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Short Report

Dihydropyranoflavones from *Pongamia pinnata*

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Das cascas do caule de *Pongamia pinnata* foram isolados dois novos compostos, 3-metoxi-(3″,4″-diidro- 3″-hidroxi-4″-acetoxi)-2″,2″-dimetilpirano-(7,8:5″,6″)-flavona e 3-metoxi-(3″,4″-diidro-4″- hidroxi-3″-acetoxi)-2″,2″-dimetilpirano-(7,8:5″,6″)-flavona, juntamente com seis compostos conhecidos, óxido de cariofileno, obovatachalcona, 8-hidroxi-6-metoxi-3-pentil-1H-isocromeno-1-ona, 6,7,2,2-dimetilcromono-8,γ,γ-dimetilalilflavonona, isolonchocarpin, ovaliflavanona A. Suas estruturas foram determinadas a partir da interpretação de dados espectroscópicos.

From the stem bark of *Pongamia pinnata*, two new compounds, 3-methoxy-(3″,4″-dihydro- 3″-hydroxy-4″-acetoxy)-2″,2″-dimethylpyrano-(7,8:5″,6″)-flavone and 3-methoxy-(3″,4″-dihydro-4″-hydroxy-3″-acetoxy)-2″,2″-dimethylpyrano-(7,8:5″,6″)-flavone, were isolated, along with six known compounds, caryophyllene oxide, obovatachalcone, 8-hydroxy-6-methoxy-3-pentyl-1H-isochromen-1-one, 6,7,2,2-dimethylchromono-8,γ,γ-dimethylallylflavanone, isolonchocarpin, ovaliflavanone A. Their structures were determined on the basis of the spectroscopic data interpretation.

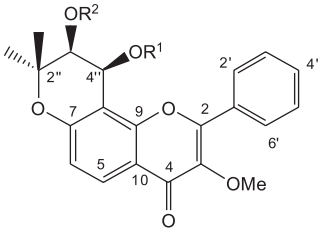
Keywords: *Pongamia pinnata*, prenylated flavonoids, flavone

Introduction

Pongamia pinnata (Linn) Pierre (Leguminosae, Papilionaceae; synonym, *Pongamia glabra* Vent), the only one species of genus *Pongamia*, is a medium sized glabrous tree, grows in the littoral regions of South Eastern Asia and Australia. All parts of the plant have been used as crude drug for the treatment of tumors, piles, skin diseases, wounds and ulcers.[1] Extracts of the plant possess significant anti-diarrhoeal, anti-fungal, anti-plasmodial, anti-ulcerogenic, anti-inflammatory, and analgesic activities.[2] Previous phytochemical investigation of this plant indicated the presence of abounding prenylated flavonoids such as furanoflavones, furanoflavonols, chromenoflavones, furanochalcones, and pyranochalcones.[3] In this paper, we reported the isolation and identification of two new flavones (**1**, **2**) from the stem bark of *Pongamia pinnata*.

Results and Discussion

The EtOH extract of *Pongmia pinnata* stem bark was submitted to successive chromatography, affording two new prenylated flavones **1** and **2** (Figure 1) along with six known compounds **3**-**8**.



1 R^1=Ac, R^2=H
2 R^1=H, R^2=Ac

Figure 1. Structures of compounds **1** and **2.**

Compound **1,** a white powder, gave a molecular ion [M^+] at *m/z* 410.13657 in the HREIMS, indicating a molecular formula $C_{23}H_{22}O_7$ (calc. 410.13655). Together with HMQC spectra, the 1D NMR (^{13}C, ^1H and DEPT, Table 1) spectra of compound **1** displayed resonances for one conjugated ketone (δ_C 173.4, C-4), a pair of *ortho*-coupling aromatic protons (δ_H 7.98, 1H, d, *J* 8.9 Hz, H-5; δ_H 6.96, 1H, d, *J* 8.9 Hz, H-6), a monosubstituted aromatic ring (δ_H 7.95, 2H, m, H-2′, 6′; δ_H 7.55, 3H, m, H-3′, 4′, 5′), two oxygenated methines (δ_H 3.93, 1H, d, *J* 4.8 Hz, H-3″; δ_C 69.3, C-3″; δ_H 6.46, 1H, d, *J* 4.8 Hz,

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H-4″; δ_C 63.2, C-4″), a acetoxy group (δ_H 1.90, 3H, s, δ_C 170.0, δ_C 21.0, OAc-4″), two methyls (δ_H 1.41, 3H, s, δ_C 26.4, Me_1-2″; δ_H 1.38, 3H, s, δ_C 21.3, Me_2-2″), a methoxy group (δ_H 3.81, 3H, s, δ_C 60.1, OCH_3-3), and an oxygenated quaternary carbon (δ_C 79.5, C-2″). With great similarity to those of 5-methoxy-(3″,4″-dihydro-3″,4″-diacetoxy)-2″,2″-dimethylpyrano-(7,8:5″,6″)-flavone,[4] these 1D NMR data suggested that compound **1** was a flavone with a acetylated dihydropyrano unit. In HMBC spectra, the correlation between H-5 (δ_H 7.98, 1H, d, *J* 8.9 Hz) and C-4 (δ_C 173.4) indicated that the ring A was unsubstituted at the C-5 and C-6 position. The observed HMBC correlations from H-3″ (δ_H 3.93, 1H, d, *J* 4.8 Hz, H-3″) to C-8 (δ_C 108.2), from H-4″ (δ_H 6.46, 1H, d, *J* 4.8Hz) to C-8 (δ_C 108.2), C-7(δ_C 158.3), and C-9 (δ_C 155.0) suggested that the dihydropyran ring is attached to A ring at C-7(oxygenated) and C-8 postion. The location of the acetoxy group at C-4″ were established by HMBC correlations from protons of Me_1-2″, Me_2-2″ to C-3″, and from H-4″ to the carbonyl of OAc-4″. The location of the methoxyl group (δ_H 3.81) at C-3 was revealed by the HMBC correlation from the protons of methoxy group to C-3 (δ_C 141.1). The substitution of a hydroxyl group at C-3″ (δ_C 72.9) was enclosed by chemical shift of C-3″ in ^{13}C NMR spectra and the HREIMS data. The molecular structure of **1** contained two chiral carbon atoms, and the relative configuration of C-3″ and C-4″ was determined as *cis* on the basis of coupling constant $J_{3'',4''}$ (4.8 Hz) and the difference (0.03 ppm) in the methyl proton signals at δ_H 1.41 and δ_H 1.38 of the 2″-*gem* dimethyl group.[5] Accordingly, compound **1** was characterized as 3-methoxy-(3″,4″-dihydro-3″-hydroxy-4″-acetoxy)-2″,2″-dimethylpyrano-(7,8:5″,6″)-flavone.

Compound **2**, a white powder, gave a molecular ion [M^+] at *m/z* 410.13665 in the HREIMS, corresponding to the molecular formula $C_{23}H_{22}O_7$ (calc. 410.13655). The NMR spectral data of **2** (Table 1) were closely comparable to those of compound **1**, with the only difference being due to the position of the acetoxy group (δ_H 2.13, 3H, s, δ_C 170.5, δ_C 21.3, OAc-3″) and hydroxyl group on the pyran ring. In HMBC spectra, the observed correlations from protons of Me_1-2″ (δ_H 1.41, 3H, s), Me_2-2″ (δ_H 1.35, 3H, s) to C-3″ (δ_C 72.9), and from H-3″ (δ_H 5.06, 1H, d, *J* 4.8 Hz) to the carbonyl of acetoxyl group suggested the acetoxy group to be located on C-3″ position. Further analysis of the NMR and MS spectra of compound **2** indicated the presence of the hydroxyl group at C-4″ position. The coupling constant $J_{3'',4''}$ was 4.8 Hz and the chemical shift difference of *gem*-dimethyl signals was 0.06 ppm in ^{1}H NMR spectrum. This evidence suggested that the relative configuration of compound **2** was *cis*-form. Thus compound **2** was characterized as 3-methoxy-(3″,4″-dihydro-4″-hydroxy-3″-acetoxy)-2″,2″-dimethylpyrano-(7,8:5″,6″)-flavone.

Additionally 6 known compounds, caryophyllene oxide (**3**),[6] obovatachalcone (**4**),[7] 8-hydroxy-6-methoxy-3-pentyl-1H-isochromen-1-one (**5**),[8] 6,7,2,2-dimethylchromono-8,γ,γ-dimethylallylflavanone(**6**),[9] isolonchocarpin (**7**),[10] ovaliflavanone A (**8**),[11] were isolated from this plant and their structures elucidated by comparing their spectroscopic data with those reported in the literature. Compounds **3, 5, 6** and **8** were isolated for the first time from this plant.

Experimental

General

Optical rotation were measured with a Jasco 1020 polarimeter. NMR spectra were obtained on a Bruker AVANCE 500 spectrometer (500 MHz for ^{1}H NMR, 125 MHz for ^{13}C NMR). EIMS and HREIMS spectra were recorded on a Finnigan MAT TSQ 700 mass spectrometer. UV spectra were obtained in a Beckman DU-640 UV spectrophotometer. A Waters Nova-pack HR C18 column (19×300mm) was used for semipreparative HPLC, along with Waters 600E Multisolvent Delivery System and a Waters 996 Photodiode Array Detector.

Plant material

The material investigated were stem bark of *Pongamia pinnata* collected in October 2002 from Hainan Province, southern China. The material was identified by Professor Si Zhang, Guangdong Key Laboratory of Marine Materia Medica, South China Sea Institute of Oceanology, Chinese Academy of Sciences. A voucher specimen is deposited at the herbarium of the South China Sea Institute of Oceanology (No. GKLMMM005).

Extraction and isolation

The dryied and powdered stem bark (6 kg) of *Pongamia pinnata* was extracted with 95% EtOH three times. After evaporation of the solvents under reduced pressure, the residue (300 g) was then extracted successively resulting four extracts: petroleum (80 g), ethyl acetate (60 g), *n*-butanol (60 g), and aqueous (80 g). The petroleum extract was fractionated into 63 fractions by open CC over silica gel with gradient mixtures of petroleum-$CHCl_3$ (6:1) to $CHCl_3$-acetone (0:1) for elution. These fractions were pooled into 17 fractions (P-1~P-17) according to their similarity on TLC. The silica gel CC of Fraction P-8 using

Table 1. ^{1}H, ^{13}C and selected HMBC NMR data for compounds **1** and **2**[a]

	1			**2**		
position	δ_H (*J* Hz)	δ_C	HMBC	δ_H (*J* Hz)	δ_C	HMBC
2		154.8			154.5	
3		141.1			141.1	
4		173.4			173.6	
5	7.98 (d, 8.9)	127.0	4, 7, 9	7.96 (d, 8.9)	126.2	4, 7, 9
6	6.96 (d, 8.9)	116.2	7, 8, 10	6.96 (d, 8.9)	116.0	7, 8, 10
7		158.3			157.3	
8		108.2			111.5	
9		155.0			155.4	
10		117.7			118.1	
1′		130.6			131.1	
2′	7.95 (m)	128.6		8.20 (m)	128.6	
3′	7.55 (m)	128.8		7.57 (m)	129.0	
4′	7.55 (m)	131.1		7.57 (m)	131.1	
5′	7.55 (m)	128.8		7.57 (m)	129.0	
6′	7.95 (m)	128.6		8.20 (m)	128.6	
2″		79.5			78.1	
3″	3.93 (d, 4.8)	69.3	$Me_{1,2}$-2″, 2″, 4″, 8	5.06 (d, 4.8)	72.9	$Me_{1,2}$-2″, 2″, 8, OAc-3″
4″	6.46 (d, 4.8)	63.2	2″, 3″, 7, 8, 9, OAc-4″	5.28 (d, 4.8)	59.6	2″, 7, 8, 9
Me_1-2″	1.41 (s)	26.4	2″, 3″	1.41 (s)	23.2	2″, 3″
Me_2-2″	1.38(s)	21.3	2″, 3″	1.35 (s)	24.9	2″, 3″
OAc-3″				2.13 (s)	170.5/21.3	
OAc-4″	1.90 (s)	170.0/21.0				
OCH_3-3	3.81 (s)	60.1	3	3.82 (s)	60.0	3

[a]spectra recorded in DMSO-d_6 (500 MHz for ^{1}H, 125 MHz for ^{13}C); TMS was used as internal standard.

petroleum-EtOAc (3:1) afforded 7 fractions (P-8-1 to P-8-7). Fraction P-8-5 were purified by CC of Pharmacia-Sephadex LH-20 with MeOH-H_2O (95: 5) and separated by reverse phase semi-preparative HPLC (ODS column, using MeOH:H_2O (66:34) 8 mL min^{-1}, flow rate, UV: 254 nm) to give compounds **1** (1.9 mg, t_R=16 min) and **2** (5.2 mg, t_R=19 min). The CC of fraction P-6 using petroleum-EtOAc (35:1) afford 50 fractions (P-6-1 to P-6-50). The fractions P-6-10, P-6-13, P-6-18, P-6-28, P-6-35 and P-6-38, purified by CC on Pharmacia-Sephadex LH-20 ($CHCl_3$-MeOH 5:1) or recrystallization, yield compounds **3** (113 mg), **4** (25 mg), **5** (16 mg), **6** (9 mg), **7** (11 mg) and **8** (13 mg), respectively.

*3-methoxy-(3″,4″-dihydro-3″-hydroxy-4″-acetoxy)-2″,2″-dimethylpyrano-(7,8:5″,6″)-flavone (**1**)*. $[\alpha]_D^{25}$ +46.5 ° (c 0.1, $CHCl_3$); UV(MeOH) λ_{max}/ nm: 255, 316; HREIMS *m/z*: 410.13657 (calc. for $C_{23}H_{22}O_7$ 410.13655); EIMS *m/z* (rel. int.,%): 410 [M^+] (76), 409 (100), 349 (21), 335 (9), 333 (17), 297 (8), 165 (17), 163 (12); ^{1}H and ^{13}C NMR spectra data: see Table 1.

*3-methoxy-(3″,4″-dihydro-4″-hydroxy-3″-acetoxy)-2″,2″-dimethylpyrano-(7,8:5″,6″)-flavone (**2**)*. $[\alpha]_D^{25}$ +15.8° (c 0.1, $CHCl_3$); UV(MeOH) λ_{max}/nm: 252, 311; HREIMS *m/z*: 410.13667 (calc. for $C_{23}H_{22}O_7$, 410.13655); EIMS *m/z* (rel. int.,%): 410 [M^+] (81), 409 (100), 349 (20), 335 (8), 333 (16), 321 (10), 297 (11), 296 (10), 295 (46), 279 (16), 165 (22), 163 (12); ^{1}H and ^{13}C NMR spectra data: see Table 1.

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Supplementary Information

Supplementary Information are available free of charge at http://jbcs.sbq.org.br, as PDF file.

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Dihydropyranoflavones from *Pongamia pinnata*

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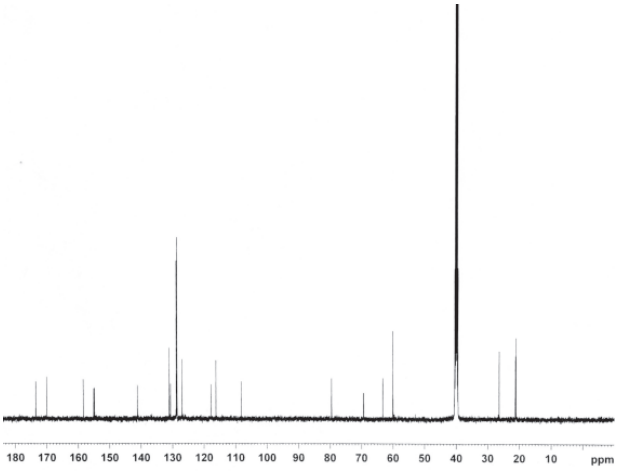

Figure S1. ^{13}C NMR spectra of 1.

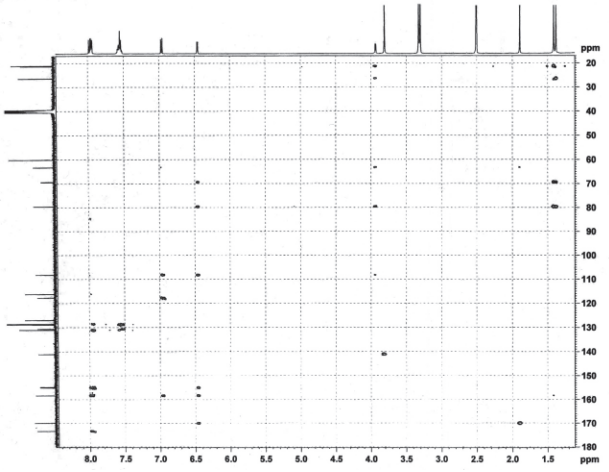

Figure S3. HMBC spectra of 1.

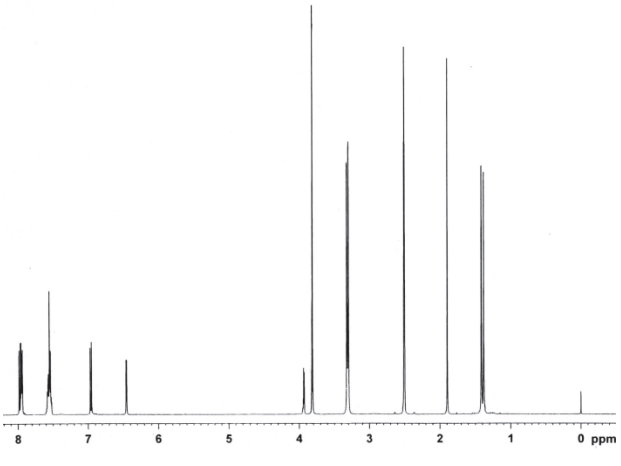

Figure S2. ^{1}H NMR spectra of 1.

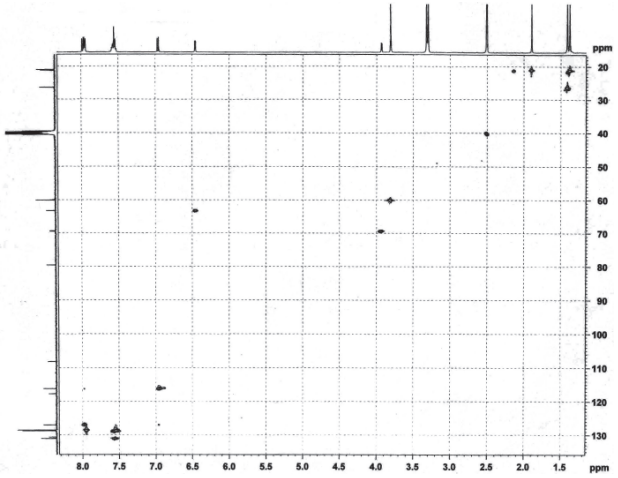

Figure S4. HSQC spectra of 1.

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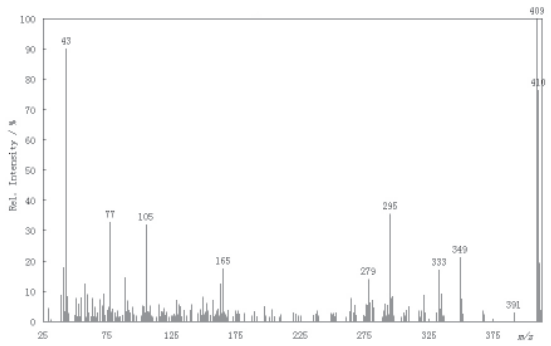

Figure S5. MS spectra of 1.

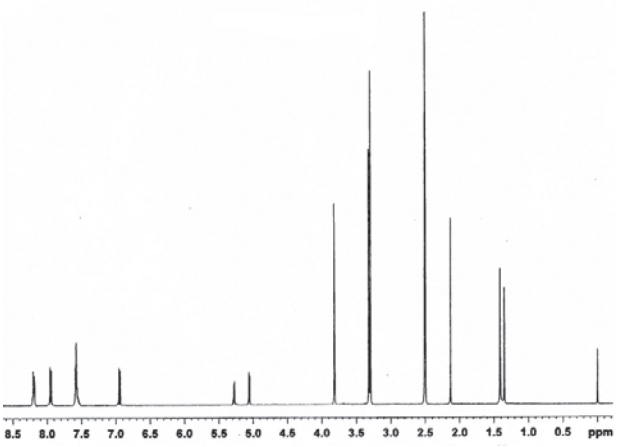

Figure S8. [1]H NMR spectra of 2.

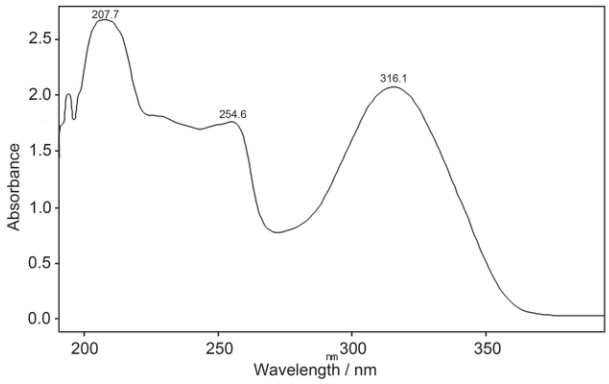

Figure S6. UV spectra of 1.

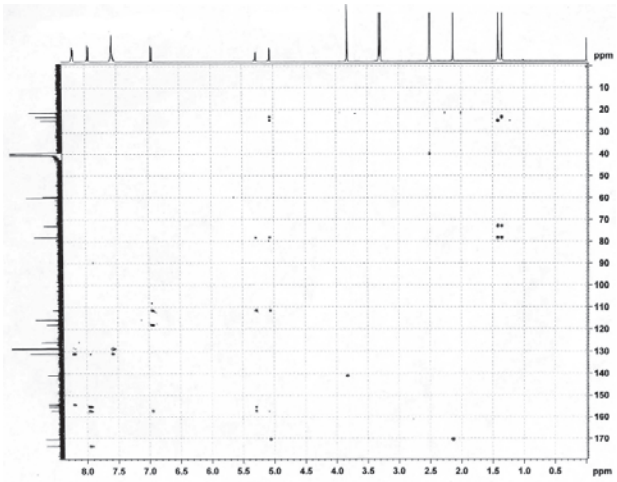

Figure S9. HMBC spectra of 2.

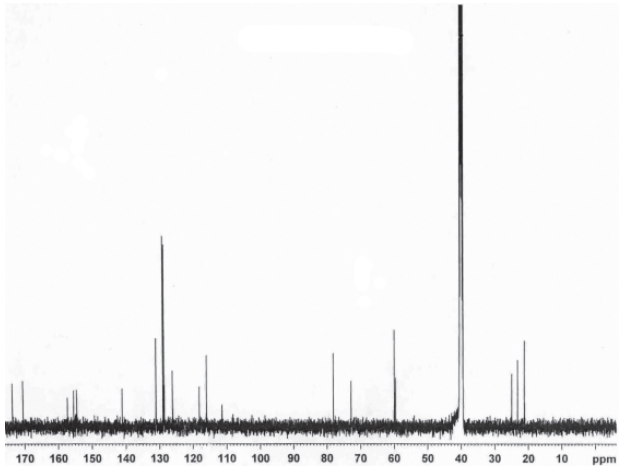

Figure S7. [13]C NMR spectra of 2.

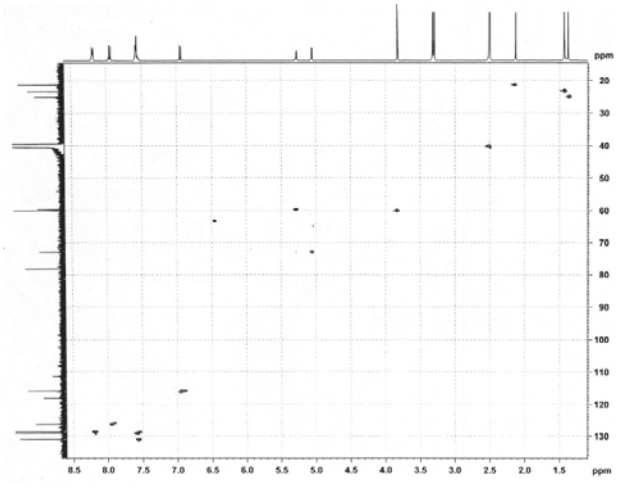

Figure S10. HSQC spectra of 2.

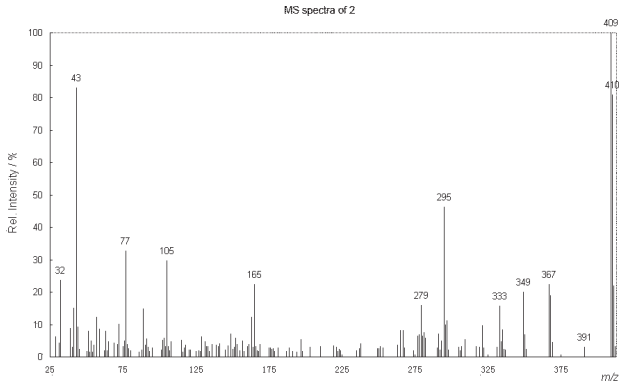

Figure S11. MS spectra of 2.

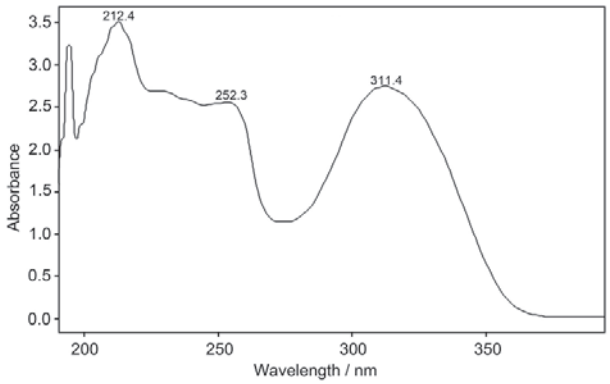

Figure S12. UV spectra of 2.