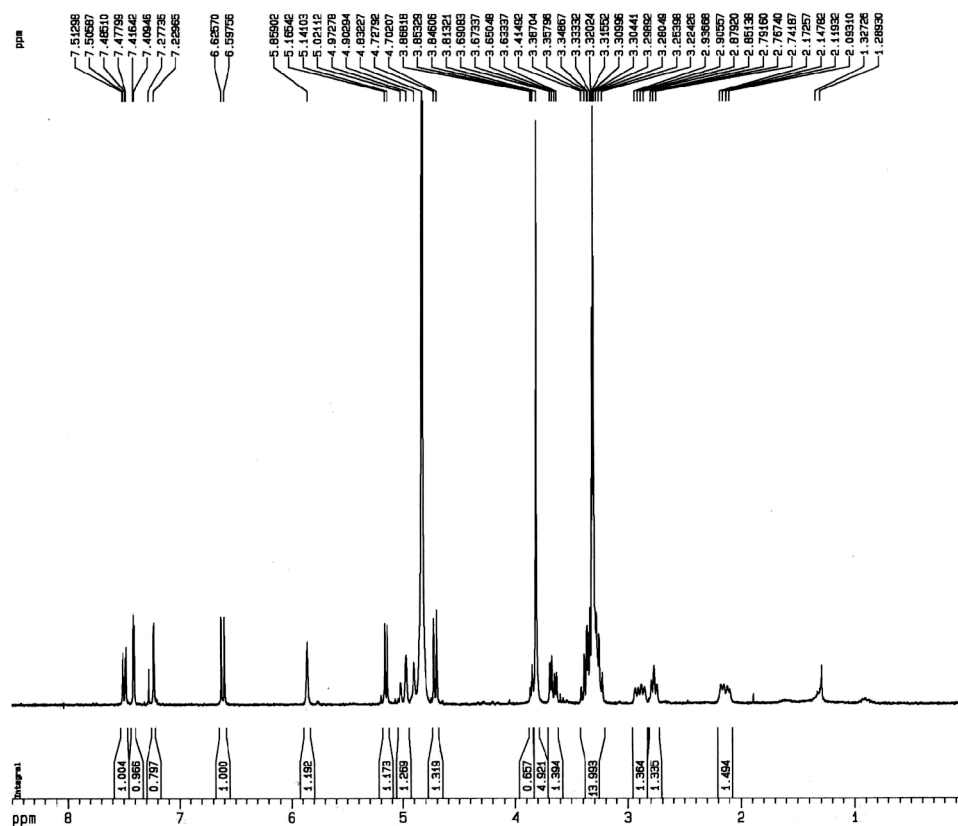


Figure 2S. ^1H NMR spectrum (300 MHz, CD_3OD) of compound 1

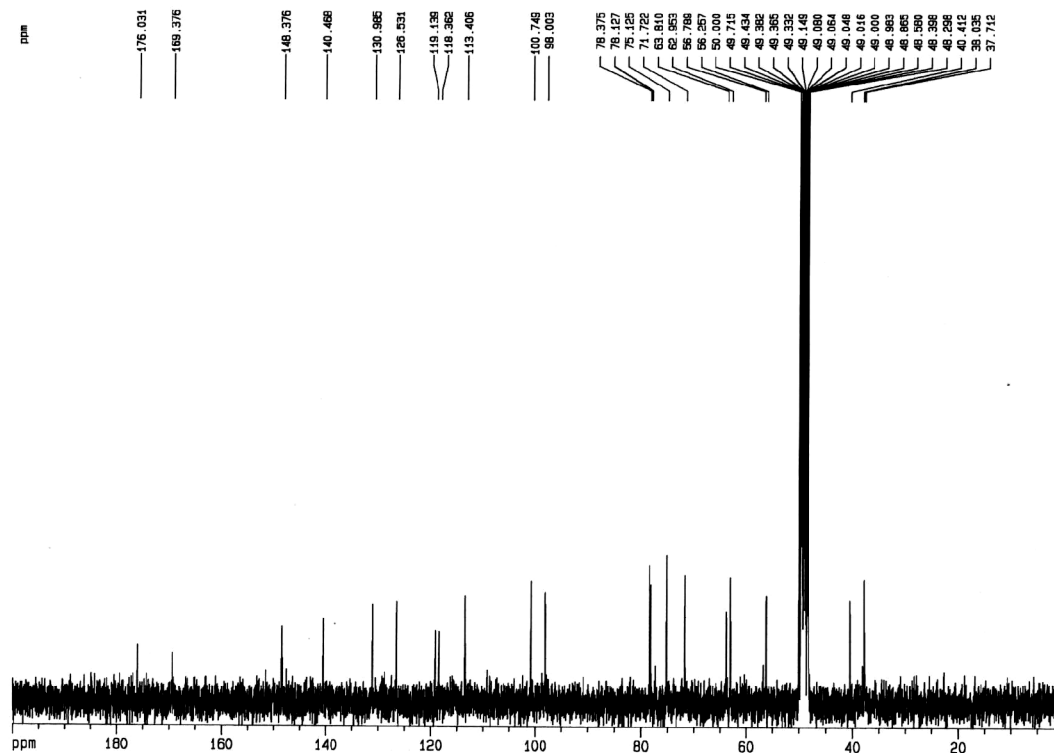


Figure 3S. ^{13}C -BB NMR spectrum (75 MHz, CD_3OD) of compound 1

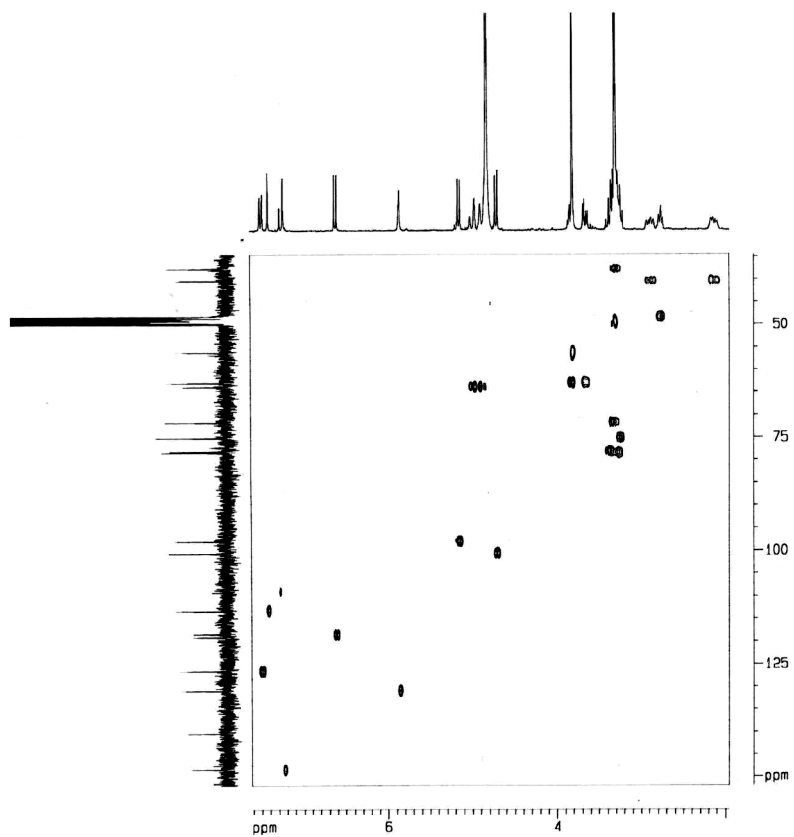


Figure 4S. HMQC spectrum of compound 1

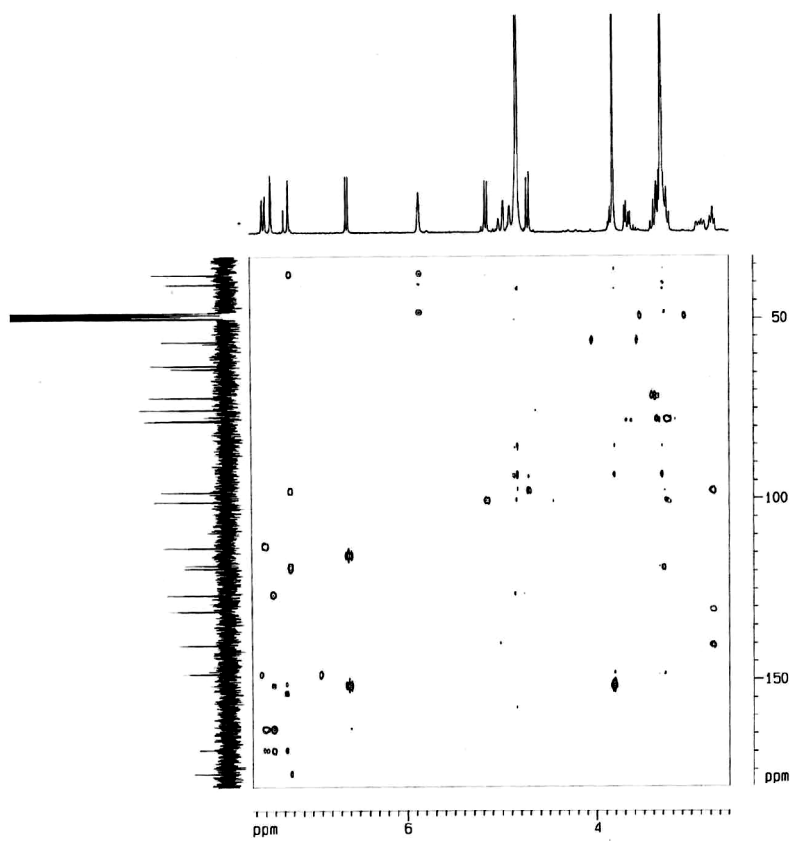


Figure 5S. HMBC spectrum of compound 1

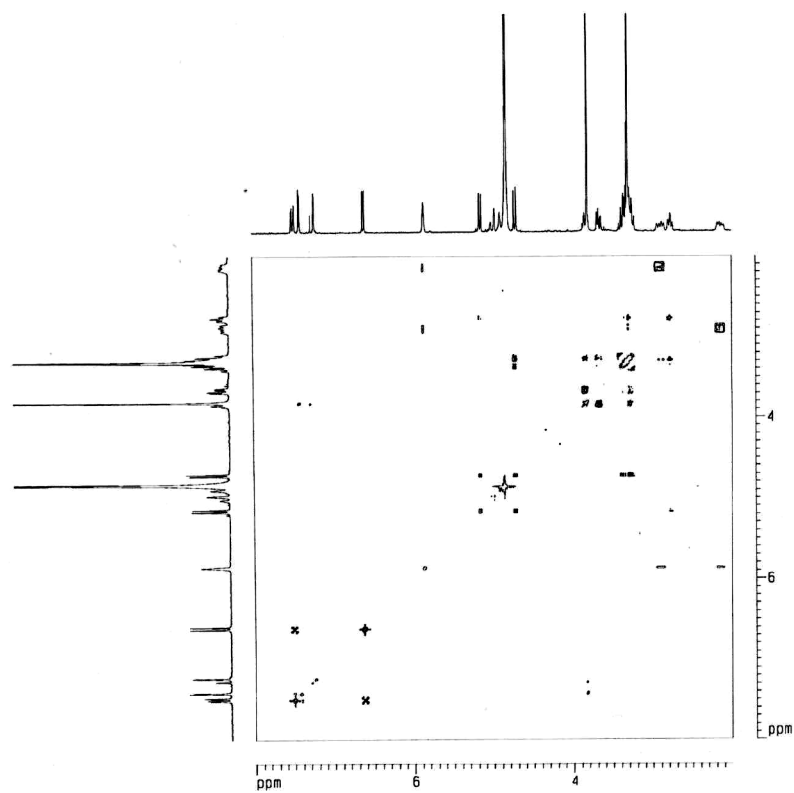


Figure 6S. NOESY spectrum of compound 1

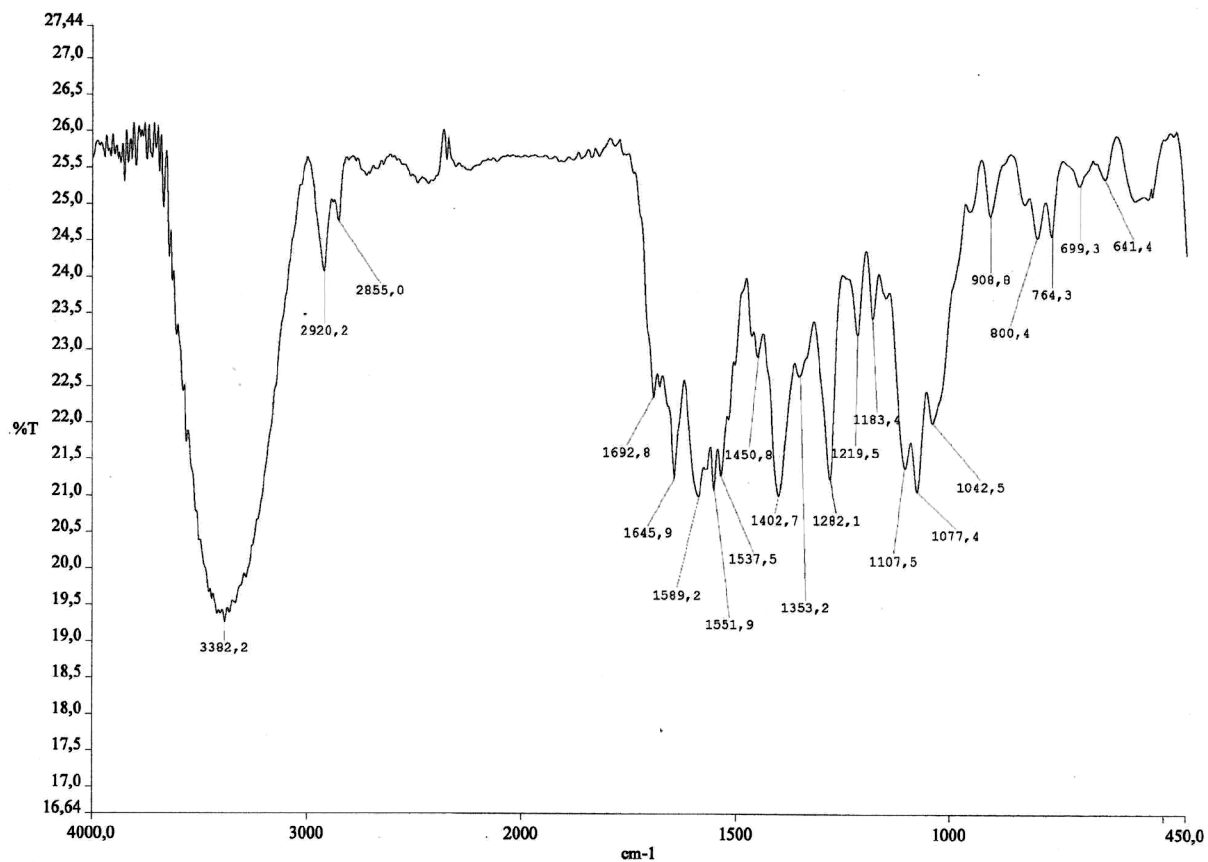


Figure 7S. I.R. spectrum of compound 1 (KBr)

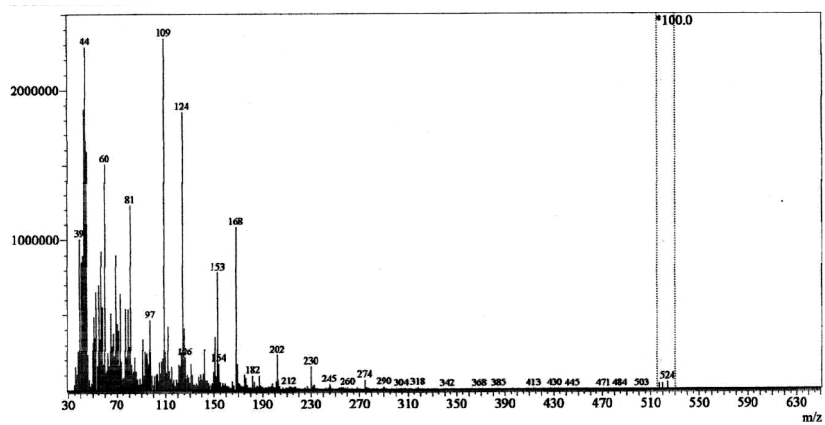


Figure 8S. Mass spectrum of compound 1 (electronic impact - 70 eV)

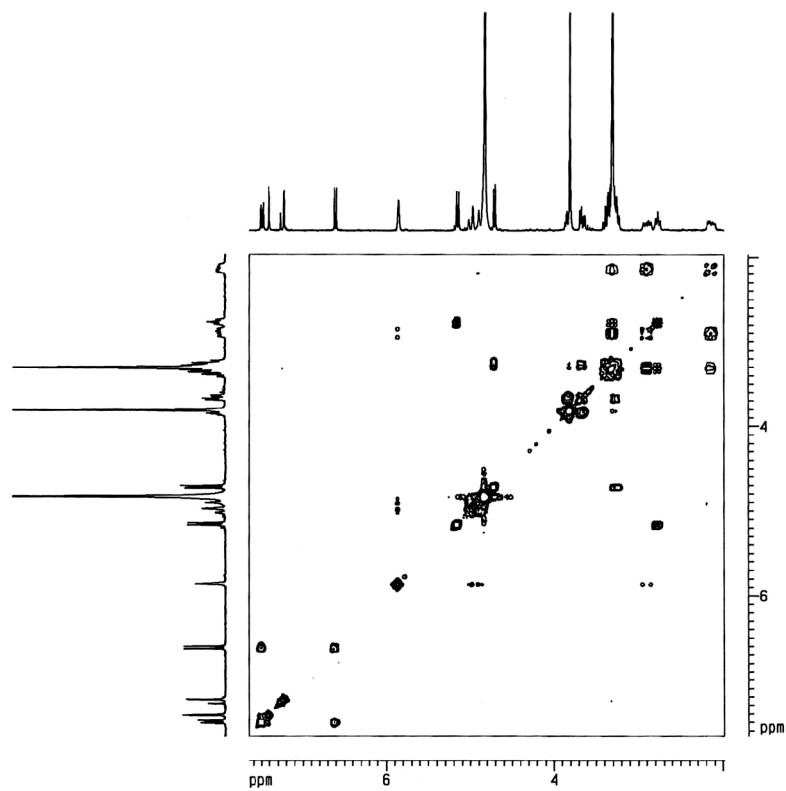


Figure 9S. COSY spectrum of compound 1

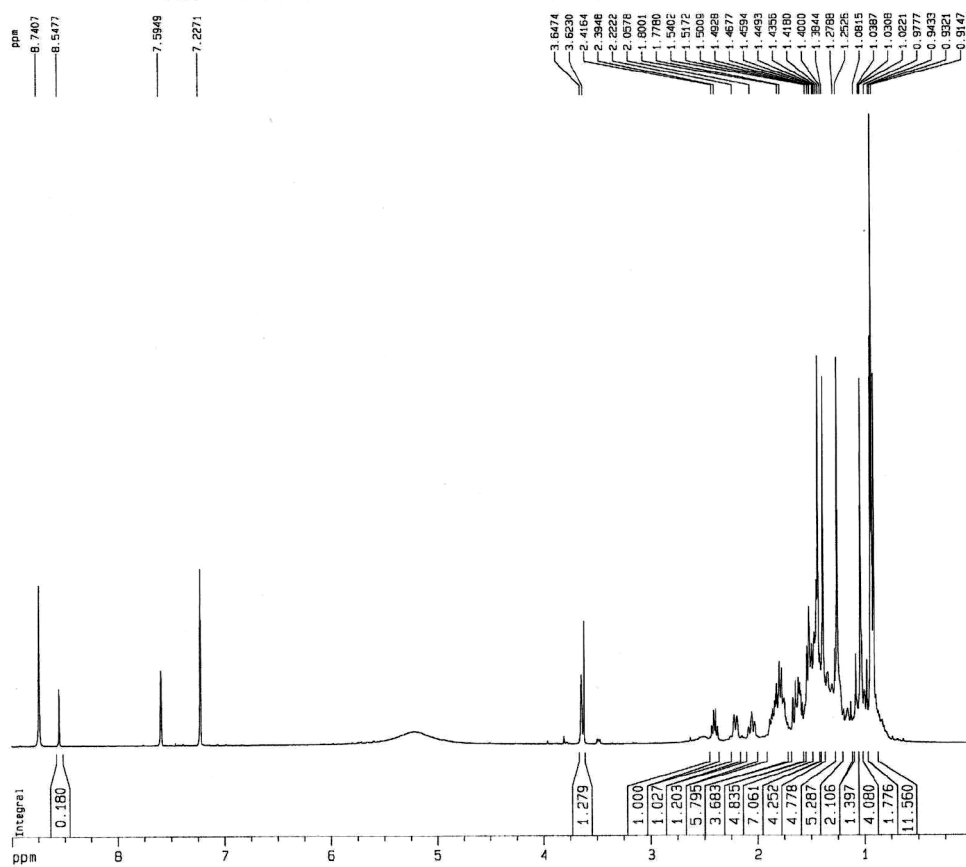


Figure 12S. ^1H NMR spectrum (500 MHz, $\text{C}_5\text{D}_5\text{N}$) of compound 4

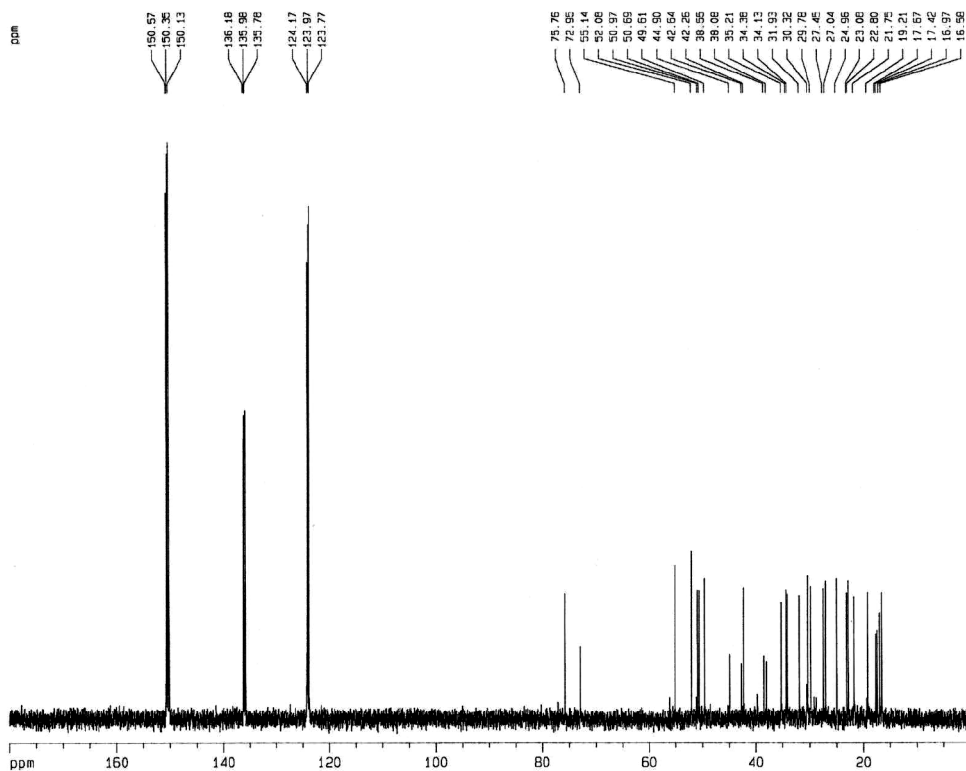


Figure 13S. ^{13}C -BB NMR spectrum (125 MHz, $\text{C}_5\text{D}_5\text{N}$) of compound 4