

## Amburosides C-H and 6-O-Protocatechuoyl Coumarin from *Amburana cearensis*

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Seis novos amburosídeos (**1-6**) e o novo protocatecuato de 6-cumarila (**7**) foram isolados das sementes e cascas do caule de *Amburana cearensis*, juntamente com cumarina, 6-hidroxicumarina, isocampferídeo, formononetina, ácido vanílico, amburosídeo A e ácido (*E*)-*o*-cumárico. As estruturas químicas dos novos compostos foram elucidadas com base na análise espectral de RMN (COSY, HSQC, HMBC e NOESY) e EM.

Six new amburosides (**1-6**) and a novel 6-coumaryl protocatechuate (**7**) were isolated from the seeds and trunk bark of *Amburana cearensis*, together with coumarin, 6-hydroxycoumarin, isokaempferide, formononetin, vanillic acid, amburoside A and (*E*)-*o*-coumaric acid. The chemical structures of the new compounds were elucidated by means of NMR (COSY, HSQC, HMBC and NOESY) and HRMS spectral analyses.

**Keywords:** *Amburana cearensis*, Fabaceae, amburosides, 6-coumaroyl-protocatechuate

### Introduction

*Amburana cearensis* A. C. Smith (syn.: *Torresea cearensis* Fr. All.- Fabaceae), known as “cumarú” or “imburana-de-cheiro”, is a native tree from northeastern Brazil, where its trunk bark and seeds are popularly used for the treatment of respiratory affections such as asthma and bronchitis.<sup>1</sup> The hydroalcohol extract from trunk bark and some of its constituents demonstrated bronchodilator, antiinflammatory and analgesic activities in pre-clinical trials.<sup>2</sup>

We have previously reported the isolation of coumarin, phenol acids, flavonoids, and the phenol glucoside amburoside A from the trunk bark of *A. cearensis*.<sup>3</sup> A cytotoxic activity assay revealed that both the flavonoids isokaempferide and kaempferol inhibit the sea egg development as well as tumor cell lines.<sup>4</sup> Amburoside A showed protective effects against CCl<sub>4</sub>-induced hepatotoxicity in rats,<sup>5</sup> and presented a significant neuroprotective effect on mesencephalic cells against 6-hydroxydopamine-induced neurotoxicity.<sup>6</sup> In addition, isokaempferide and amburoside A also showed effects on rodent inflammatory processes and myeloperoxidase activity in human neutrophils.<sup>7</sup>

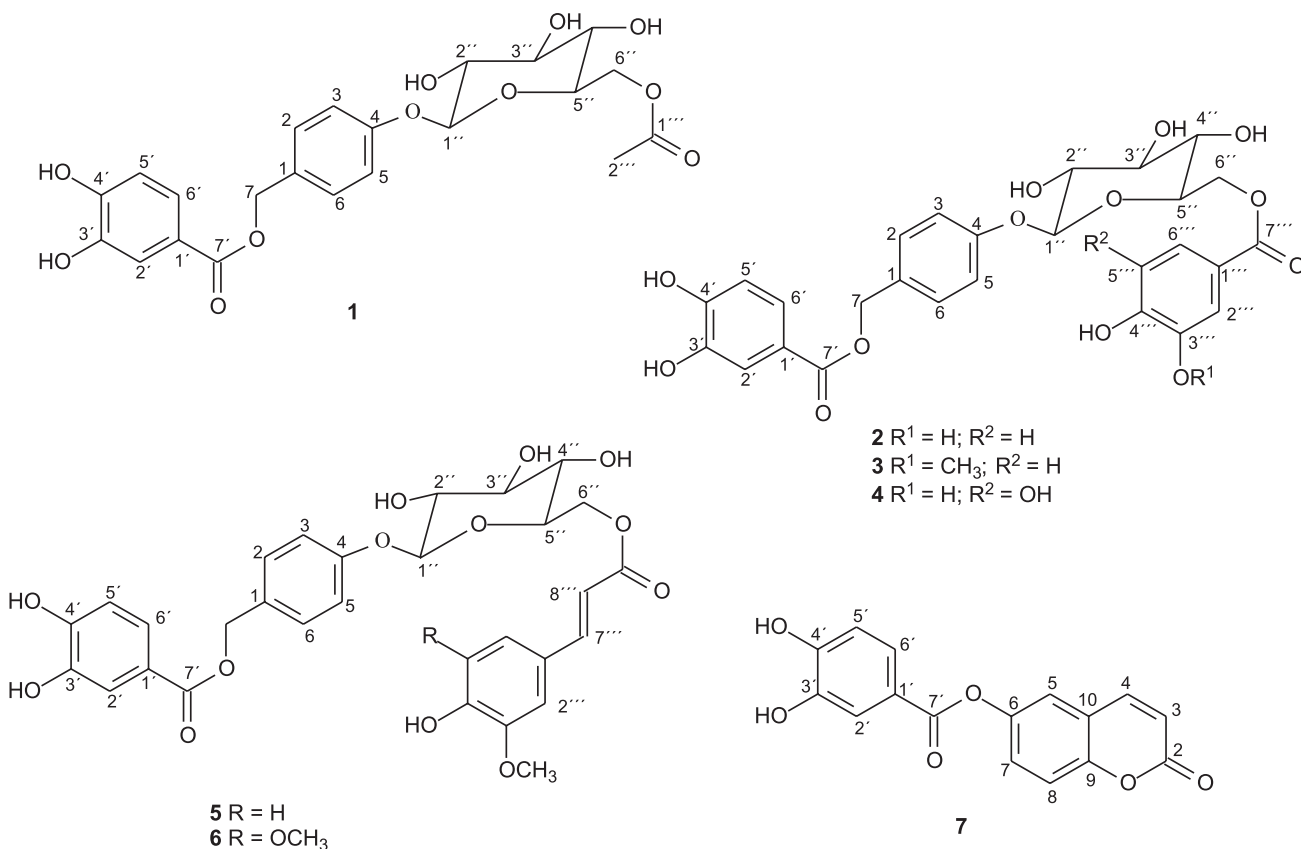
Continuing with the earlier phytochemical and pharmacological studies on *A. cearensis*, it is reported now the isolation and structural elucidation of six new amburosides C-H (**1-6**) from the seeds, besides a new 6-coumaryl protocatechuate (**7**) from the trunk bark. In addition, known compounds were characterized as coumarin, 6-hydroxycoumarin, isokaempferide, formononetin, amburoside A, (*E*)-*o*-coumaric acid and vanillic acid. The structure elucidation was performed by spectroscopic techniques such as IR, HRMS, 1D and 2D NMR, and comparison to spectral data from literature.

### Results and Discussion

Compound **1**, a brown solid, showed a molecular formula of C<sub>22</sub>H<sub>24</sub>O<sub>11</sub> as established from its HRESIMS quasi-molecular ion at *m/z* 487.1302 [M + Na]<sup>+</sup>. The <sup>1</sup>H NMR spectrum showed singlet signals relative to one methyl group at δ 2.02 (s) and to an oxymethylene at δ 5.23 (s). An axial anomeric proton at δ 4.91 (d, *J* 7.4 Hz, H-1'), and signals in the region of δ 4.91-3.39, suggested the presence of a sugar unit, whose β-D-anomeric configuration was judged based on the large value of its coupling constant (*J* 7.4 Hz). The presence of an A<sub>2</sub>B<sub>2</sub> system in the aromatic region at δ 7.37 (*d*, *J* 8.6, 2H) and 7.08 (*d*, *J* 8.6, 2H), suggested the existence of a *para*-disubstituted benzene ring. A second aromatic ring was characterized as an ABX

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spin system, corresponding to a 1,2,4 substitution by the signals at  $\delta$  7.43 (*d*,  $J$  2.1), 7.41 (*dd*,  $J$  8.4; 2.1) and 6.79 (*d*,  $J$  8.4). The  $^{13}C$  NMR spectral data of **1** confirmed the  $\beta$ -glucopyranosyl unit by the signals at  $\delta$  102.3 (C-1''), 75.0 (C-2''), 78.0 (C-3''), 72.0 (C-4''), 75.6 (C-5'') and 64.8 (C-6''),<sup>8</sup> together with 14 further signals ascribable for the aglycone, that were assigned two carbonyls, a methyl group, an oxymethylene and twelve aromatic carbons, including the symmetric *para*-substituted benzene ring.

On the basis of HMBC and HSQC spectral analysis, the structure of compound **1** was further determined and all proton and carbon signals were fully assigned. In the HMBC spectrum, the anomeric hydrogen at  $\delta$  4.91 (H-1'') did correlate with the oxygenated aromatic carbon at  $\delta$  159.0 (C-4), which in turn, also correlated with the hydrogens at  $\delta$  7.08 (H-3/5) and 7.37 (H-2/6). These findings indicated that the  $\beta$ -D-glucosyl moiety was located at C-4 of the *para*-substituted aromatic ring. The acetyl group was attached to C-6'' of the glucose unit, as evidenced by the HMBC cross-peaks of oxymethylene protons at  $\delta$  4.39 and 4.24 (2H-6'') and the methyl protons at  $\delta$  2.02 (CH<sub>3</sub>-2''') with the carbonyl at  $\delta$  172.9 (C-1'''). On the other hand, the long-range correlation observed between the oxymethylene singlet at  $\delta$  5.23 (H-7) with the other carbonyl group at  $\delta$  168.3 (C-7'), and with the two equivalent hydrogenated aromatic carbons at  $\delta$  130.8 (C-2/6), allowed the attachment of the ester

function to the C-1 of the *para*-substituted aromatic ring. In addition, the aromatic hydrogen at  $\delta$  7.43 (H-2') showed correlation with the ester carbonyl carbon at  $\delta$  168.3 (C-7'), with the oxygenated carbons at  $\delta$  146.3 (C-3') and 151.9 (C-4'), and with the hydrogenated carbon at  $\delta$  123.9 (C-6'), characterizing the presence of the protocatechuoyl unit. Thus, the structure of compound **1** was established as the new phenol glucoside 4-*O*- $\beta$ -D-(6''-*O*-acetylglucopyranosyl)-benzyl protocatechuate, named amburoside D.

Compound **2**, a brown solid, showed a *quasi*-molecular ion at  $m/z$  581.1286 [M + Na]<sup>+</sup> in the HRESIMS, in accordance with a molecular formula C<sub>27</sub>H<sub>26</sub>O<sub>13</sub>. As observed for compound **1**, the  $^1H$  NMR spectrum of compound **2** showed the characteristic doublet for the anomeric hydrogen at  $\delta$  4.92 (*d*,  $J$  7.4, H-1'') relative to the  $\beta$ -D-glucosyl unit, besides the oxy-benzylic methylene group at  $\delta$  5.18 (*s*, H-7). The COSY spectrum revealed the presence of two ABX type systems relative to the two protocatechuoyl units through the typical signals at  $\delta$  6.79 (*d*,  $J$  8.1, H-5'), 7.41 (*dd*,  $J$  8.1; 2.0, H-6') and 7.43 (*d*,  $J$  2.0, H-2'), and 6.83 (*d*,  $J$  8.2, H-5'''), 7.45 (*dd*,  $J$  8.2; 2.0, H-6''') and 7.47 (*d*,  $J$  2.0, H-2'''). The remaining resonances in the aromatic region were those of an A<sub>2</sub>B<sub>2</sub> system at  $\delta$  7.26 (*d*,  $J$  8.6, H-2/6) and 7.06 (*d*,  $J$  8.6, H-3/5), consistent with a *para*-disubstituted aromatic ring also observed for compound **1**.

The HMBC spectrum revealed cross-peaks between the anomeric proton at  $\delta$  4.92 (*d*, *J* 7.4, H-1'') with the carbon at  $\delta$  158.4 (C-4) of the *para*-disubstituted benzene ring. Moreover, the concomitant correlations of the oxybenzylic hydrogens at  $\delta$  5.18 (*s*, 2H-7) with the carbonyl at  $\delta$  168.3 (C-7') and the carbons at  $\delta$  130.9 (C-2/6), and of the methylene group of the glucosyl unit at  $\delta$  4.63 (*m*, C-6'') with the other carbonyl at  $\delta$  168.2 (C-7'''), determined the ester linkage of the protocatechuoyl units with those carbons. Thus, compound **2** was determined to be the new 4-*O*- $\beta$ -D-(6''-*O*-protocatechuoylglucopyranosyl)-benzyl protocatechuate, named amburoside E.

Compound **3** was also isolated as a brown solid. The HRESIMS of **3** exhibited a *quasi*-molecular ion peak at  $m/z$  571.1487 [M-H]<sup>-</sup>, consistent with the molecular formula C<sub>28</sub>H<sub>28</sub>O<sub>13</sub>. The multiplicity pattern of each coupled proton system in the <sup>1</sup>H NMR spectrum of **3** were very similar to those observed for **2**, except by the presence of an additional methoxyl group at  $\delta$  3.83 (*s*, OCH<sub>3</sub>-3'''). This observation was confirmed by the <sup>13</sup>C NMR data of **3** that showed the presence of an extra methoxyl group at  $\delta$  56.7 (OCH<sub>3</sub>-3'''), thus suggesting the methylation of a hydroxy group of the protocatechuoyl unit of compound **2**. The HMBC experiment confirmed the linkage of the vanilloyl moiety to C-6'' of the glycosyl unit by the correlation of the methoxyl group at  $\delta$  3.83 (OCH<sub>3</sub>-3''') with the carbon at  $\delta$  148.9 (C-3'''), and the correlation of the hydrogen at  $\delta$  7.54 (H-2'') with this carbon at  $\delta$  148.9 (C-3''') and with the carbonyl group at  $\delta$  168.0 (C-7'''). In addition, the methylene group of the acylated glucosyl unit at  $\delta$  4.68 (H-6'') showed correlation with the carbonyl group at  $\delta$  168.0 (C-7'''). Hence, the structure of compound **3** was determined as 4-*O*- $\beta$ -D-(6''-*O*-vannilloylglucopyranosyl)-benzyl protocatechuate, named amburoside G.

Compound **4** was isolated as a dark brown solid, and showed [M + Na]<sup>+</sup> peak at  $m/z$  597.1256 in the HRESIMS spectrum, corresponding to the molecular formula C<sub>27</sub>H<sub>26</sub>O<sub>14</sub>. Its <sup>1</sup>H NMR data resemble those of compound **2**, except for the absence of an AMX system and the appearance of a signal at  $\delta$  7.10 (*s*, H-2'''/6'') integrating for two hydrogens suggesting the presence of a galloyl group in **4**. This was supported by the <sup>13</sup>C NMR spectrum which displayed two absorptions of oxygenated aromatic carbons at  $\delta$  146.7 (C-3'''/5''). The location of the galloyl group as attached to the glucosyl unit was determined by the HMBC long range correlations of the oxymethylene group of the glucose moiety at  $\delta$  4.60 and 4.40 (2H-6'') with the carbonyl at  $\delta$  168.3 (C-7'''). From the above evidences, compound **4** was characterized as the new 4-*O*- $\beta$ -D-(6''-*O*-galloylglucopyranosyl)-benzyl protocatechuate, named amburoside C.

Compound **5** was isolated as a dark brown solid and exhibited a [M + Na]<sup>+</sup> peak at  $m/z$  621.1626 in agreement with the molecular formula of C<sub>30</sub>H<sub>30</sub>O<sub>13</sub> by HRESIMS. Its <sup>1</sup>H NMR spectrum displayed signals relative to the protocatechuoyl, benzyl and glucosyl units, as observed for compounds **1-4**. Moreover, a feruloyl unit was suggested for this compound by the additional olefinic proton signals with a *trans* relationship at  $\delta$  6.37 (*d*, *J* 16.0, H-8'') and 7.61 (*d*, *J* 16.0, H-7''), besides one methoxyl group at  $\delta$  3.86 (OCH<sub>3</sub>-3'''). This observation was confirmed by the <sup>13</sup>C NMR spectrum, that showed two absorptions relative to the olefinic methine carbons at  $\delta$  115.4 (C-8'') and 147.2 (C-7''), and the methoxyl group at  $\delta$  56.6 (OCH<sub>3</sub>-3'''). The position of the feruloyl group in **5** was predicted to be linked to the hydroxy group C-6'' of the glucose unit from HMBC cross-peaks of hydrogens at  $\delta$  4.52 (H-6''), 7.04 (H-6'') and 7.61 (H-7'') with the carbonyl group at  $\delta$  169.1 (C-9''). Thus, compound **5** was characterized as the new 4-*O*- $\beta$ -D-(6''-*O*-feruloylglucopyranosyl)-benzyl protocatechuate, named amburoside F.

The HRESIMS of compound **6** exhibited a *quasi*-molecular ion [M-H]<sup>-</sup> at  $m/z$  627.1766 corresponding to the molecular formula C<sub>31</sub>H<sub>31</sub>O<sub>14</sub>. Comparison of its <sup>1</sup>H and <sup>13</sup>C NMR spectra with those of **5** indicated their structural similarity, except for the existence of an extra methoxyl group and the disappearance of the AMX system. In the <sup>1</sup>H NMR spectrum, a two-proton singlet at  $\delta$  6.90 (*s*, H-2'''/6''), a 6H singlet at  $\delta$  3.86 (OCH<sub>3</sub>-H-3'''/5'') characteristic of substitution pattern of the symmetric benzene ring, and the two doublets at  $\delta$  6.42 (*d*, *J* 15.9, H-8'') and 7.62 (*d*, *J* 15.9, H-7''), were indicative of the presence of a sinapoyl moiety. Furthermore, the interlinkage site of the sinapoyl group was established on the basis of the concomitant HMBC correlations between the glucosyl methylene group at  $\delta$  4.51 (H-6'') and the olefinic hydrogens at  $\delta$  6.42 (H-8'') and 7.62 (H-7'') with the carbonyl group at  $\delta$  169.0 (C-9''). The structure of **6** was finally established to be the new 4-*O*- $\beta$ -D-(6''-*O*-sinapoylglucopyranosyl)-benzyl protocatechuate, named amburoside H.

Compound **7** was isolated as a redish solid (mp 257.6-258.3 °C). Its molecular formula C<sub>16</sub>H<sub>10</sub>O<sub>6</sub> was deduced from the HRESIMS spectrum, which presented a [M+H]<sup>+</sup> ion at  $m/z$  299.0550 (calculated for C<sub>16</sub>H<sub>11</sub>O<sub>6</sub>, 299.0552). Its FT-IR spectrum exhibited a broad and strong absorption at 3315 cm<sup>-1</sup> characteristic of hydroxyl groups and a sharp and intense band at 1699 cm<sup>-1</sup> relative to the conjugated C=O stretching.

Its <sup>1</sup>H NMR spectrum displayed a pair of doublets at  $\delta$  8.02 and 6.49 (1H, *J* 9.6 Hz, H-4 and H-3), typical of olefin hydrogens of coumarin compounds, and two AMX

systems whose signals were differentiated by  $^1\text{H}$ ,  $^1\text{H}$  COSY analysis as A- [ $\delta$  7.40 (*d*,  $J$  9.0 Hz, H-8); 7.25 (*dd*,  $J$  9.0 and 2.9 Hz, H-7) and 7.21 (*d*,  $J$  2.9 Hz, H-5)]; and B- [ $\delta$  7.69 (*dd*,  $J$  8.5 and 2.0 Hz, H-6'); 7.48 (*d*,  $J$  2.0 Hz, H-2') and 7.06 (*d*,  $J$  8.5 Hz, H-5')]. Thus, a spin system was related to the aromatic ring of a monosubstituted coumarin skeleton, and the other one was attributed to a protocatechuoyl moiety.

The  $^{13}\text{C}$  NMR-CPD spectrum showed 16  $\text{sp}^2$  carbon signals, two of them at  $\delta$  166.6 (C-7') and 160.0 (C-2) compatible with carbonyls of a benzoyl ester and of a coumarin. The unequivocal assignment of two coincident signals at  $\delta$  122.3 (C-1' / 2') was only possible by  $^{13}\text{C}$  NMR-GATED spectrum, which displayed one resonance as a pair of doublets ( $^1J_{\text{C,H}}$  122.0 Hz and  $^3J_{\text{C,H}}$  7.2 Hz, C-2') and another as a doublet ( $^3J_{\text{C,H}}$  7.2 Hz, C-1').

The HMBC spectrum characterized the coumarin-skeleton through the correlations of the olefin hydrogen signals at  $\delta$  6.49 (H-3) and 8.02 (H-4) with the lactone carbonyl signal at  $\delta$  160.0 (C-2), and the aromatic carbons at  $\delta$  149.0 (C-9) and 119.5 (C-10), besides other correlations observed for the aromatic hydrogens at  $\delta$  7.21 (H-5), 7.25 (H-7). The existence of the protocatechuate moiety was evidenced by correlations of aromatic hydrogens at  $\delta$  7.48 (H-2'), 7.06 (H-5'm) and 7.69 (H-6') with the non-hydrogenated carbons at  $\delta$  122.3 (C-1'), 142.6 (C-3') and 153.5 (C-4'), as well with the ester carbonyl at  $\delta$  166.6 (C-7').

On the basis of coumarin biosynthesis, it was initially proposed the protocatechuate-group linked to coumarin at C-7 position.<sup>9,10</sup> However, the spectroscopic evidences led us to reject this proposition and to suggest the ester bridge through the oxygenated C-6. The placement of substituent at C-6 was supported by the dipolar coupling observed on 2D-NOESY spectrum between the hydrogens at  $\delta$  8.02 (*d*,  $J$  9.6 Hz, H-4) and 7.21 (*d*,  $J$  2.9 Hz, H-5).  $^3J$  HMBC correlations of the hydrogens at  $\delta$  7.25 (H-7) and the carbons at  $\delta$  114.6 (C-5) and 149.0 (C-9) reinforced the structural proposition. Moreover, 7-hydroxy coumarin and its derivatives show a  $^{13}\text{C}$  NMR chemical shift at about  $\delta$  102-104 assigned to C-8.<sup>11</sup> No signal was observed in this range on the  $^{13}\text{C}$  NMR spectrum of **7**, the most shielded hydrogenated carbon of **7** appears at  $\delta$  114.6. Thus, compound **7** was named as the new protocatechuoyl derivative of 6-hydroxy-coumarin.

In addition, the precursor of compound **7**, 6-hydroxycoumarin was isolated from seeds of *A. cearensis* and characterized by physical and spectroscopic methods. Comparative analysis of 6-hydroxycoumarin (mp 221.7-222.9 °C,  $t_{\text{R}}$  = 11.7 min) with an authentic sample of umbelliferone and with literature data (mp 230-233 °C,  $t_{\text{R}}$  = 13.1 min) allowed to distinguish them.<sup>12,13</sup>

According to biogenetic rules, monooxygenated-coumarins are preferentially substituted at C-7 position yielding umbelliferone and its derivatives, due to the common precursor of this chemical class, *p*-coumaric acid, which is biosynthetized by the shikimate pathway from either tyrosine-deamination or *p*-hydroxylation of cinnamic acid.<sup>9,10</sup> Nevertheless, despite a large occurrence of simple coumarins oxygenated at the C-7 position, 6-hydroxycoumarin was also found in some species like *Bidens parviflora* (Asteraceae), *Paeonia suffruticosa* (Paeoniaceae) and *Hydrangea chinensis* (Hydrangeaceae).<sup>13-15</sup>

Among the isolated compounds, isokampferide and coumarin have been isolated from the trunk bark, however formononetin and (*E*)-*o*-coumaric acid are being reported for first time for the genus *Amburana*. Vanillic acid and amburoside A already described for the trunk bark, are now being identified from seeds.<sup>3,17</sup>

## Experimental

### General experimental procedures

NMR experiments were performed on a Bruker DRX-500 spectrometer, operating at 500 MHz for  $^1\text{H}$ , and at 125 MHz for  $^{13}\text{C}$ . NMR samples were dissolved in deuterated solvents (Cambridge Isotope Laboratories, Andover, USA) and referenced to their residual undeuterated portions for protons and to the central peak of the deuterated carbons. IR spectra were recorded on a Perkin-Elmer FT-IR 1000 spectrometer (Waltham, USA), using KBr pellets. High resolution mass spectra were recorded on an UltrTOF-Q mass spectrometer Bruker Daltonics, Billerica, USA and LC-IT-TOF model 225-07100-34-SHIMADZU, by positive or negative ionization modes of the ESI source. Melting points (uncorrected) were determined from a Mettler Toledo FP82HT apparatus (Columbus, USA), adopting a heating rate of 2 °C  $\text{min}^{-1}$ . Column chromatography was performed either over silica gel 60 (VETEC, 70-230 mesh) or Sephadex LH-20 (Amersham Pharmacia Biotech, Uppsala, Sweden). TLC was performed on precoated silica gel aluminum sheets (Merck) and monitored by UV detection and exposure to iodine vapour. Reverse phase chromatography was performed on C18 Sep-Pak® Waters cartridge. HPLC analysis were performed on a Waters 1525 chromatograph (Milford, USA) equipped with a binary pump and photodiode-array detector (Waters-2996 PDA), using Waters X-Terra RP-18 columns (analytical: 250  $\times$  4.6 mm, 5  $\mu\text{m}$ ; semi-preparative: 250  $\times$  10 mm, 10  $\mu\text{m}$ ) at 35 °C in a thermostatic oven. HPLC grade methanol was purchased from Tedia Co (Fairfield, USA)

and the HPLC grade water (18 mΩ) was obtained by a Milli-Q purification system (Millipore, Bedford, USA).

### Plant material

The trunk barks of *Amburana cearensis* A. C. Smith were collected at the Quixeramobim County (Ceará State, Northeast of Brazil) in September 2002 while the seeds were purchased from a herbal store in Fortaleza City in June 2001. Voucher specimens (# 837 and 847) were deposited at the Herbário Prisco Bezerra (EAC) and identified by Dr. Afrânio G. Fernandes, from the Department of Biology, Federal University of Ceará.

### Extraction and isolation

Around 1.5 kg of powdered seeds of *A. cearensis* were previously macerated in hexane (1.8 L) for 24 h, yielding a yellowish oil (312 g) containing a precipitate that after being separated, was characterized as coumarin (53.5 g,

mp 68 °C).<sup>10</sup> Then, 1.1 kg of the remaining residue were extracted in small aliquots (100 g) with ethanol on a Soxhlet apparatus, affording a dark brown extract (144.0 g, 9.6%  $m_{\text{ext}}/m_{\text{seed}}$ ), after pooling together all ethanol solutions and vacuum distillation of the solvent. Powdered dried trunk barks (4.2 kg) of *A. cearensis* were subjected to the same extraction procedure yielding also a brown ethanol extract (228.0 g, 5.4%  $m_{\text{ext}}/m_{\text{trunk}}$ ).

The ethanol extract of the trunk barks (228.0 g) was suspended in H<sub>2</sub>O-MeOH (2:1) and subjected to partition with EtOAc (3 × 150 mL). The EtOAc fraction (64.5 g) was purified by chromatography on silica gel 60 by elution with CHCl<sub>3</sub>:EtOAc mixtures of increasing polarity to yield 4 fractions. Purification of the fraction 2 (15.4 g; eluted with CHCl<sub>3</sub>/EtOAc 50:50) on Sephadex LH-20 (CHCl<sub>3</sub>:MeOH 50:50) yielded compounds formononetin (3.0 mg, mp 240.2-242.9 °C)<sup>16</sup>, isokaempferide (171.4 mg, mp 290.7-292.1 °C) and 6-coumaryl protocatechuate **7** (7 mg, mp 257.6-258.3 °C). The ethanol extract from seeds (144.0 g) was suspended in H<sub>2</sub>O-MeOH (2:1) and

**Table 1.** <sup>1</sup>H NMR data assignments for compounds **1-7** (500 MHz, CD<sub>3</sub>OD, *J* in Hz)

Position	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>	<b>7<sup>a</sup></b>
2	7.37 d ( <i>J</i> 8.6)	7.26 d ( <i>J</i> 8.6)	7.05 d ( <i>J</i> 8.6)	7.29 d ( <i>J</i> 8.6)	7.28 d ( <i>J</i> 8.7)	7.30 d ( <i>J</i> 8.6)	
3	7.08 d ( <i>J</i> 8.6)	7.06 d ( <i>J</i> 8.6)	7.05 d ( <i>J</i> 8.6)	7.06 d ( <i>J</i> 8.6)	7.07 d ( <i>J</i> 8.7)	7.08 d ( <i>J</i> 8.6)	6.49 d ( <i>J</i> 9.6)
4							8.02 d ( <i>J</i> 9.6)
5	7.08 d ( <i>J</i> 8.6)	7.06 d ( <i>J</i> 8.6)	7.05 d ( <i>J</i> 8.6)	7.06 d ( <i>J</i> 8.6)	7.07 d ( <i>J</i> 8.7)	7.08 d ( <i>J</i> 8.6)	7.21 d ( <i>J</i> 2.9)
6	7.37 d ( <i>J</i> 8.6)	7.26 d ( <i>J</i> 8.6)	7.05 d ( <i>J</i> 8.6)	7.29 d ( <i>J</i> 8.6)	7.28 d ( <i>J</i> 8.7)	7.30 d ( <i>J</i> 8.6)	
7	5.23 s	5.18 s	5.17 s	5.19 s	5.09 s	5.10 s	7.25 dd ( <i>J</i> 9.0; 2.9)
8							7.40 d ( <i>J</i> 9.0)
2'	7.43 d ( <i>J</i> 2.1)	7.43 d ( <i>J</i> 2.0)	7.43 d ( <i>J</i> 2.0)	7.43 d ( <i>J</i> 1.7)	7.40 d ( <i>J</i> 2.0)	7.39 d ( <i>J</i> 1.9)	7.48 d ( <i>J</i> 2.0)
5'	6.79 d ( <i>J</i> 8.4)	6.79 d ( <i>J</i> 8.1)	6.79 d ( <i>J</i> 8.2)	6.79 d ( <i>J</i> 8.0)	6.77 d ( <i>J</i> 8.2)	6.76 d ( <i>J</i> 8.2)	7.06 d ( <i>J</i> 8.5)
6'	7.41 dd ( <i>J</i> 8.4; 2.1)	7.41 dd ( <i>J</i> 8.1; 2.0)	7.41 dd ( <i>J</i> 8.2; 2.0)	7.41 dd ( <i>J</i> 8.0; 1.7)	7.37 dd ( <i>J</i> 8.2; 2.0)	7.36 dd ( <i>J</i> 8.2; 1.9)	7.69 dd ( <i>J</i> 8.5; 2.0)
1''	4.91 d ( <i>J</i> 7.4)	4.92 d ( <i>J</i> 7.4)	4.93 d ( <i>J</i> 7.4)	4.90 d ( <i>J</i> 7.4)	4.90 d ( <i>J</i> 7.4)	4.93 d ( <i>J</i> 7.4)	
2''	3.46	3.50 m	3.50 m	3.49 m	3.50 m	3.47 m	
3''	3.47	3.51 m	3.51 m	3.49 m	3.51 m	3.49 m	
4''	3.39	3.45 m	3.44 m	3.45 m	3.43 m	3.45 m	
5''	3.64	3.77 m	3.78 m	3.76 m	3.76 m	3.73 m	
6''	4.39 m 4.24 m	4.63 m 4.38 m	4.68 m 4.40 m	4.60 m 4.40 m	4.52 m 4.38 m	4.51 m 4.40 m	
2'''	2.02 s	7.47 d ( <i>J</i> 2.0)	7.54 d ( <i>J</i> 1.8)	7.10 s	7.16 d ( <i>J</i> 1.8)	6.90 s	
5'''		6.83 d ( <i>J</i> 8.2)	6.87 d ( <i>J</i> 8.2)		6.79 d ( <i>J</i> 8.2)		
6'''		7.45 d ( <i>J</i> 8.2; 2.0)	7.59 dd ( <i>J</i> 8.2; 1.8)	7.10 s	7.04 dd ( <i>J</i> 8.2; 1.8)	6.90 s	
7'''					7.61 d ( <i>J</i> 16)	7.62 d ( <i>J</i> 15.9)	
8'''					6.37 d ( <i>J</i> 16.0)	6.42 d ( <i>J</i> 15.9)	
3'''-OMe			3.83 s		3.86 s	3.86 s	
5'''-OMe						3.86	

<sup>a</sup>spectral data in DMSO-d<sub>6</sub>.

successively extracted with  $\text{CHCl}_3$  and EtOAc. The EtOAc fraction (5.3 g) was subjected to CC over Sephadex LH-20 (MeOH 100%) affording 5 pooled fractions after TLC analysis. Fraction (3) (61.8 mg) was chromatographed using reverse phase cartridge (500 mg) and  $\text{H}_2\text{O}:\text{MeOH}$  (50:50) as eluent to give compound **4** (9.3 mg). Fraction (1) was applied to Sephadex LH-20 (MeOH 100%) to yield three sub-fractions. The sub-fraction (1) and (3) (1.6 g) was fractionated over silica gel 60 and eluted with

hexane:EtOAc mixtures (80:20, 75:25, 70:30, 60:40, 50:50, 25:75, 400 mL) to yield 7 sub-fractions. Sub-fraction (1), (3) and (4) (37.0 mg) was chromatographed by HPLC/PDA (semi-preparative Waters X-Terra RP-18 column), utilizing a mixture of  $\text{H}_3\text{PO}_4$  (pH 3.0) and MeOH as mobile phase, which varied from 20 to 60% of the latter ( $t_{\text{R}} = 7.56; 10.30$  and  $11.24$  min) corresponding to vanillic acid (14.3 mg, yellowish solid,

**Table 2.**  $^{13}\text{C}$  NMR data assignments for compounds **1-7** (125 MHz,  $\text{CD}_3\text{OD}$ ,  $J$  in Hz)

Position	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>	7 <sup>a</sup>
1	132.1	131.9	131.9	131.9	131.9	132.0	
2	130.8	130.9	130.7	130.9	130.9	130.8	160.0
3	118.0	118.0	117.9	117.9	118.0	118.1	116.8
4	159.0	158.4	158.4	159.0	158.9	159.0	143.8
5	118.0	118.0	117.9	117.9	118.0	118.1	114.6
6	130.8	130.9	130.7	130.9	130.9	130.8	153.6
7	67.2	67.2	67.2	67.3	67.2	67.1	121.3
8	-	-	-	-	-	-	117.7
9	-	-	-	-	-	-	149.0
10	-	-	-	-	-	-	119.5
1'	122.9	122.8	122.8	122.8	122.8	122.8	122.3
2'	117.6	117.6	117.6	117.6	117.6	117.6	122.3
3'	146.3	146.3	146.3	146.3	146.3	146.3	142.6
4'	151.9	151.9	151.4	151.9	151.9	151.9	153.5
5'	116.1	116.0	116.1	116.1	116.0	116.1	117.1
6'	123.9	123.9	123.9	123.9	123.9	123.8	127.4
7'	168.3	168.3	168.3	168.3	168.2	168.2	166.6
1''	102.3	102.3	102.4	102.4	102.3	102.4	-
2''	75.0	75.0	75.0	75.1	75.0	75.0	-
3''	78.0	78.1	78.1	78.1	78.1	78.1	-
4''	72.0	72.1	72.2	72.0	72.0	72.0	-
5''	75.6	75.8	75.8	75.8	75.7	75.7	-
6''	64.8	65.0	65.2	65.0	64.7	64.7	-
1'''	172.9	122.7	122.6	121.6	127.8	126.8	-
2'''	20.9	117.8	114.0	110.4	111.8	107.2	-
3'''	-	146.4	148.9	146.7	149.5	149.7	-
4'''	-	152.0	153.2	140.1	150.8	140.0	-
5'''	-	116.6	116.2	146.7	116.6	149.7	-
6'''	-	124.0	125.4	110.4	124.4	107.2	-
7'''	-	168.2	168.0	168.3	147.2	147.5	-
8'''	-	-	-	-	115.4	115.9	-
9'''	-	-	-	-	169.1	169.0	-
3'''-OMe	-	-	56.7	-	56.6	57.1	-
5'''-OMe	-	-	-	-	-	57.1	-

<sup>a</sup>Spectral data in  $\text{DMSO}-d_6$ .

mp 204.9-205.8 °C)<sup>10</sup>, 6-hydroxy-coumarin (5.0 mg, white powder, mp 221.7-222.9 °C)<sup>9,14</sup> and (*E*)-*o*-coumaric acid (9.7 mg, white powder, mp 213.6-215.9 °C).<sup>15</sup> Fraction (1), (3) and (5) (65.3 mg) was submitted to the same chromatographic process. The chromatogram at 254 nm showed 5 peaks ( $t_R$  = 6.65; 9.36; 11.06; 12.50 and 13.57 min) corresponding respectively to amburoside A (19.7 mg, mp 197.8-199.1 °C)<sup>10,13</sup>, amburoside D (**1**, 3.4 mg), amburoside E (**2**, 7.8 mg), amburoside F (**5**, 13.1 mg), and a mixture of amburoside G (**3**, 5.7 mg) and amburoside H (**6**, 4.0 mg), which was posteriorly reinjected and purified.

The known compounds were identified by <sup>1</sup>H and <sup>13</sup>C NMR, in addition to comparison with literature data. Furthermore, 6-hydroxycoumarin ( $t_R$  = 11.7 min) and an authentic sample of umbelliferone ( $t_R$  = 13.1 min) were compared by HPLC (analytical Waters X-Terra RP-18 column), using the same elution gradient, but changing the running time (20 min) and flow rate (1.0 mL min<sup>-1</sup>).

#### Amburoside D (**1**)

Brown solid, mp 110.2-114.3 °C; [ $\alpha$ ]<sub>D</sub><sup>20</sup>: -34° (*c* 0.22, MeOH); IR (KBr)  $\nu_{\max}$ /cm<sup>-1</sup>: 3419 (O-H), 1700 (C=O), 1618, 1513, 1445, 1376, 1291, 1227 and 1077 (C-OH and C-O-C). HRESIMS (positive ion mode)  $m/z$  487.1302 [M+Na]<sup>+</sup>, (calc. for C<sub>22</sub>H<sub>24</sub>O<sub>11</sub>Na, 487.1216). <sup>1</sup>H (500 MHz, CD<sub>3</sub>OD) and <sup>13</sup>C NMR (125 MHz, CD<sub>3</sub>OD), see Tables 1 and 2.

#### Amburoside E (**2**)

Brown solid, mp 126.1-129.0 °C; [ $\alpha$ ]<sub>D</sub><sup>20</sup>: -26° (*c* 0.12, MeOH); IR (KBr)  $\nu_{\max}$ /cm<sup>-1</sup>: 3407 (O-H), 1691 (C=O), 1609, 1516, 1445, 1376, 1297, 1228, 1073 (C-OH and C-O-C) and 763. HRESIMS (positive ion mode)  $m/z$  581.1286 [M+Na]<sup>+</sup>, (calc. for C<sub>27</sub>H<sub>26</sub>O<sub>13</sub>Na, 581.1271); <sup>1</sup>H (500 MHz, CD<sub>3</sub>OD) and <sup>13</sup>C NMR (125 MHz, CD<sub>3</sub>OD) see Tables 1 and 2.

#### Amburoside G (**3**)

Brown solid; mp 102.6-106.9 °C; [ $\alpha$ ]<sub>D</sub><sup>20</sup>: -27° (*c* 0.22, MeOH); IR (KBr)  $\nu_{\max}$ /cm<sup>-1</sup>: 3427 (O-H), 1691 (C=O), 1607, 1515, 1455, 1381, 1295, 1230, 1071 (C-OH and C-O-C) and 764. HRESIMS (negative ion mode)  $m/z$  571.1487 [M-H]<sup>-</sup>, (calc. for C<sub>28</sub>H<sub>27</sub>O<sub>13</sub>, 571.1481); <sup>1</sup>H (500 MHz, CD<sub>3</sub>OD) and <sup>13</sup>C NMR (125 MHz, CD<sub>3</sub>OD) see Tables 1 and 2.

#### Amburoside C (**4**)

Brown solid; mp 193.2-197.8 °C; [ $\alpha$ ]<sub>D</sub><sup>20</sup>: -44° (*c* 0.19, MeOH); IR (KBr)  $\nu_{\max}$ /cm<sup>-1</sup>: 3403 (O-H), 1700 (C=O), 1653, 1609, 1509, 1450, 1369, 1295, 1226 and 1072 (C-OH and C-O-C). HRESIMS (positive ion mode)

$m/z$  597.1256 [M+Na]<sup>+</sup>, (calc. for C<sub>27</sub>H<sub>26</sub>O<sub>14</sub>Na, 597.1220); <sup>1</sup>H (500 MHz, CD<sub>3</sub>OD) and <sup>13</sup>C NMR (125 MHz, CD<sub>3</sub>OD) (see Tables 1 and 2).

#### Amburoside F (**5**)

Brown solid; mp 108.7-112.2 °C; [ $\alpha$ ]<sub>D</sub><sup>20</sup>: -33° (*c* 0.60, MeOH). IR (KBr)  $\nu_{\max}$ /cm<sup>-1</sup>: 3410 (O-H), 1689 (C=O), 1604, 1514, 1447, 1378, 1295, 1227, 1120, 1076 (C-OH and C-O-C) and 765 HRESIMS (positive ion mode)  $m/z$  621.1626 [M+Na]<sup>+</sup> (calc. for C<sub>30</sub>H<sub>30</sub>O<sub>14</sub>Na, 621.1584); <sup>1</sup>H (500 MHz, CD<sub>3</sub>OD) and <sup>13</sup>C NMR (125 MHz, CD<sub>3</sub>OD) see Tables 1 and 2.

#### Amburoside H (**6**)

Brown solid; mp 126.9-131.4 °C; [ $\alpha$ ]<sub>D</sub><sup>20</sup>: -27° (*c* 0.20, MeOH). IR (KBr)  $\nu_{\max}$ /cm<sup>-1</sup>: 3428 (O-H), 1700 (C=O), 1653, 1610, 1515, 1455, 1225, 1114, 1077 (C-OH and C-O-C) and 824. HRESIMS (negative ion mode)  $m/z$  627.1766 [M-H]<sup>-</sup> (calc. for C<sub>31</sub>H<sub>30</sub>O<sub>14</sub>, 627.1716); <sup>1</sup>H (500 MHz, CD<sub>3</sub>OD) and <sup>13</sup>C NMR (125 MHz, CD<sub>3</sub>OD) see Tables 1 and 2.

#### 6-coumaryl protocatechuate (**7**)

Red solid (mp 257.6-258.3 °C); IR (KBr)  $\nu_{\max}$ /cm<sup>-1</sup>: 3315 (O-H), 1699 (C=O), 1609, 1565, 1519, 1440, 1305, 1259, 1224 and 1179 (-C=O and O=C-O-); HRESIMS (positive ion mode)  $m/z$  299.0550 [M+H]<sup>+</sup> (calc. for C<sub>16</sub>H<sub>11</sub>O<sub>6</sub>, 299.0552); <sup>1</sup>H (500 MHz, DMSO-*d*<sub>6</sub>) and <sup>13</sup>C NMR (125 MHz, DMSO-*d*<sub>6</sub>) see Tables 1 and 2.

## Supplementary Information

Supplementary data (IR, HRMS, <sup>1</sup>H and <sup>13</sup>C NMR spectra of compounds **1-7**) are available free of charge at <http://jbcs.sbq.org.br>, as PDF file.

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## Amburosidés C-H and 6-O-protocatechuoyl Coumarin from *Amburana cearensis*

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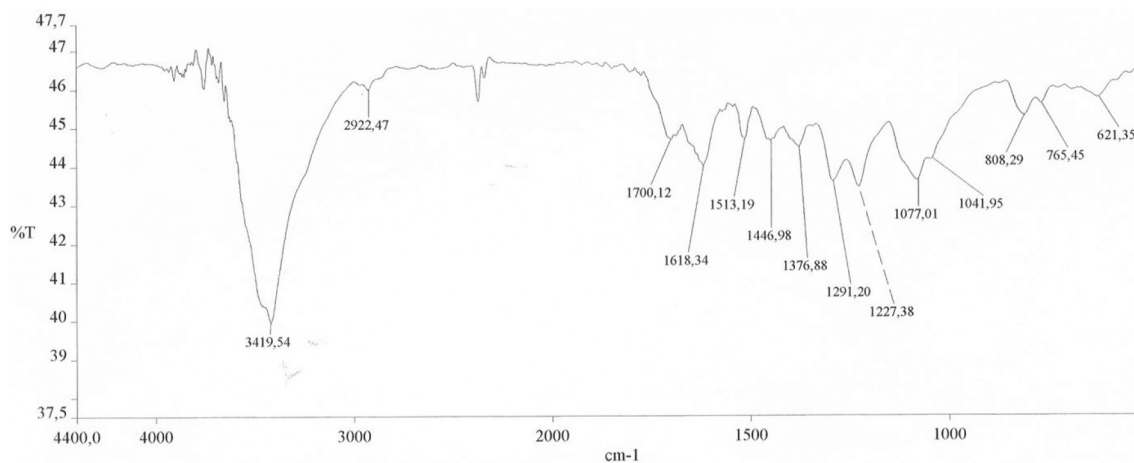


Figure S1. Infrared spectrum of compound 1 (KBr pellets).

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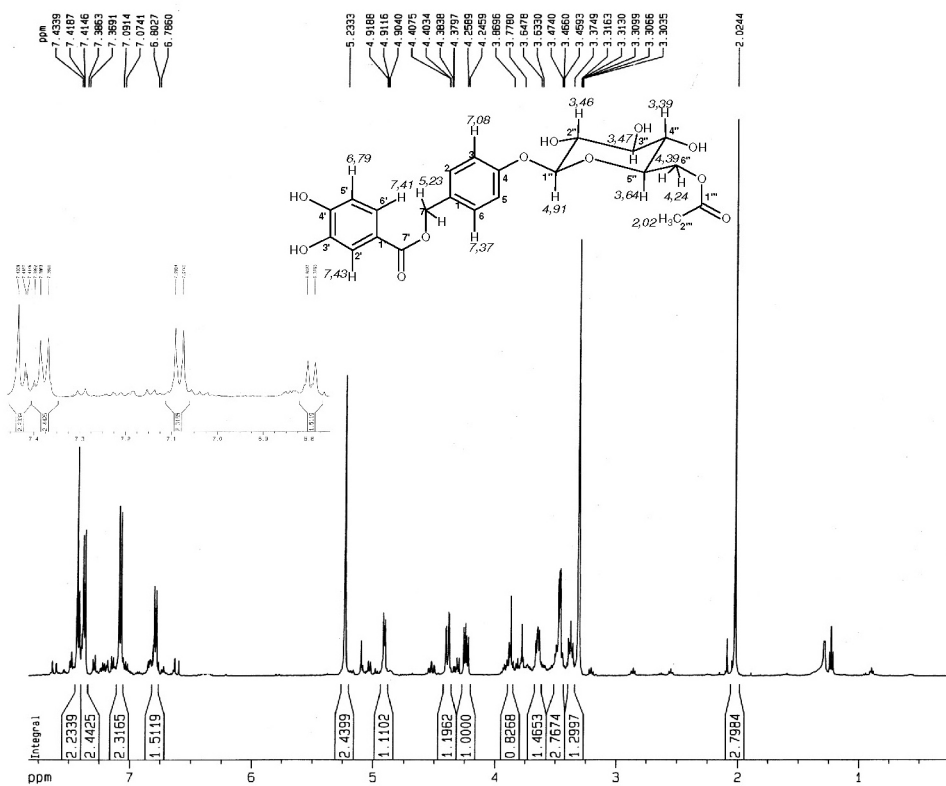


Figure S2.  $^1\text{H}$  NMR spectrum of **1** ( $\text{CD}_3\text{OD}$ , 500 MHz).

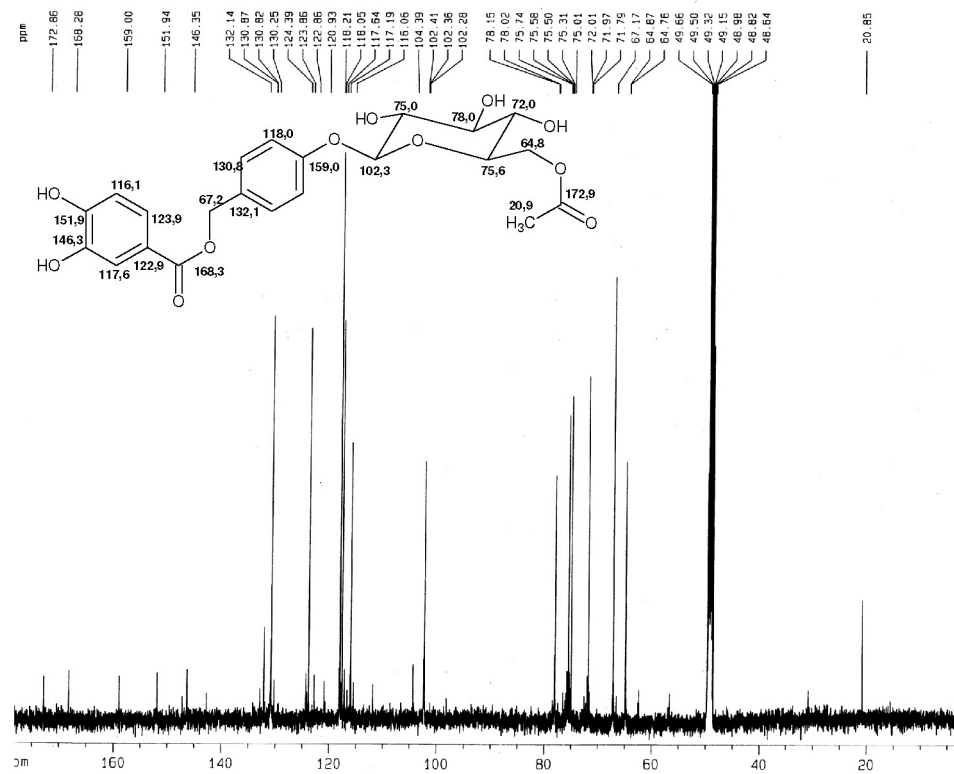


Figure S3.  $^{13}\text{C}$  NMR spectrum of **1** ( $\text{CD}_3\text{OD}$ , 125 MHz).

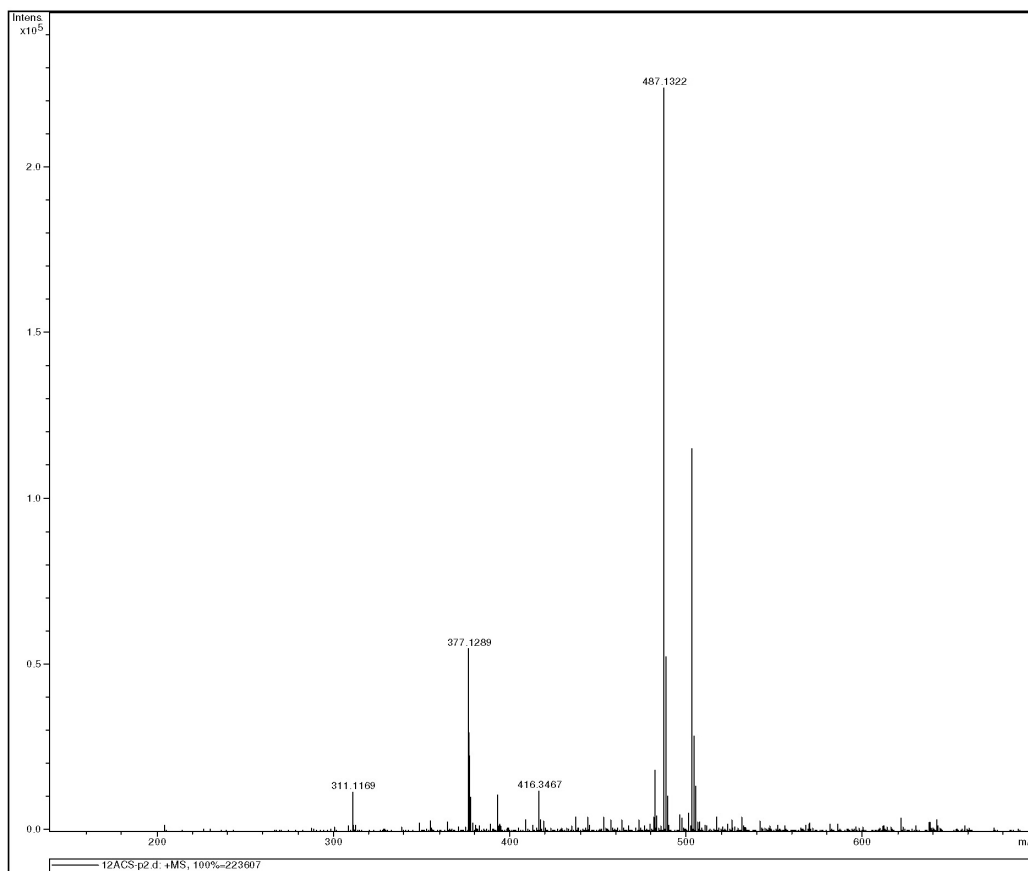


Figure S4. High resolution electrospray ionization mass spectrum of **1**.

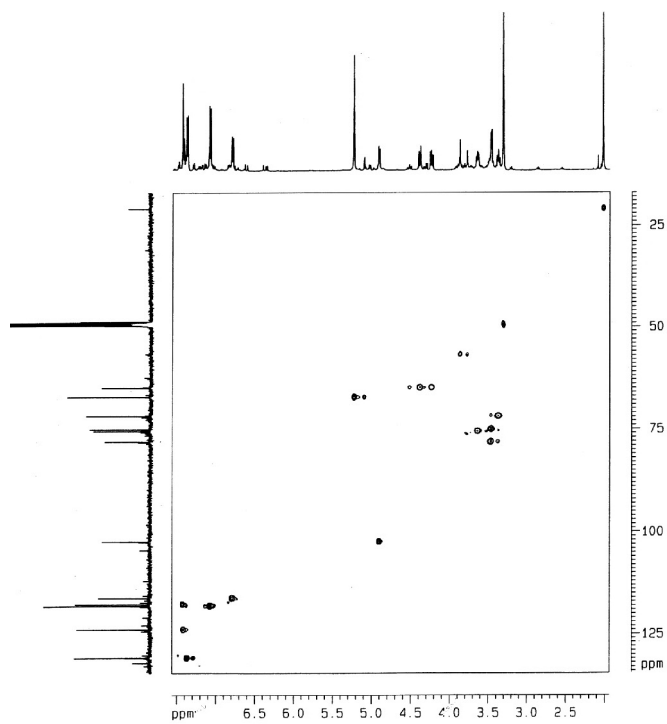


Figure S5. <sup>1</sup>H, <sup>13</sup>C HSQC-NMR spectrum of **1** (CD<sub>3</sub>OD, 500 × 125 MHz).

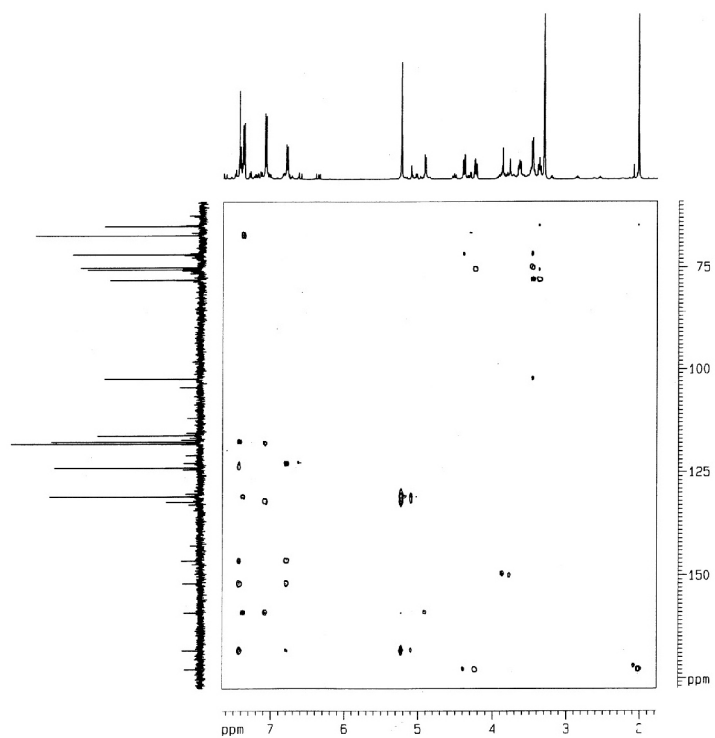


Figure S6.  $^1\text{H}$ ,  $^{13}\text{C}$  HMBC-NMR spectrum of **1** ( $\text{CD}_3\text{OD}$ ,  $500 \times 125$  MHz).

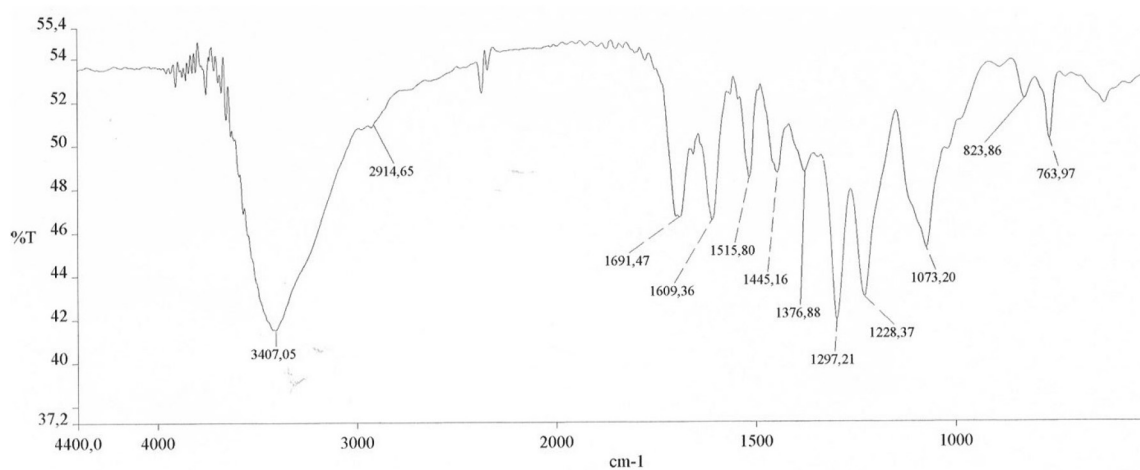


Figure S7. Infrared spectrum of compound **2** (KBr pellets).

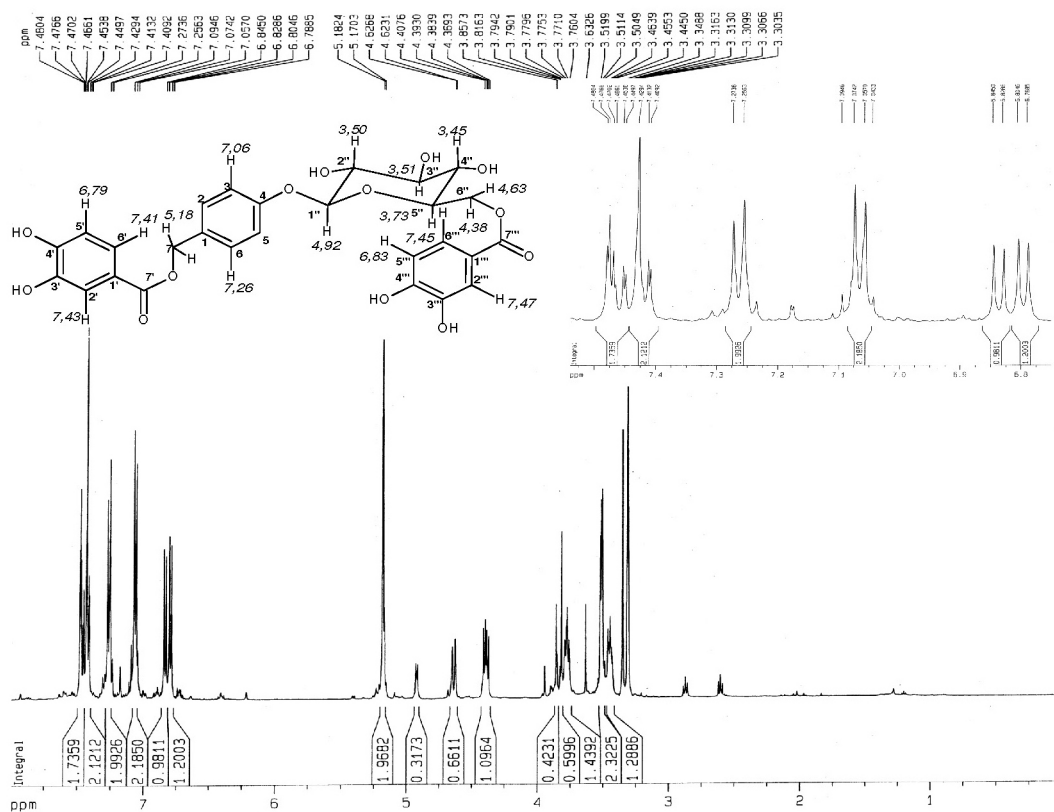


Figure S8.  $^1\text{H}$  NMR spectrum of 2 ( $\text{CD}_3\text{OD}$ , 500 MHz).

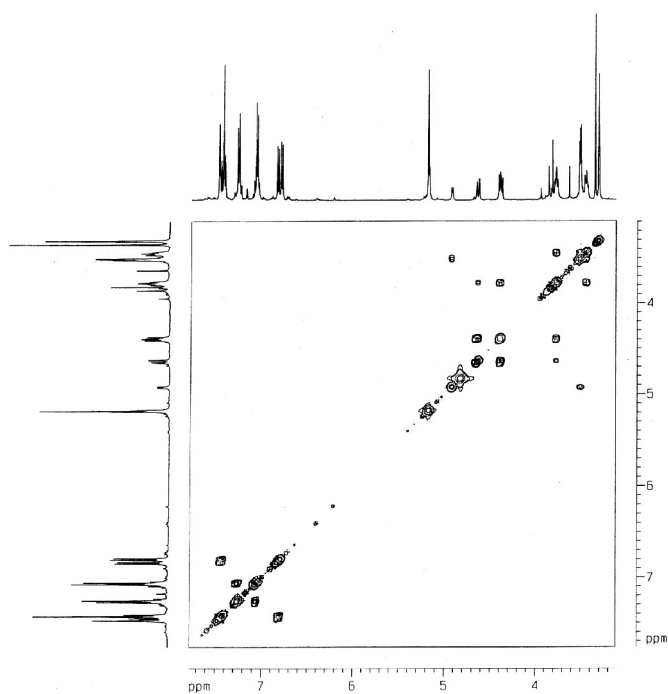


Figure S9.  $^1\text{H}$ ,  $^1\text{H}$  COSY-NMR spectrum of 2 ( $\text{CD}_3\text{OD}$ , 500  $\times$  500 MHz).

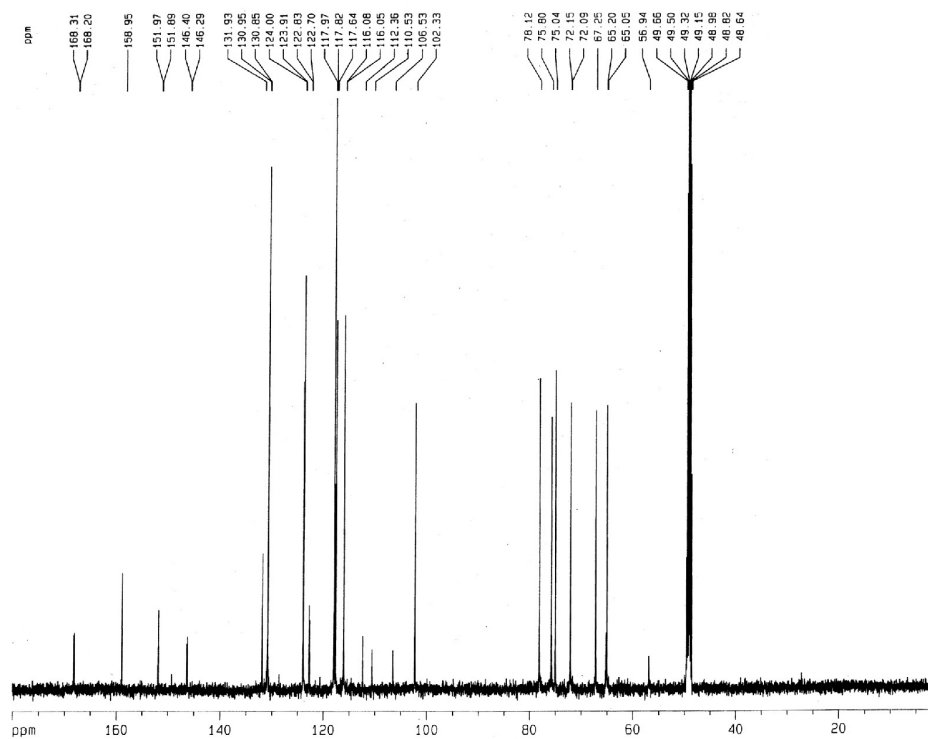


Figure S10.  $^{13}\text{C}$  NMR spectrum of **2** ( $\text{CD}_3\text{OD}$ , 125 MHz).

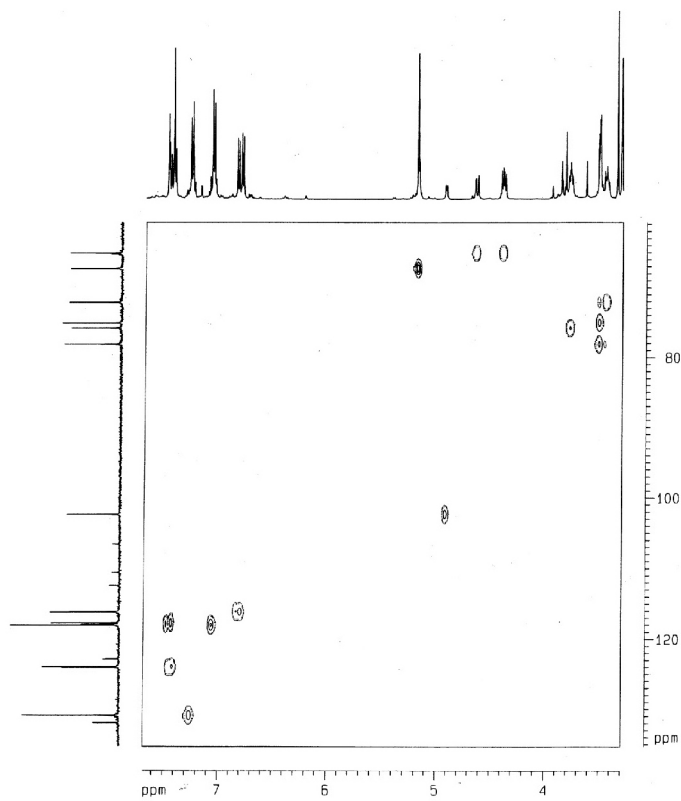
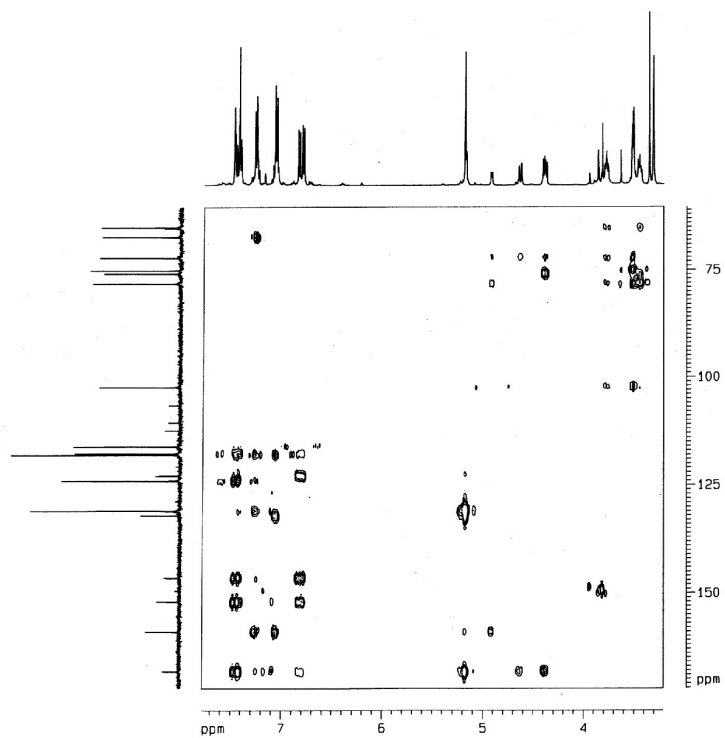
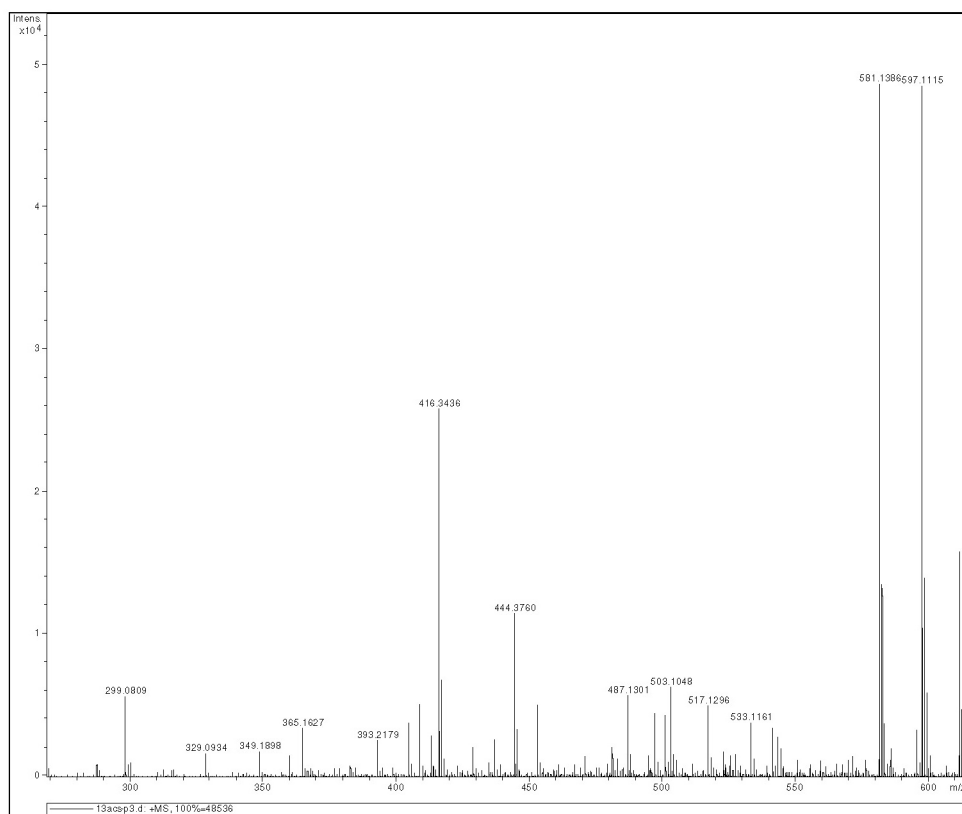


Figure S11.  $^1\text{H}$ ,  $^{13}\text{C}$  HSQC-NMR spectrum of **2** ( $\text{CD}_3\text{OD}$ , 500  $\times$  125 MHz).



**Figure S12.**  $^1\text{H}$ ,  $^{13}\text{C}$  HMBC-NMR spectrum of **2** ( $\text{CD}_3\text{OD}$ ,  $500 \times 125$  MHz).



**Figure S13.** High resolution electrospray ionization mass spectrum of **2**.

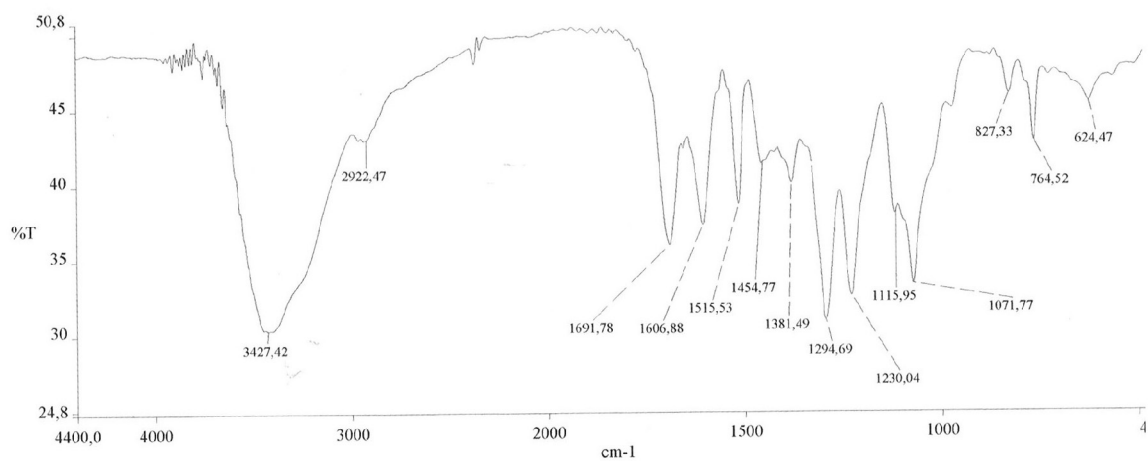


Figure S14. Infrared spectrum of compound **3** (KBr pellets).

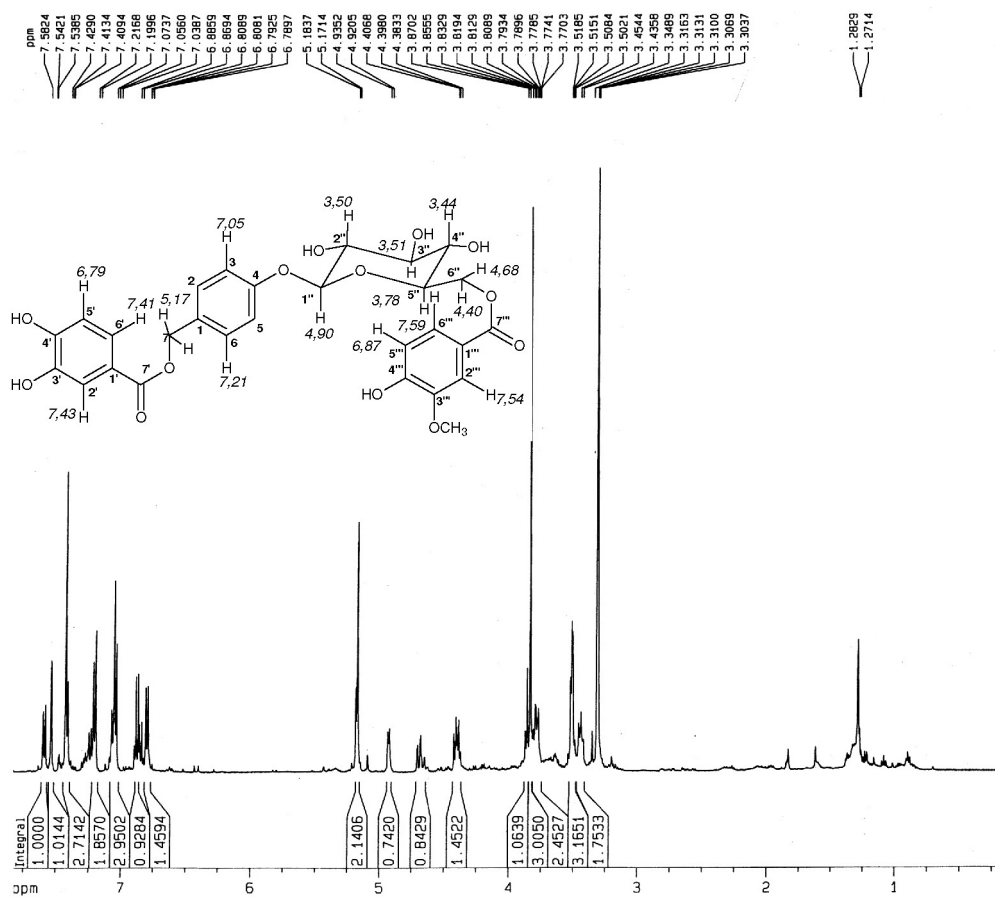


Figure S15.  $^1\text{H}$  NMR spectrum of **3** ( $\text{CD}_3\text{OD}$ , 500 MHz).



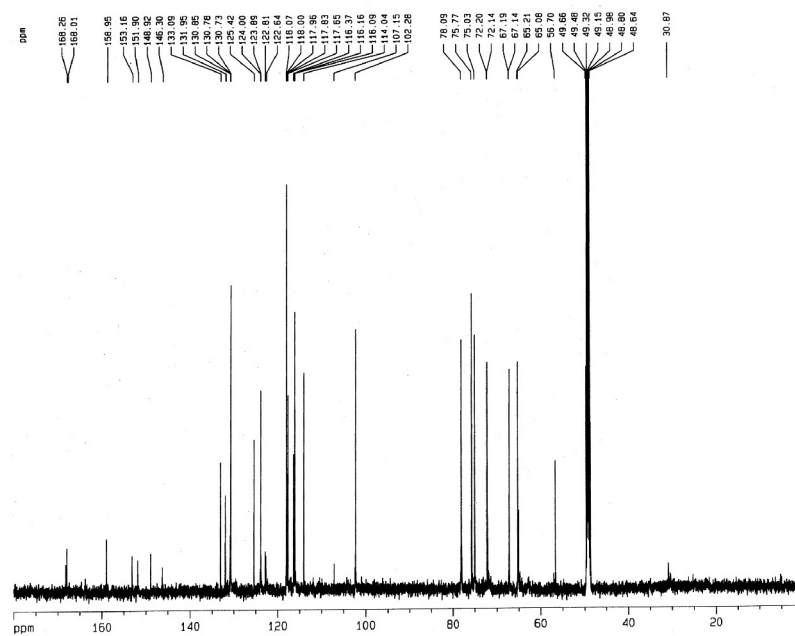


Figure S16.  $^{13}\text{C}$  NMR spectrum of **3** ( $\text{CD}_3\text{OD}$ , 125 MHz).

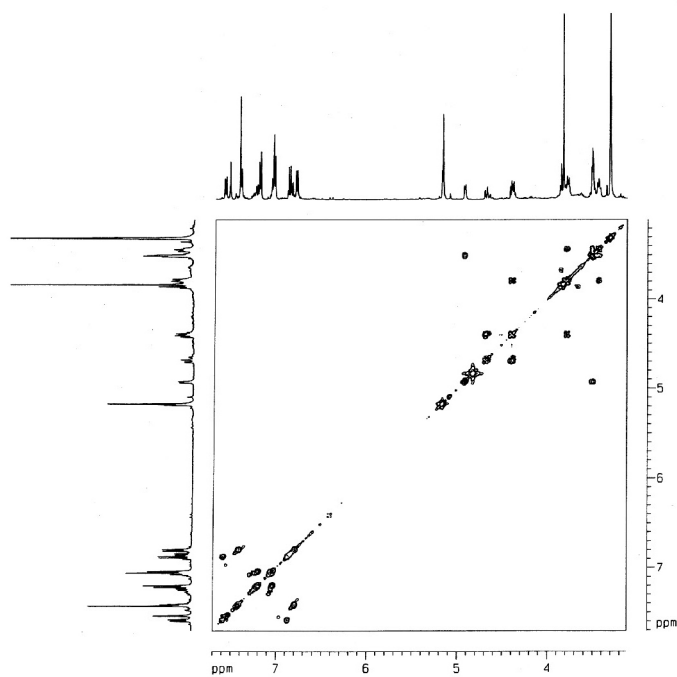


Figure S17.  $^1\text{H}$ ,  $^1\text{H}$  COSY-NMR spectrum of **3** ( $\text{CD}_3\text{OD}$ , 500  $\times$  500 MHz).

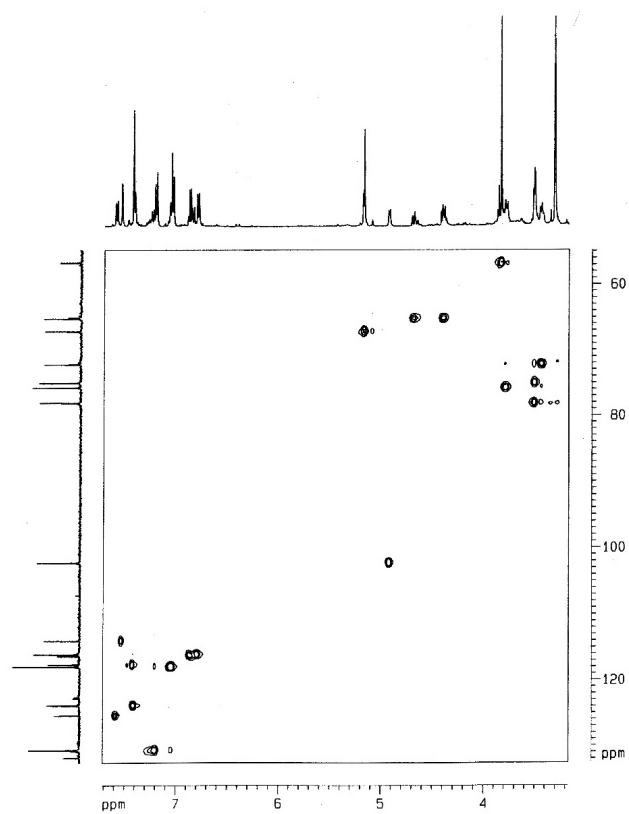


Figure S18.  $^1\text{H}$ ,  $^{13}\text{C}$  HSQC-NMR spectrum of 3 ( $\text{CD}_3\text{OD}$ ,  $500 \times 125$  MHz).

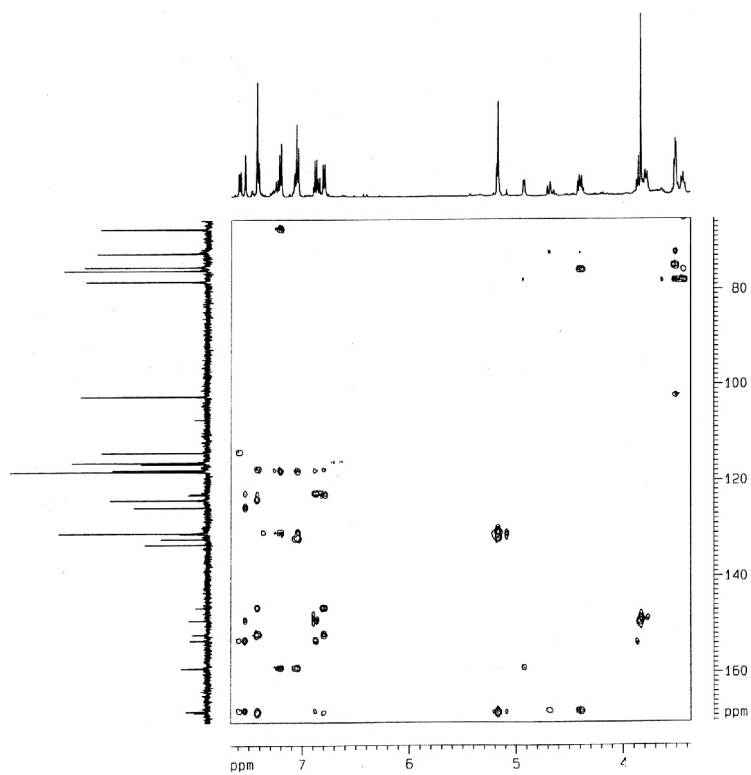
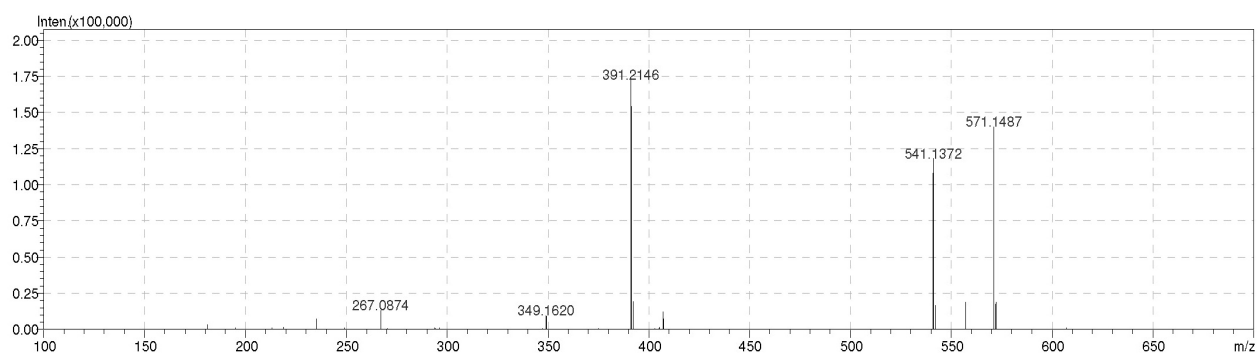
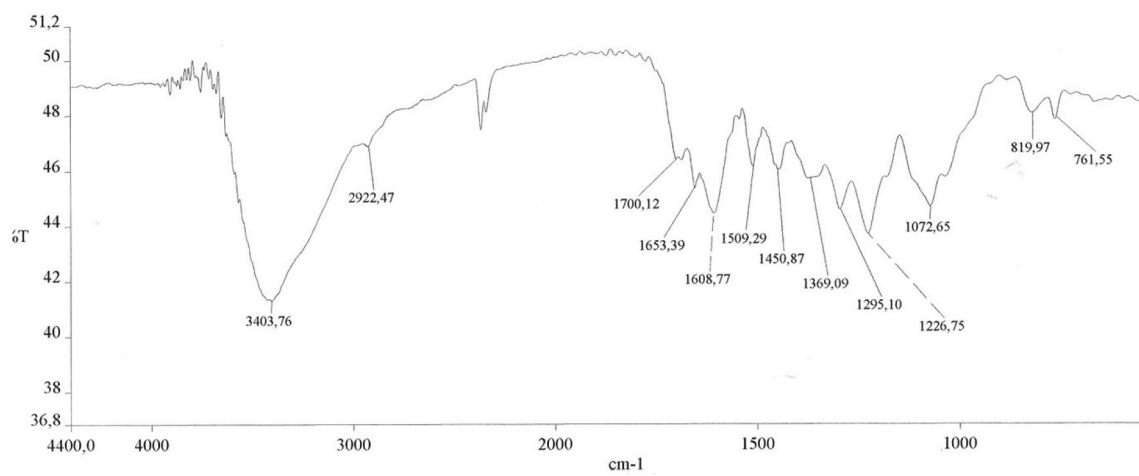


Figure S19.  $^1\text{H}$ ,  $^{13}\text{C}$  HMBC-NMR spectrum of 3 ( $\text{CD}_3\text{OD}$ ,  $500 \times 125$  MHz).



**Figure S20.** High resolution electrospray ionization mass spectrum of **3**.



**Figure S21.** Infrared spectrum of compound **4** (KBr pellets).

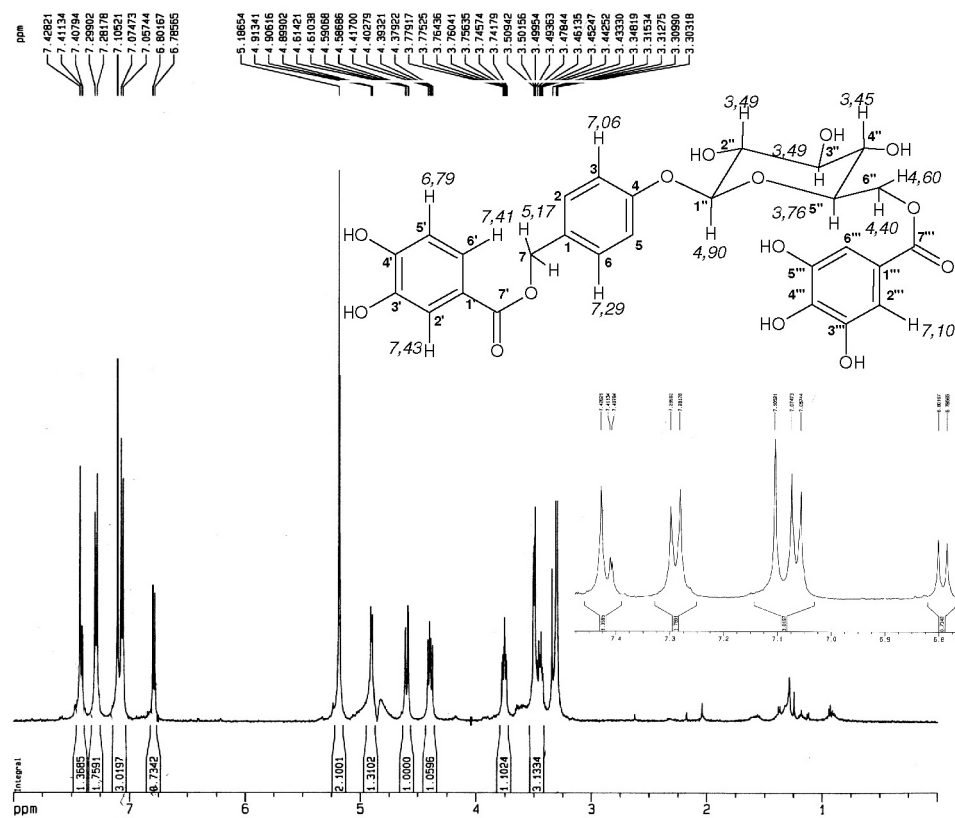


Figure S22.  $^1\text{H}$  NMR spectrum of **4** ( $\text{CD}_3\text{OD}$ , 500 MHz).

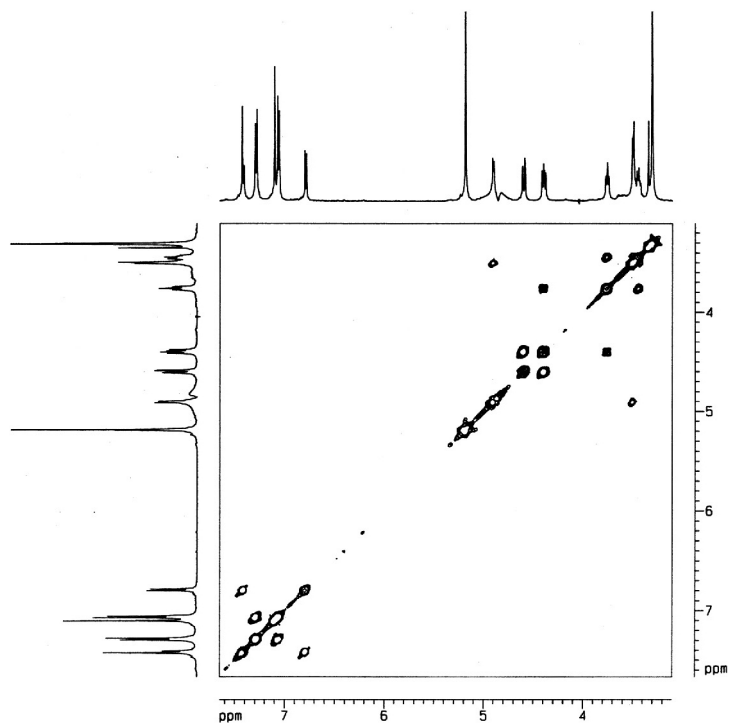


Figure S23.  $^1\text{H}$ ,  $^1\text{H}$  COSY-NMR spectrum of **4** ( $\text{CD}_3\text{OD}$ , 500  $\times$  500 MHz).

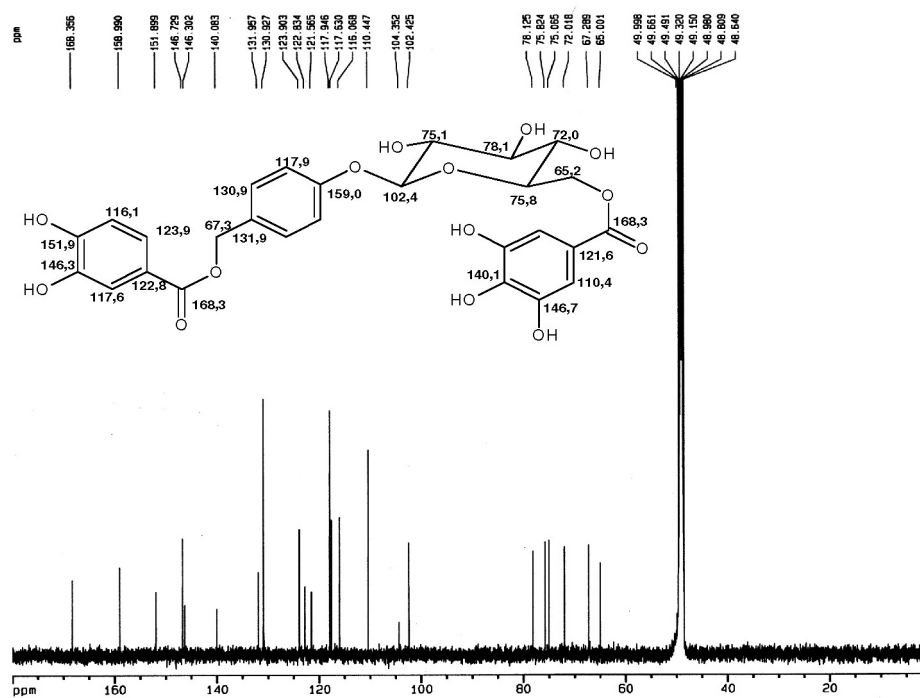


Figure S24.  $^{13}\text{C}$  NMR spectrum of 4 ( $\text{CD}_3\text{OD}$ , 125 MHz).

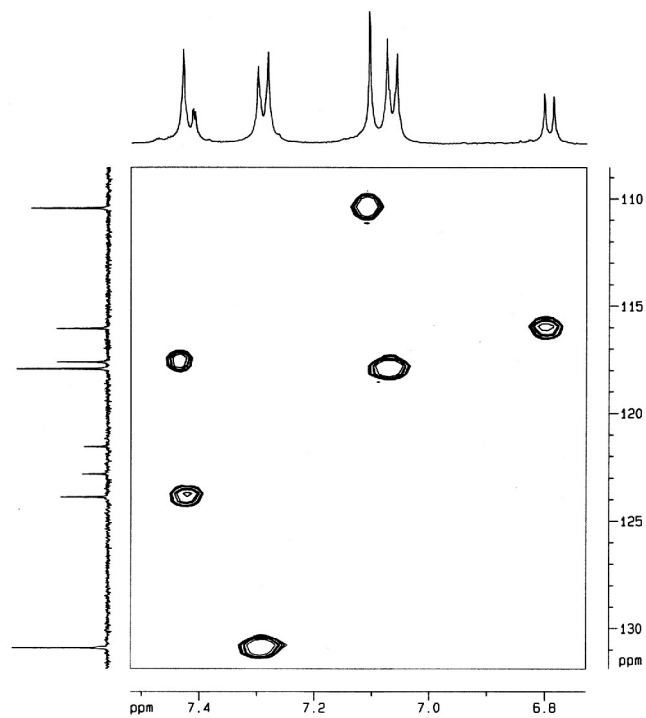


Figure S25.  $^1\text{H}$ ,  $^{13}\text{C}$  HSQC-NMR spectrum of 4 ( $\text{CD}_3\text{OD}$ , 500  $\times$  125 MHz).

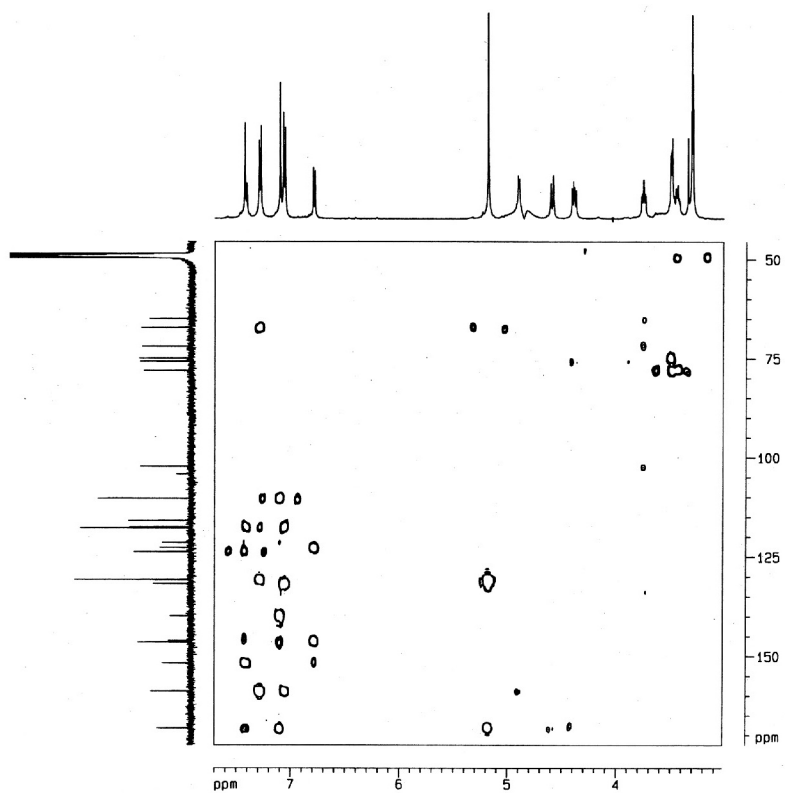


Figure S26.  $^1\text{H}$ ,  $^{13}\text{C}$  HMBC-NMR spectrum of **4** ( $\text{CD}_3\text{OD}$ ,  $500 \times 125$  MHz).

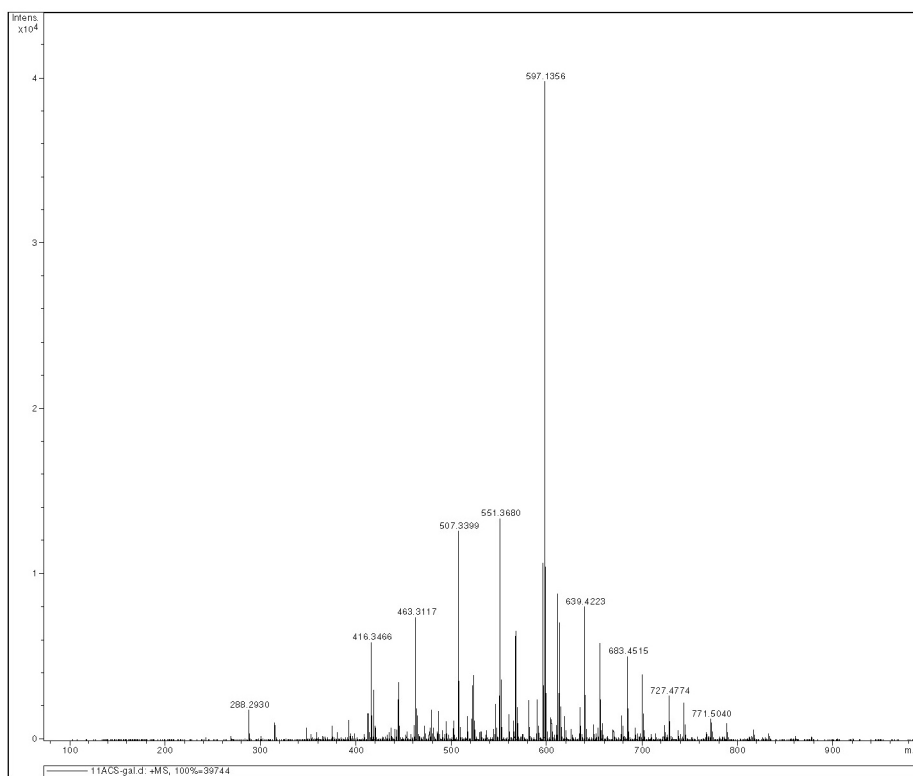


Figure S27. High resolution electrospray ionization mass spectrum of **4**.

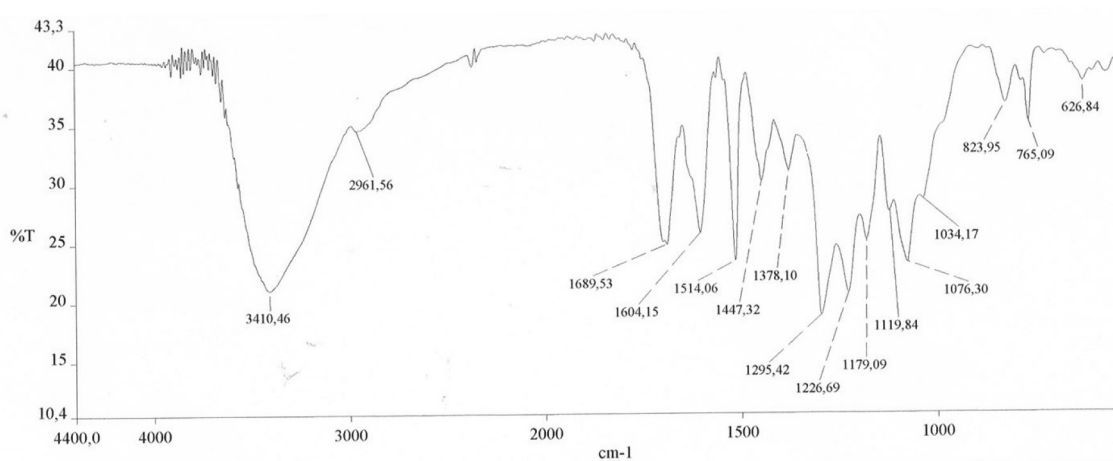


Figure S28. Infrared spectrum of compound **5** (KBr pellets).

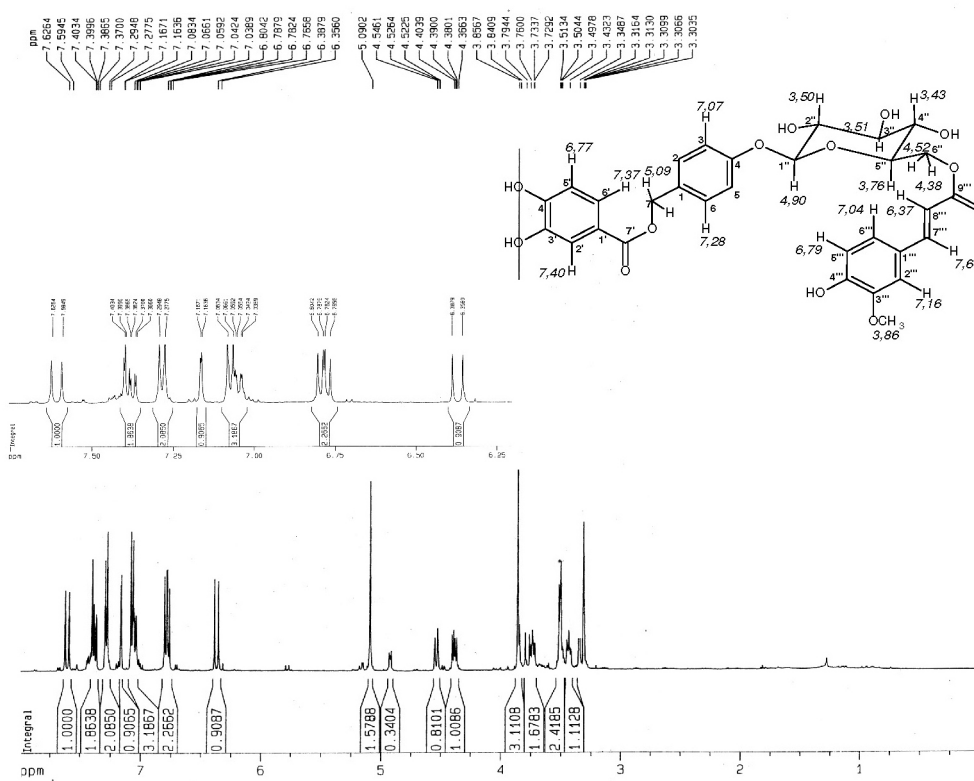


Figure S29.  $^1\text{H}$  NMR spectrum of **5** ( $\text{CD}_3\text{OD}$ , 500 MHz).

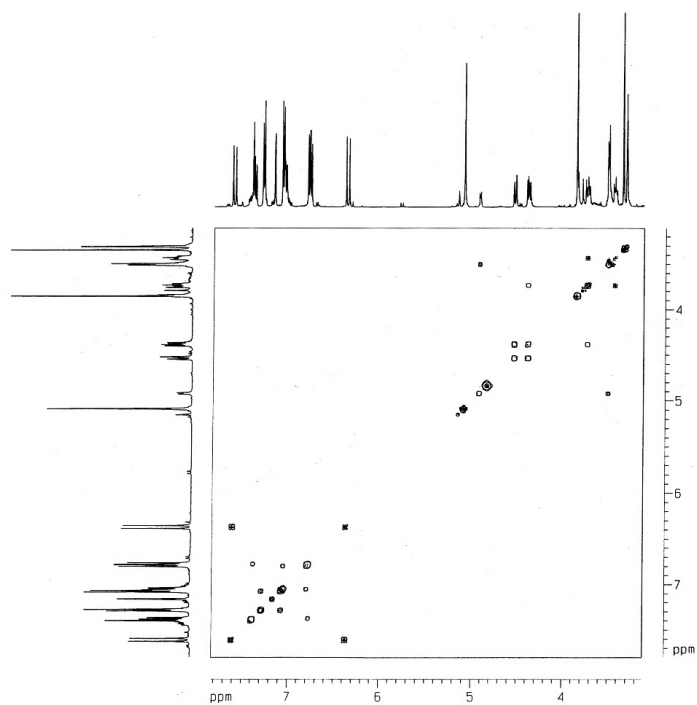


Figure S30.  $^1\text{H}$ ,  $^1\text{H}$  COSY-NMR spectrum of **5** ( $\text{CD}_3\text{OD}$ ,  $500 \times 500$  MHz).

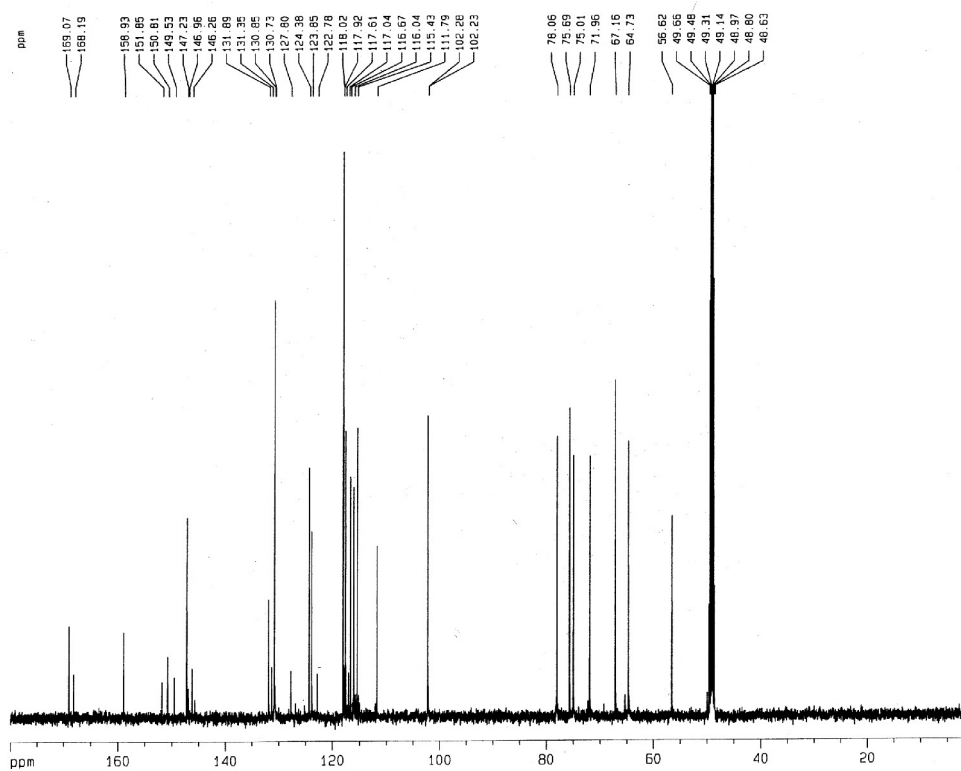
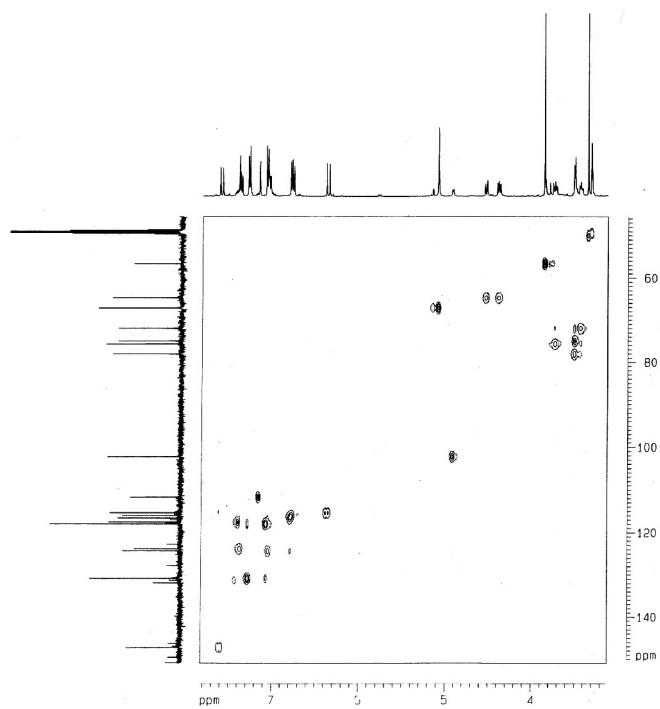
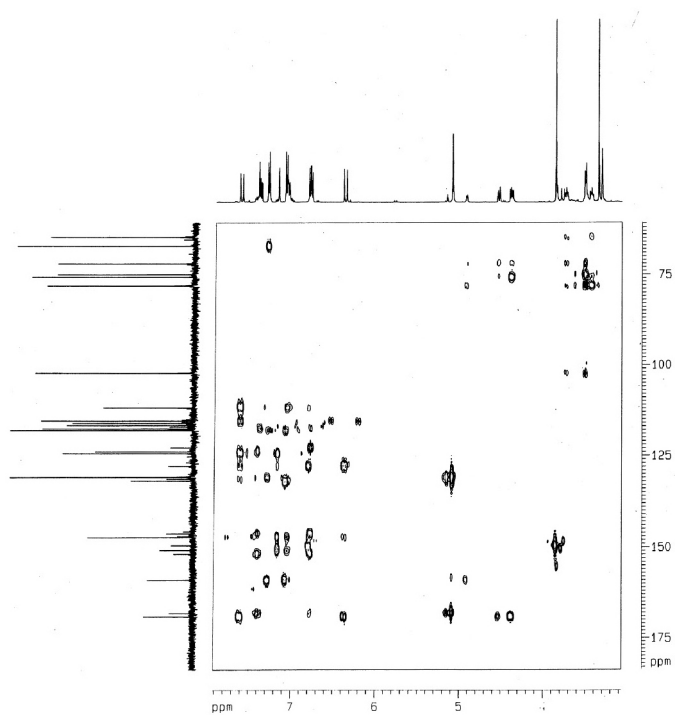


Figure S31.  $^{13}\text{C}$  NMR spectrum of **5** ( $\text{CD}_3\text{OD}$ , 125 MHz).





**Figure S32.**  $^1\text{H}$ ,  $^{13}\text{C}$  HSQC-NMR spectrum of **5** ( $\text{CD}_3\text{OD}$ ,  $500 \times 125$  MHz).



**Figure S33.**  $^1\text{H}$ ,  $^{13}\text{C}$  HMBC-NMR spectrum of **5** ( $\text{CD}_3\text{OD}$ ,  $500 \times 125$  MHz).

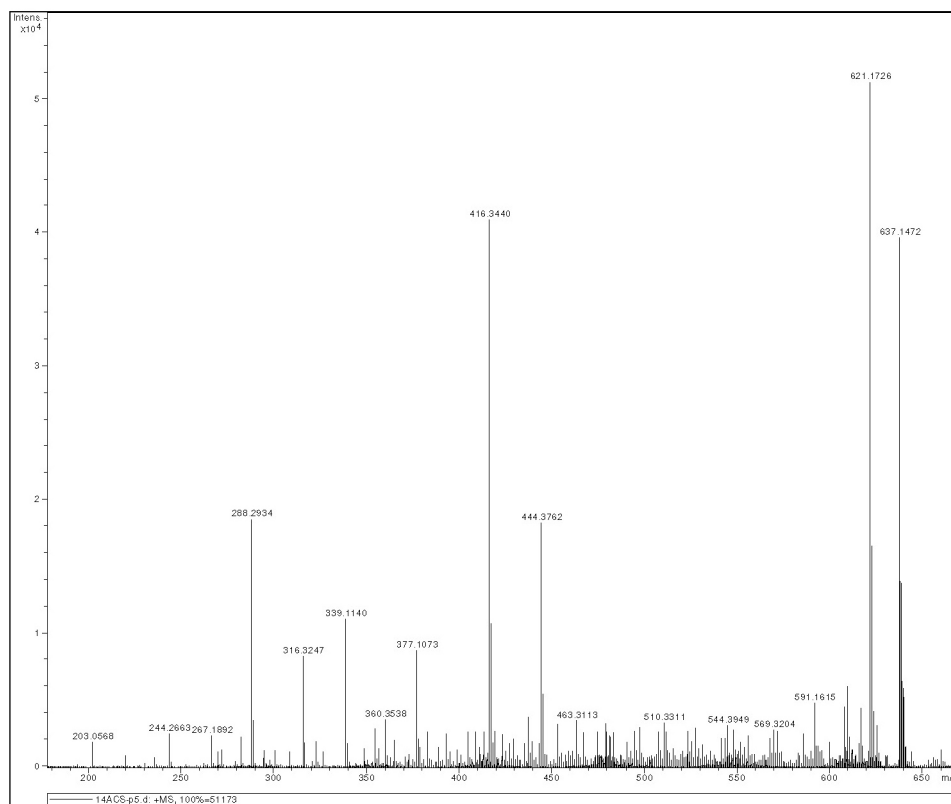


Figure S34. High resolution electrospray ionization mass spectrum of **5**.

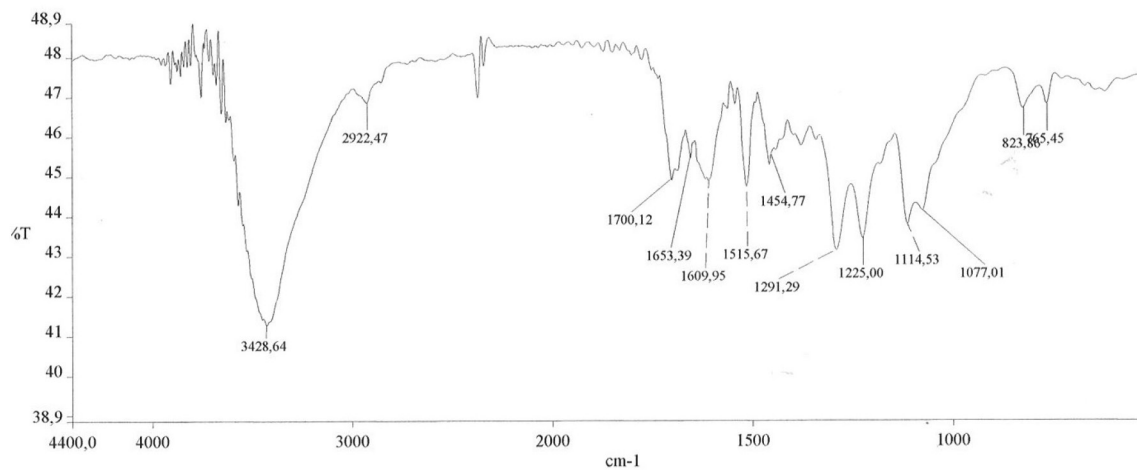


Figure S35. Infrared spectrum of compound **6** (KBr pellets).

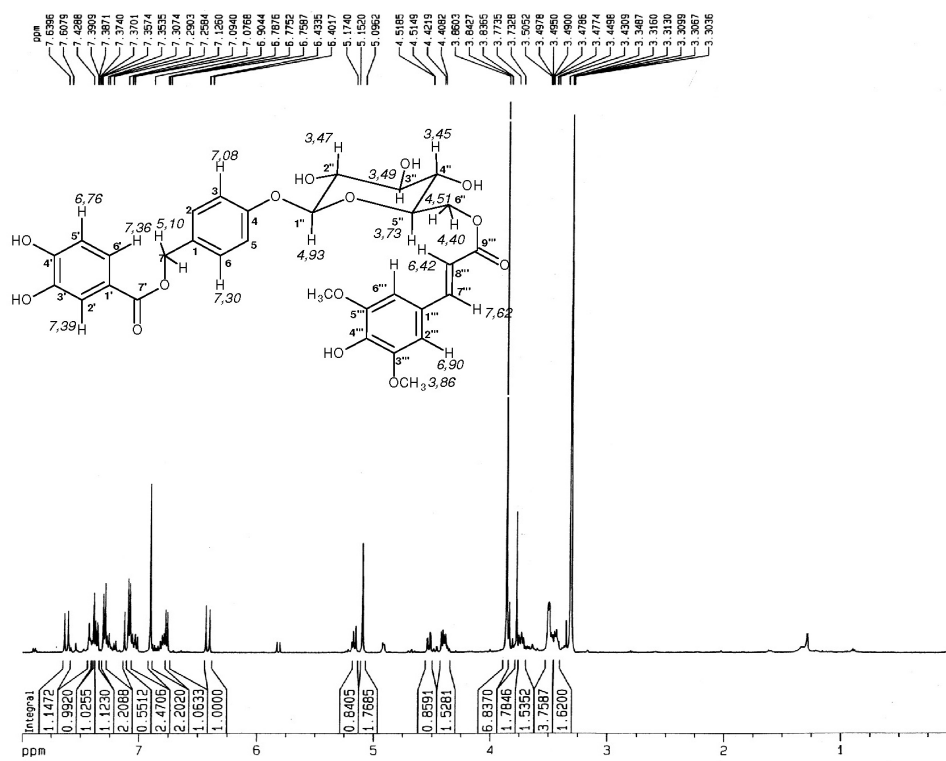


Figure S36.  $^1\text{H}$  NMR spectrum of **6** ( $\text{CD}_3\text{OD}$ , 500 MHz).

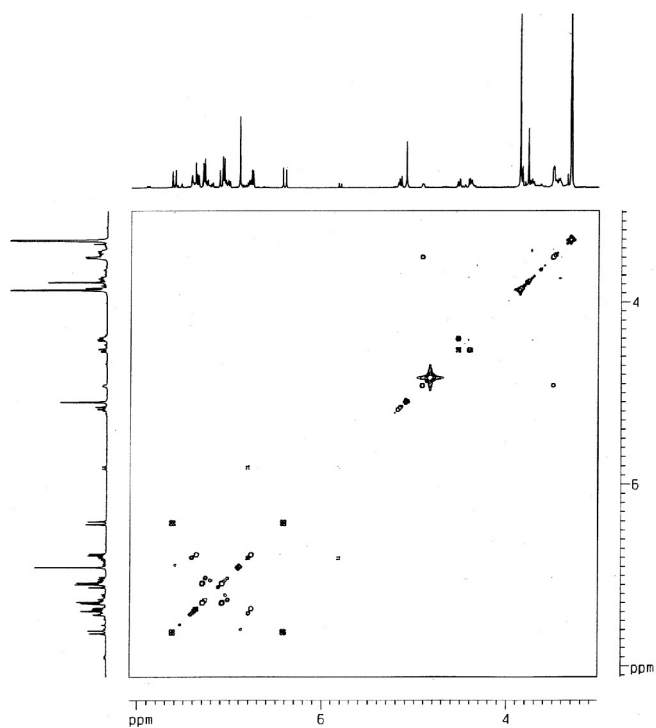


Figure S37.  $^1\text{H}$ ,  $^1\text{H}$  COSY-NMR spectrum of **6** ( $\text{CD}_3\text{OD}$ , 500  $\times$  500 MHz).

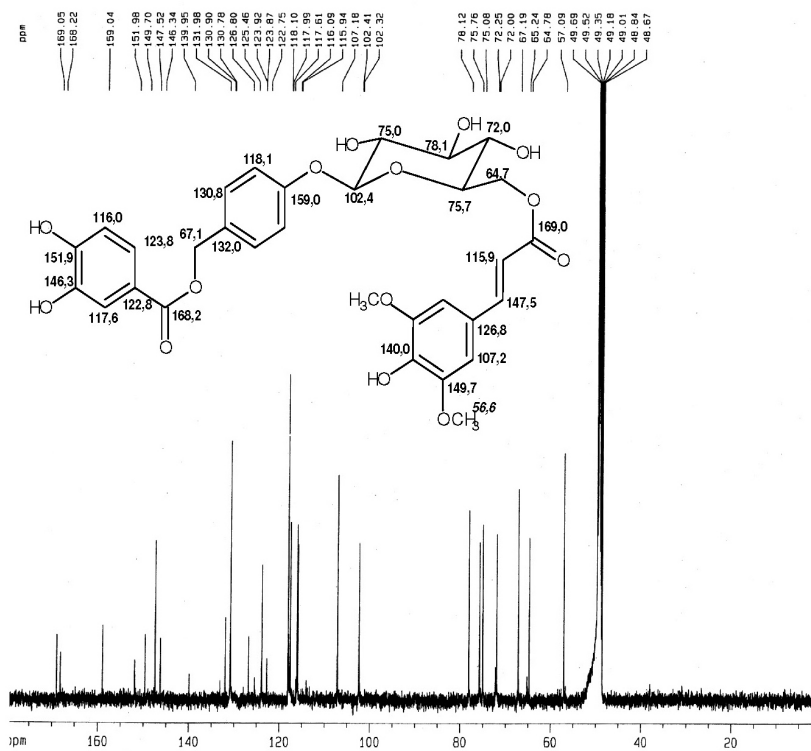


Figure S38.  $^{13}\text{C}$  NMR spectrum of **6** ( $\text{CD}_3\text{OD}$ , 125 MHz).

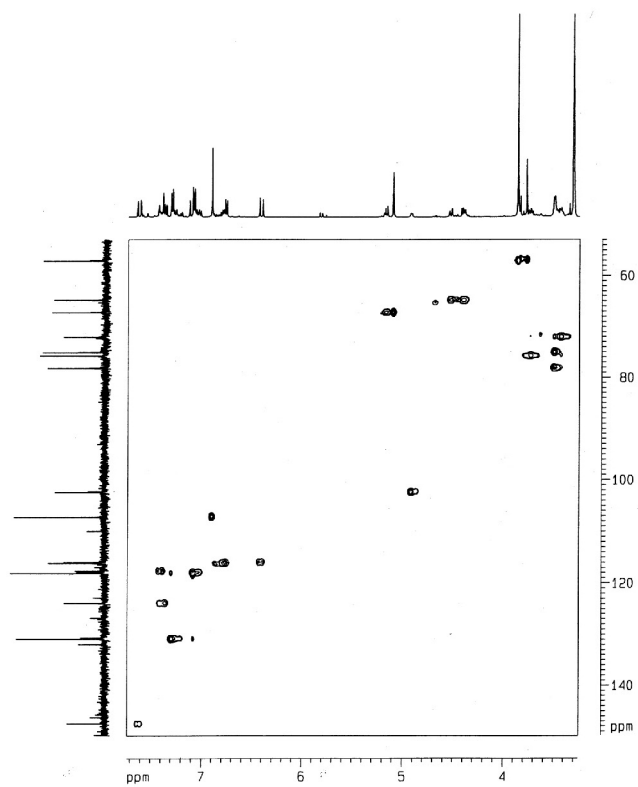
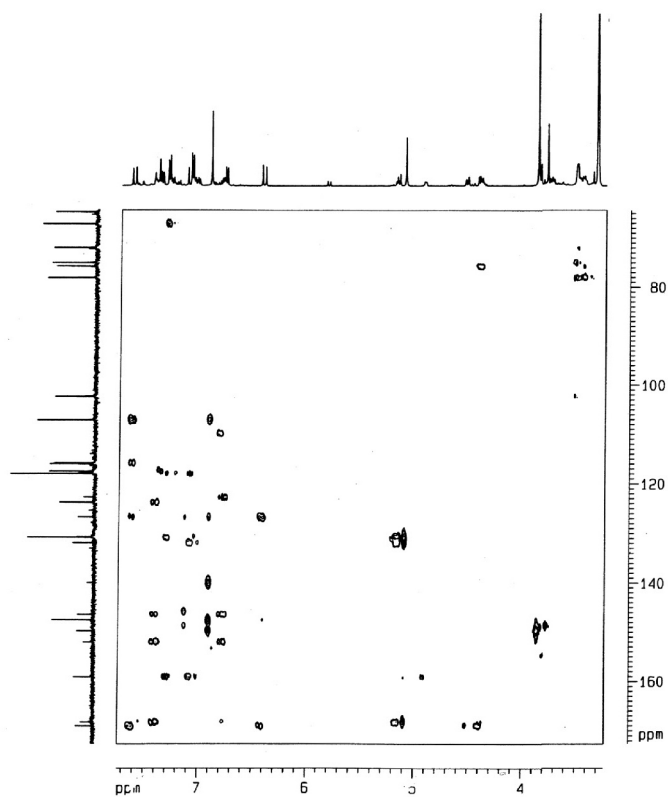
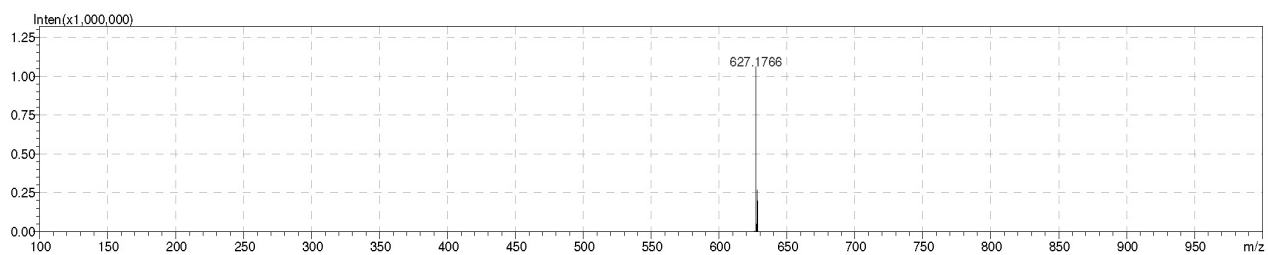


Figure S39.  $^1\text{H}$ ,  $^{13}\text{C}$  HSQC-NMR spectrum of **6** ( $\text{CD}_3\text{OD}$ , 500  $\times$  125 MHz).



**Figure S40.** <sup>1</sup>H, <sup>13</sup>C HMBC-NMR spectrum of **6** (CD<sub>3</sub>OD, 500 × 125 MHz).



**Figure S41.** High resolution electrospray ionization mass spectrum of **6**.

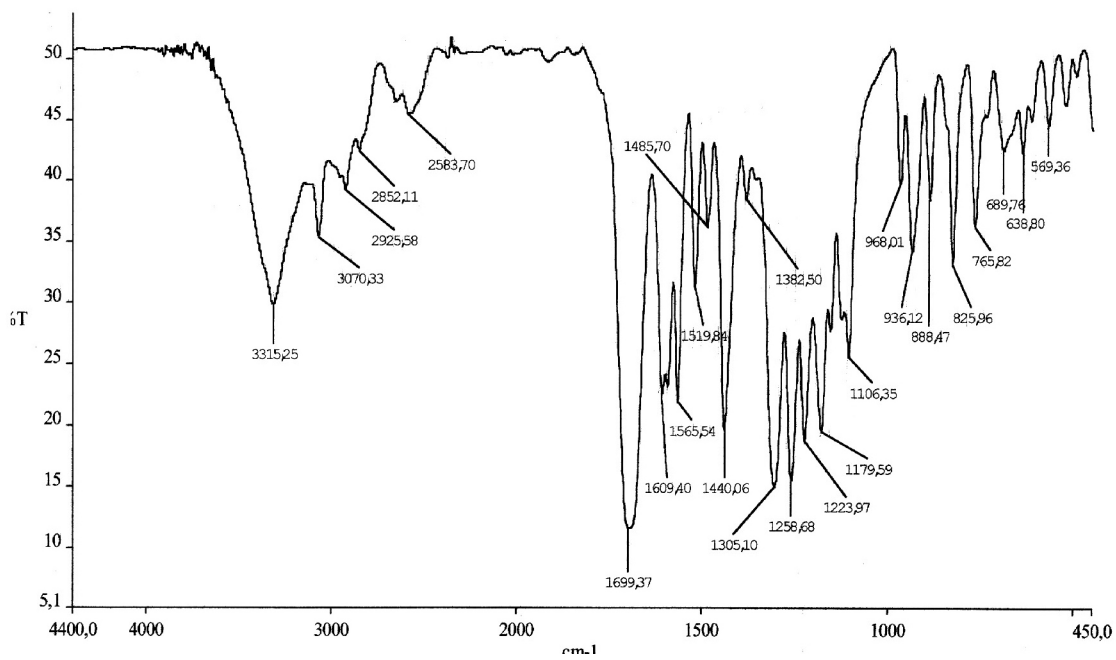


Figure S42. Infrared spectrum of compound 7 (KBr pellets).

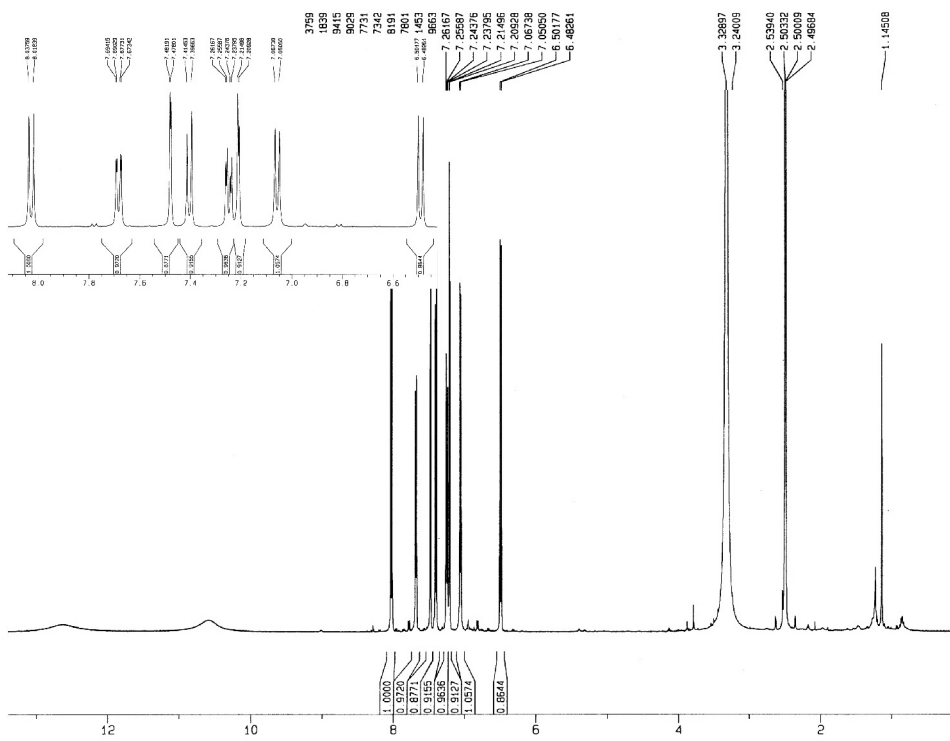


Figure S43. <sup>1</sup>H NMR spectrum of 7 (DMSO-*d*<sub>6</sub>, 500 MHz).

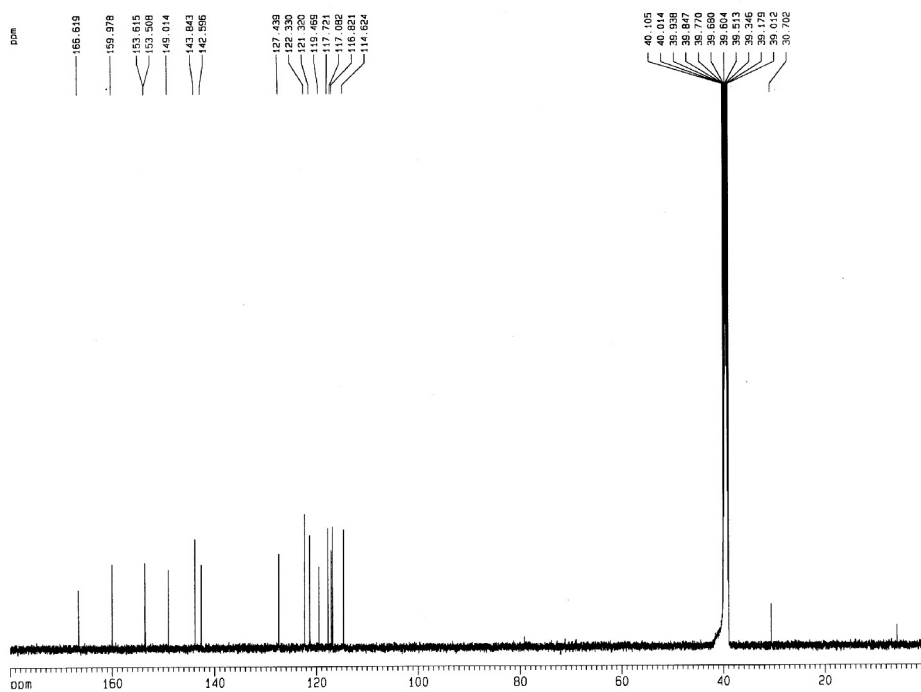


Figure S44.  $^{13}\text{C}$  NMR spectrum of **7** ( $\text{DMSO-}d_6$ , 125 MHz).

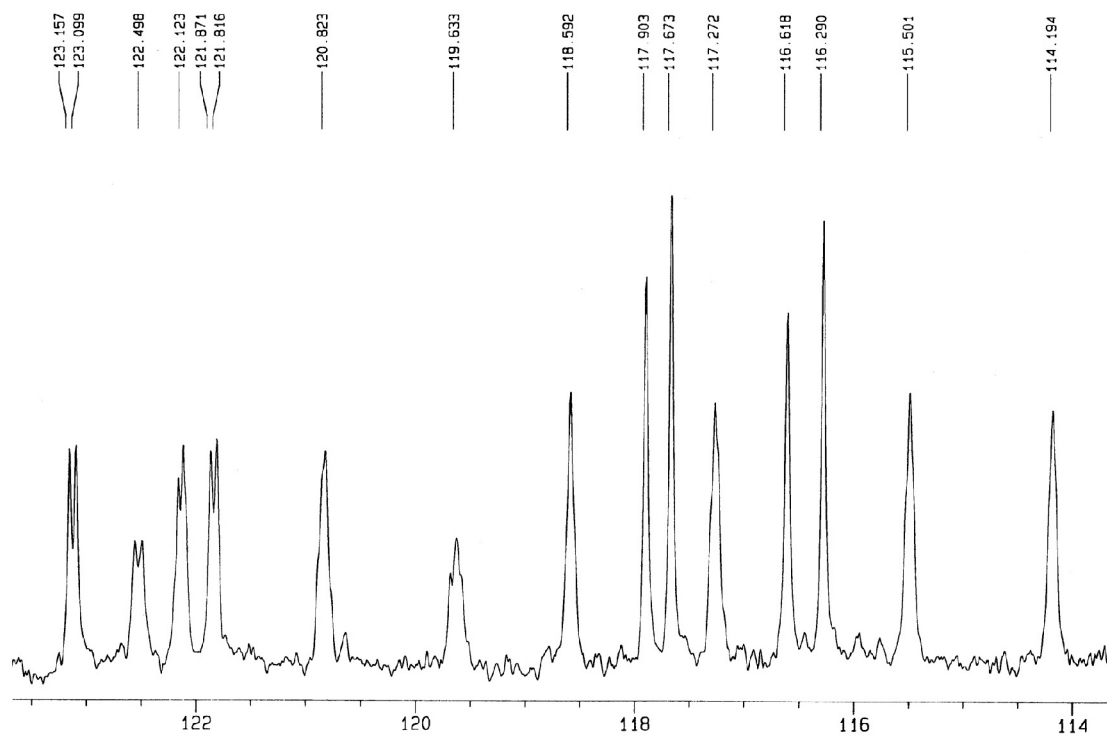


Figure S45. Expansion of the  $^{13}\text{C}$  NMR-GATED spectrum of **7** ( $\delta$  123.5-113.5).

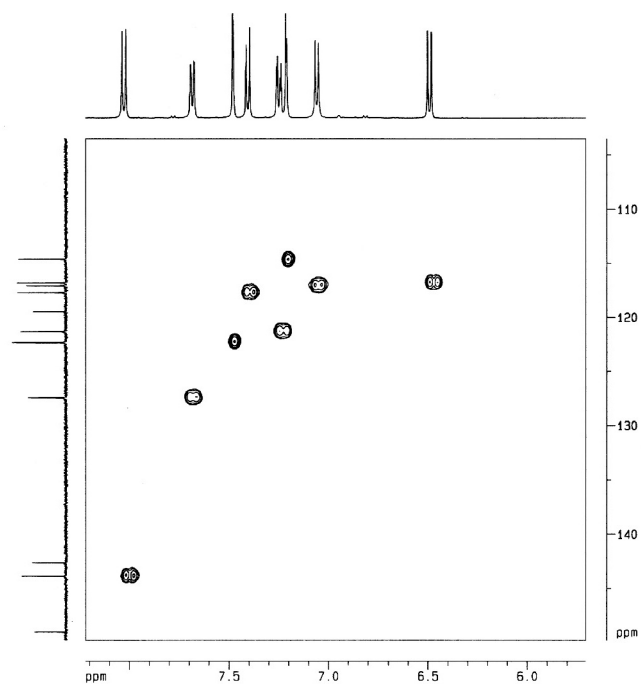


Figure S46.  $^1\text{H}$ ,  $^{13}\text{C}$  HSQC-NMR spectrum of 7 (DMSO- $d_6$ , 500  $\times$  125 MHz).

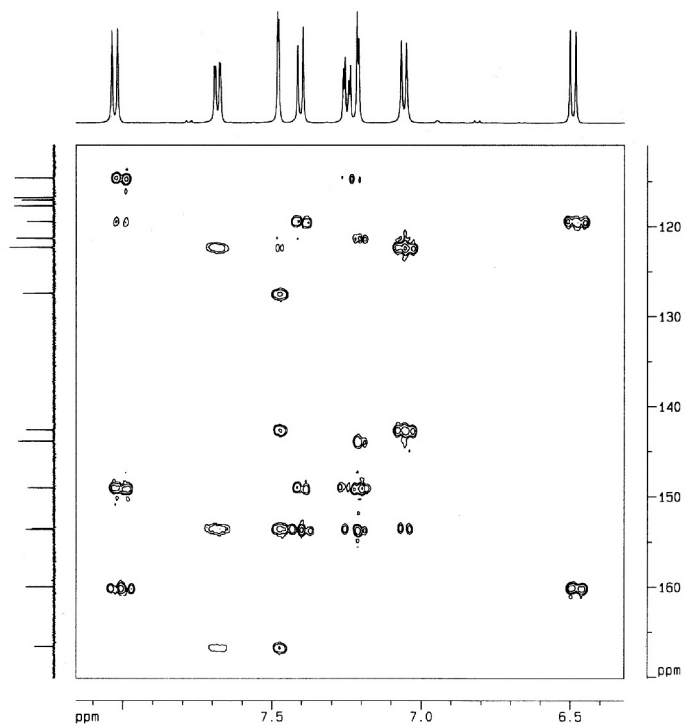


Figure S47.  $^1\text{H}$ ,  $^{13}\text{C}$  HMBC-NMR spectrum of 7 (DMSO- $d_6$ , 500  $\times$  125 MHz).



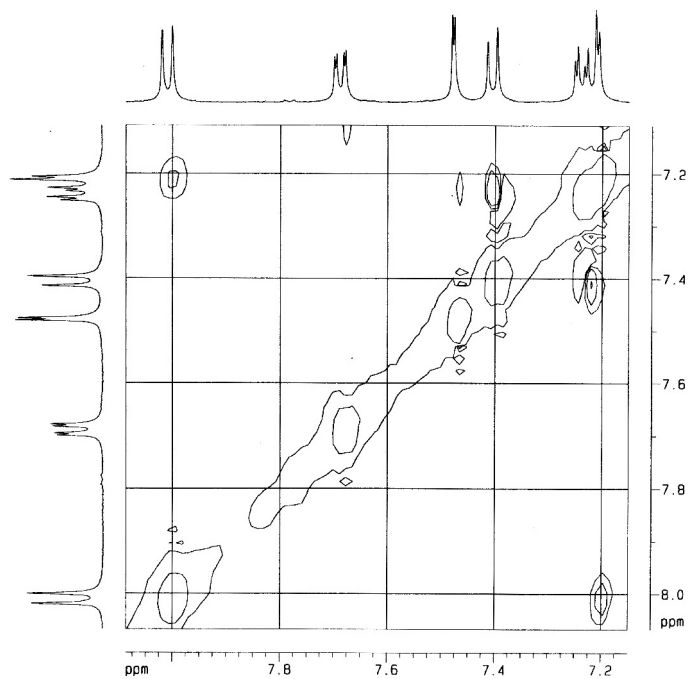


Figure S48.  $^1\text{H}$ ,  $^1\text{H}$  NOESY-NMR spectrum of **7** ( $\text{DMSO-}d_6$ ,  $500 \times 500$  MHz).

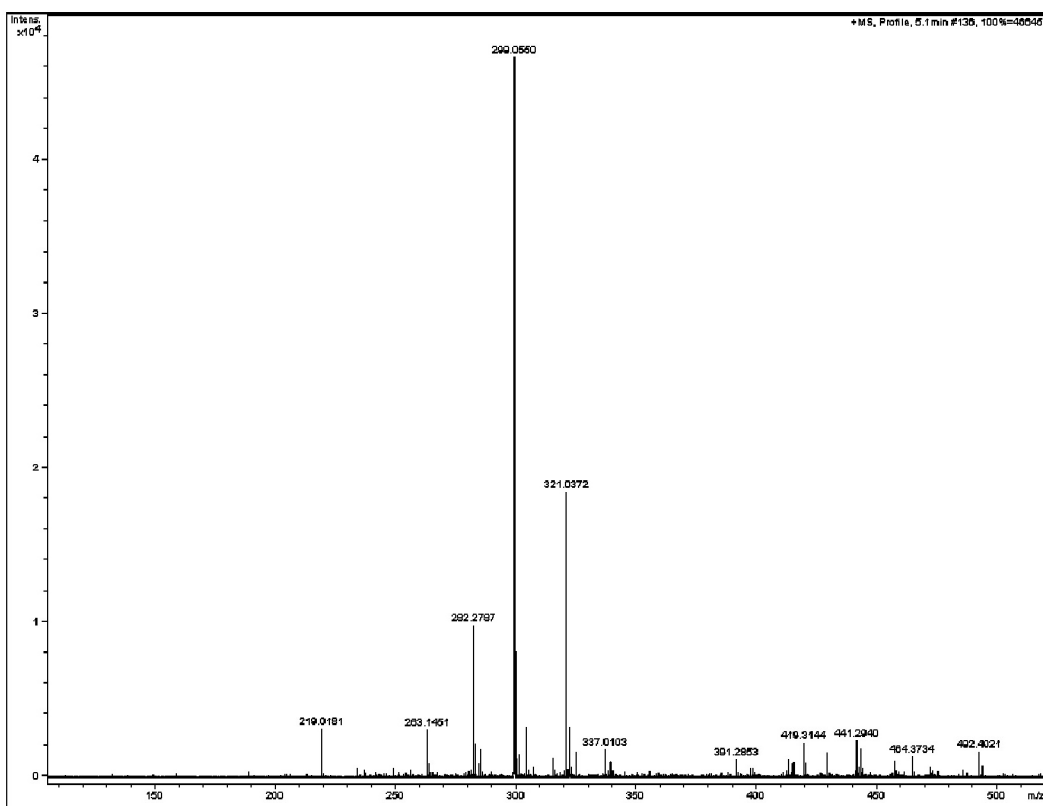


Figure S49. High resolution electrospray ionization mass spectrum of **7**.