

Supporting Information

Rec. Nat. Prod. 5:3 (2011) 238-241

Lignans and an Abundant Flavone Glycoside with Free-Radical Scavenging Activity from the Roots of the Endemic Species *Stachys mialhesi* de Noé

Souheila Laggoune¹, Ignacio Brouard², Francisco Leon², Claude-Alain
Calliste³, Jean-luc Duroux³, Jaime Bermejo², Zahia Kabouche¹ and Ahmed
Kabouche^{1*}

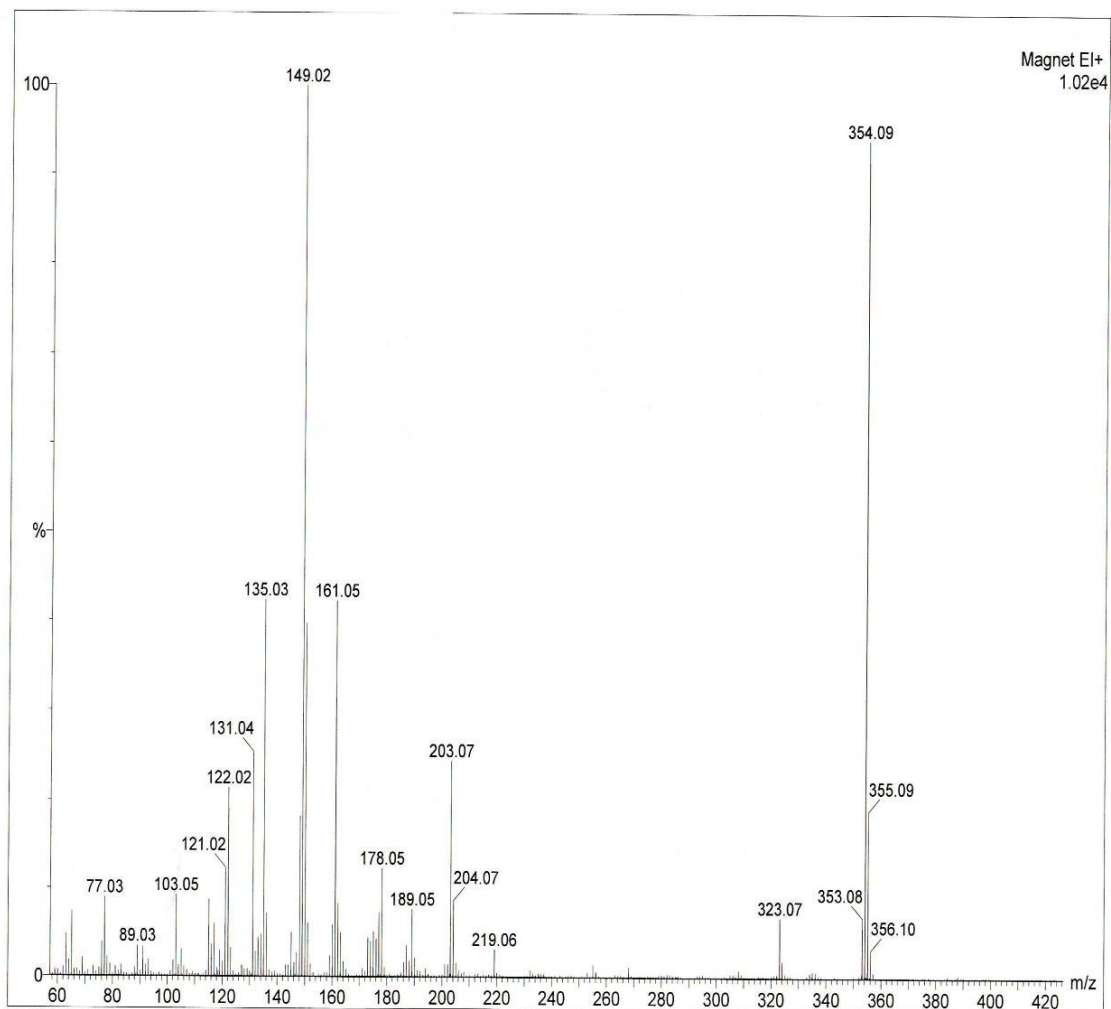
¹Laboratoire d'Obtention de Substances Thérapeutiques (L.O.S.T), Université Mentouri -
Constantine, Route de Ain El Bey, 25000 Constantine, Algérie

²Instituto de Productos Naturales y Agrobiología-C.S.I.C.-Instituto Universitario de Bio-
Orgánica "Antonio González", Universidad de La Laguna, Av. Astrofísico F. Sánchez 3,
38206 La Laguna, Tenerife, Spain

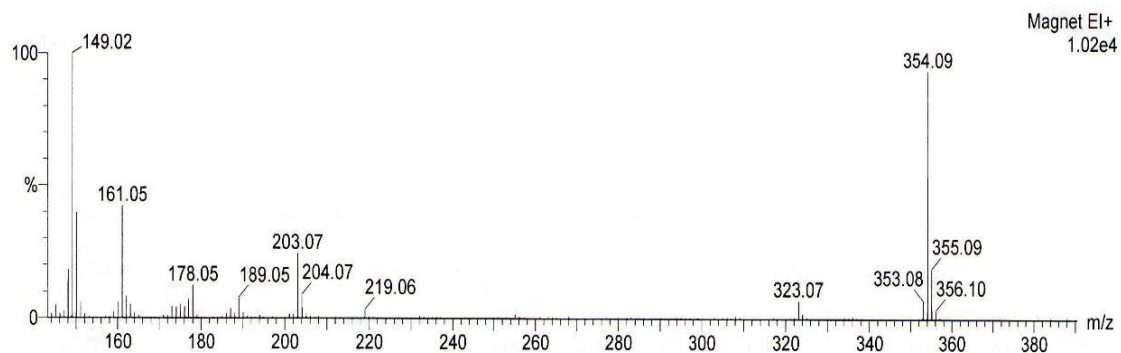
³Laboratoire de Biophysique, UPRES EA-1085, Biomolécules et cibles cellulaires tumorales
Faculté de Pharmacie, 2 rue du Dr. Marcland, 87025 Limoges Cedex, France

| Table of Contents | Page |
|---|------|
| S1: HRESI-MS Spectrum of Compound 3 ((+)-sesamin) | 3 |
| S2: Expansion of HRESI-MS Spectrum of Compound 3 ((+)-sesamin) | 4 |
| S3: ¹ H-NMR (500 MHz, CDCl ₃) Spectrum of Compound 3 ((+)-sesamin) | 5 |
| S4: Expansion of the ¹ H-NMR Spectrum of Compound 3 ((+)-sesamin) | 6 |
| S5: Expansion of the ¹ H-NMR Spectrum of Compound 3 ((+)-sesamin) | 7 |
| S6: ¹³ C-NMR + DEPT (125 MHz, CDCl ₃) Spectrum of Compound 3 ((+)-sesamin) | 8 |
| S7: Expansion of the ¹³ C-NMR + DEPT Spectrum of Compound 3 ((+)-sesamin) | 9 |
| S8: COSY (500 MHz) Spectrum of Compound 3 ((+)-sesamin) | 10 |
| S9: Expansion of the COSY Spectrum of Compound 3 ((+)-sesamin) | 11 |

| | |
|--|----|
| S10: HSQC (500 MHz) Spectrum of Compound 3 ((+)-sesamin) | 12 |
| S11: Expansion of the HSQC Spectrum of Compound 3 ((+)-sesamin) | 13 |
| S12: Expansion of ESIMS Spectrum of compound 4 ((±)-paulownin) | 14 |
| S13: Expansion of HRESI-MS Spectrum of Compound 4 ((±)-paulownin) | 15 |
| S14: ¹ H-NMR (500 MHz, CDCl ₃) Spectrum of Compound 4 ((±)-paulownin) | 16 |
| S15: Expansion of the ¹ H-NMR Spectrum of Compound 4 ((±)-paulownin) | 17 |
| S16: Expansion of the ¹ H-NMR Spectrum of Compound 4 ((±)-paulownin) | 18 |
| S17: ¹³ C-NMR + DEPT (125 MHz, CDCl ₃) Spectrum of Compound 4 ((±)-paulownin) | 19 |
| S18: Expansion of the ¹³ C-NMR + DEPT Spectrum of Compound 4 ((±)-paulownin) | 20 |
| S19: COSY (400 MHz) Spectrum of Compound 4 ((±)-paulownin) | 21 |
| S20: HSQC (500 MHz) Spectrum of Compound 4 ((±)-paulownin) | 22 |
| S21: Expansion of the HSQC Spectrum of Compound 4 ((±)-paulownin) | 23 |
| S22: HMBC (500 MHz) Spectrum of Compound 4 ((±)-paulownin) | 24 |
| S23: HRESIMS Spectrum of Compound 5 (Isoscutellarein-7- <i>O</i> -(2"- <i>O</i> -6"- <i>O</i> -acetyl-β-D-allopyranosyl-β-D-glucopyranoside) | 25 |
| S24: ¹ H-NMR (400 MHz, DMSO- <i>d</i> ₆) Spectrum of Compound 5 | 26 |
| S25: Expansion of the ¹ H-NMR Spectrum of Compound 5 | 27 |
| S26: ¹³ C-NMR + DEPT (125 MHz, DMSO- <i>d</i> ₆) Spectrum of Compound 5 | 28 |
| S27: Expansion of the ¹³ C-NMR + DEPT Spectrum of Compound 5 | 29 |
| S28: COSY (500 MHz) Spectrum of Compound 5 | 30 |
| S29: Expansion of the COSY Spectrum of Compound 5 | 31 |
| S30: HSQC (500 MHz) Spectrum of Compound 5 | 32 |
| S31: Expansion of the HSQC Spectrum of Compound 5 | 33 |
| S32: HMBC (500 MHz) Spectrum of Compound 5 | 34 |
| S33: Expansion of the HMBC Spectrum of Compound 5 | 35 |

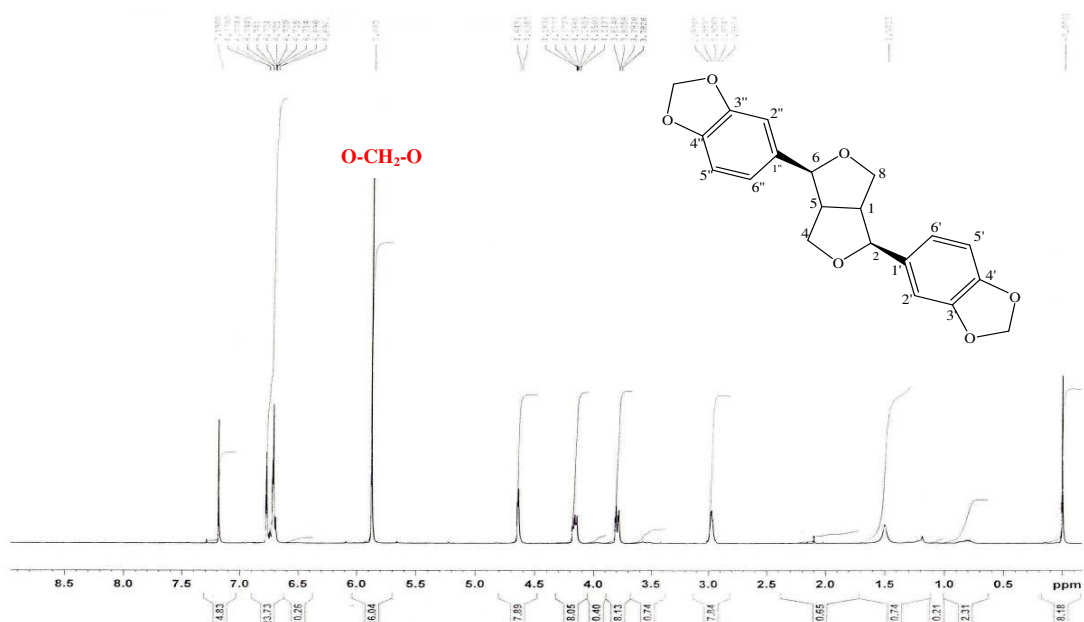


S1: HRESI-MS Spectrum of Compound 3 ((+)-sesamin)



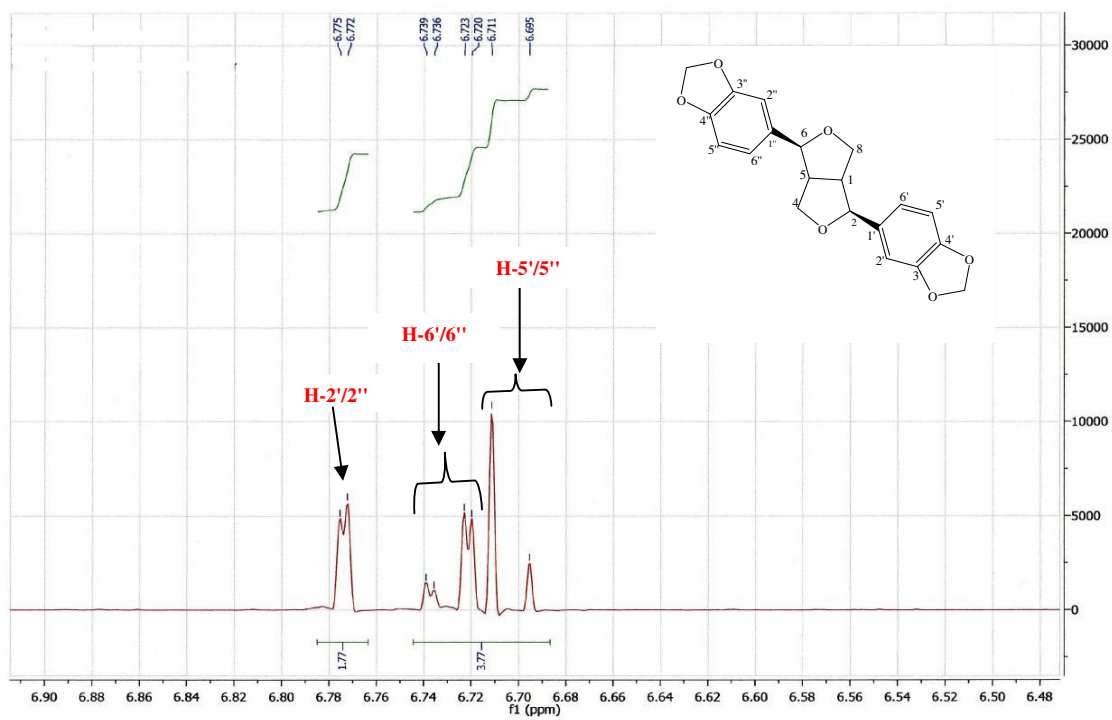
| Mass | RA | Calc. Mass | mDa | PPM | DBE | Formula |
|----------|-------|------------|------|------|------|-------------|
| 355.1180 | 6.14 | 355.1182 | -0.2 | -0.5 | 11.5 | C20 H19 O6 |
| 355.1139 | 8.66 | 355.1123 | 1.6 | 4.5 | 20.5 | C27 H15 O |
| 355.1017 | 15.27 | 355.1029 | -1.2 | -3.4 | 7.5 | C16 H19 O9 |
| 355.0976 | 16.07 | 355.0970 | 0.6 | 1.6 | 16.5 | C23 H15 O4 |
| 355.0894 | 18.86 | 355.0877 | 1.7 | 4.9 | 3.5 | C12 H19 O12 |
| 355.0813 | 15.90 | 355.0818 | -0.5 | -1.3 | 12.5 | C19 H15 O7 |
| 355.0772 | 13.27 | 355.0759 | 1.3 | 3.6 | 21.5 | C26 H11 O2 |
| 354.1313 | 10.58 | 354.1315 | -0.2 | -0.5 | 7.0 | C17 H22 O8 |
| 354.1273 | 15.87 | 354.1256 | 1.7 | 4.8 | 16.0 | C24 H18 O3 |
| 354.1151 | 47.75 | 354.1162 | -1.1 | -3.1 | 3.0 | C13 H22 O11 |
| 354.1110 | 59.19 | 354.1103 | 0.7 | 1.9 | 12.0 | C20 H18 O6 |

S2: Expansion of HRESI-MS Spectrum of Compound 3 ((+)-sesamin)



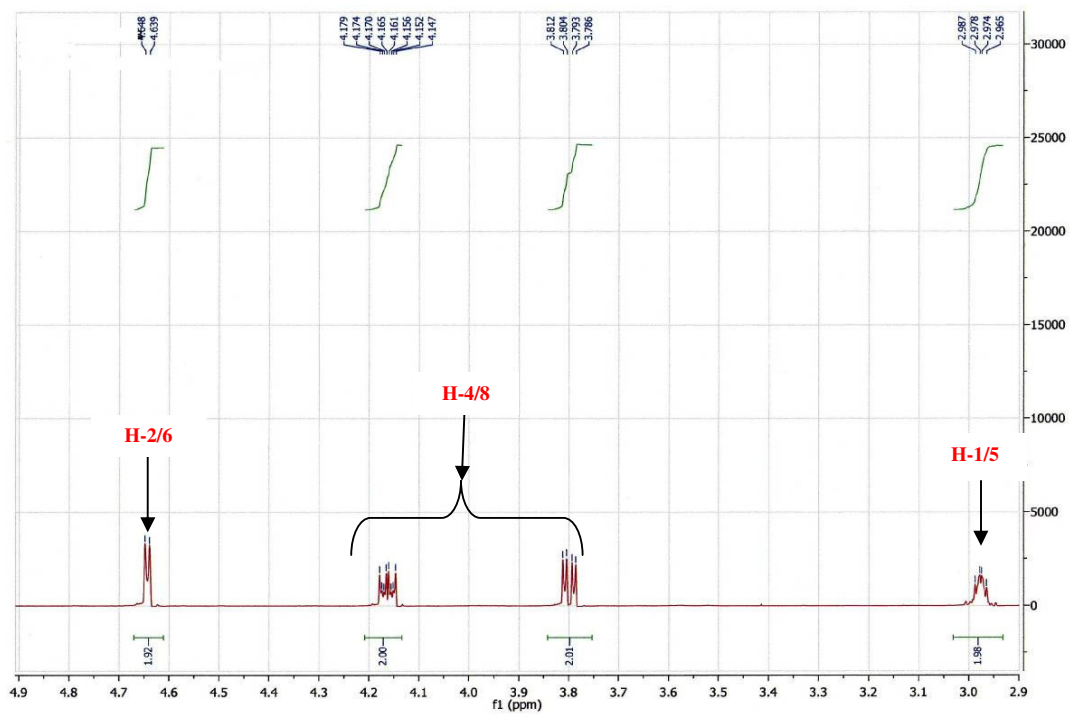
S3: ¹H-NMR (500 MHz, CDCl₃) Spectrum of Compound **3** (+)-sesamin)

(+)-*Sesamin* (**3**): Pink needle Crystals. ¹H-NMR (CDCl₃, 500 MHz), δ: 2.98 (2H, m, H-1/5), 3.80 (2H, dd, H-4/8), 4.16 (2H, dd, H-4/8), 4.64 (2H, d, H-2/6), 5.88 (4H, s, [(-O-CH₂-O)-2]), 6.70 *d* (2H, d, H-5'/5"), 6.72 (2H, dd, H-6'/6"), 6.77 (d, 2H, H-2'/2"). ¹³C-NMR (CDCl₃, 125 MHz), δ: 54.3 (C-1/5), 85.7 (C-2/6), 71.7 (C-4/8), 101.0 (C-[-(O-CH₂-O)-2]), 135.0 (C-1'/1"), 106.4 (C-2'/2"), 147.9 (C-3'/3"), 147.1 (C-4'/4"), 108.1 (C-5'/5"), 119.3 (C-6'/6"). EIMS: *m/z* = 354 [M]⁺ for formula C₂₀H₁₈O₆ [3,4].

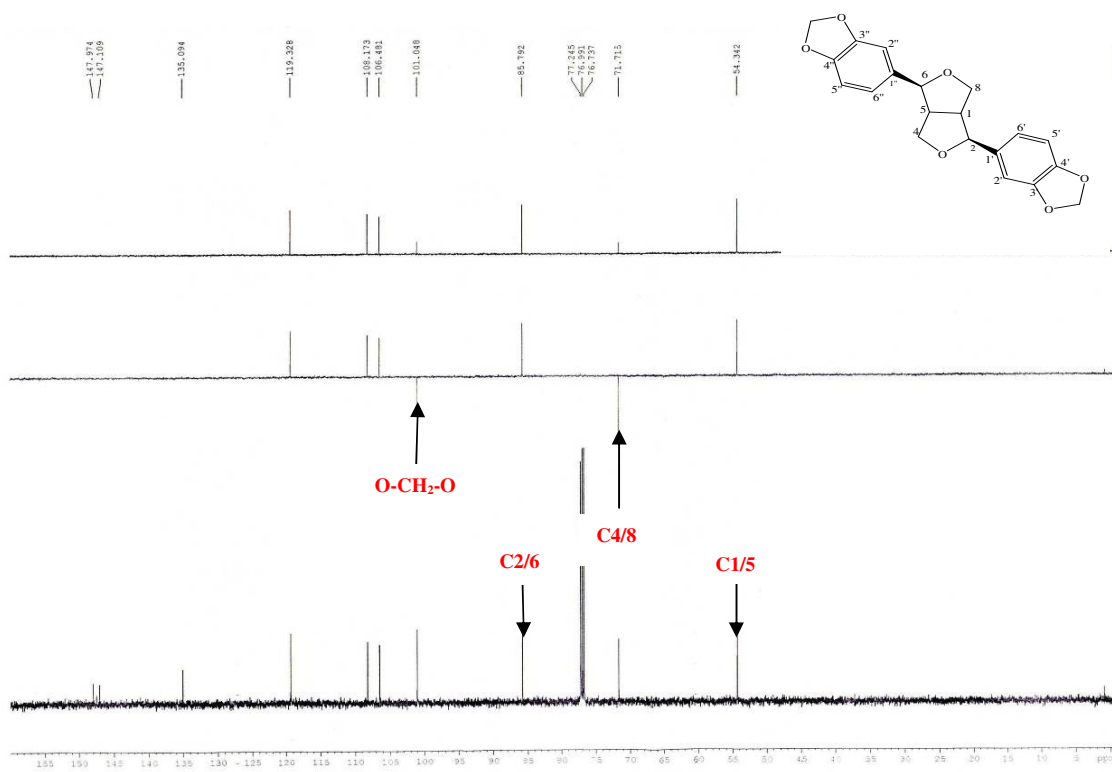


S4: $^1\text{H-NMR}$ Spectrum of Compound **3** ((+)-sesamin) (From 6.48 to 6.90 ppm)

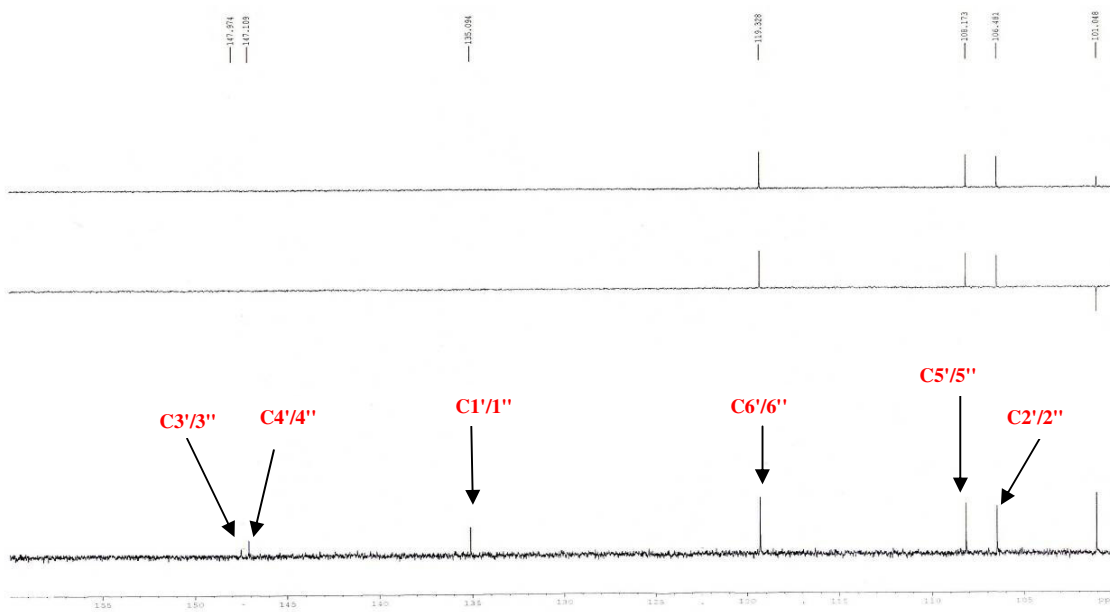
*



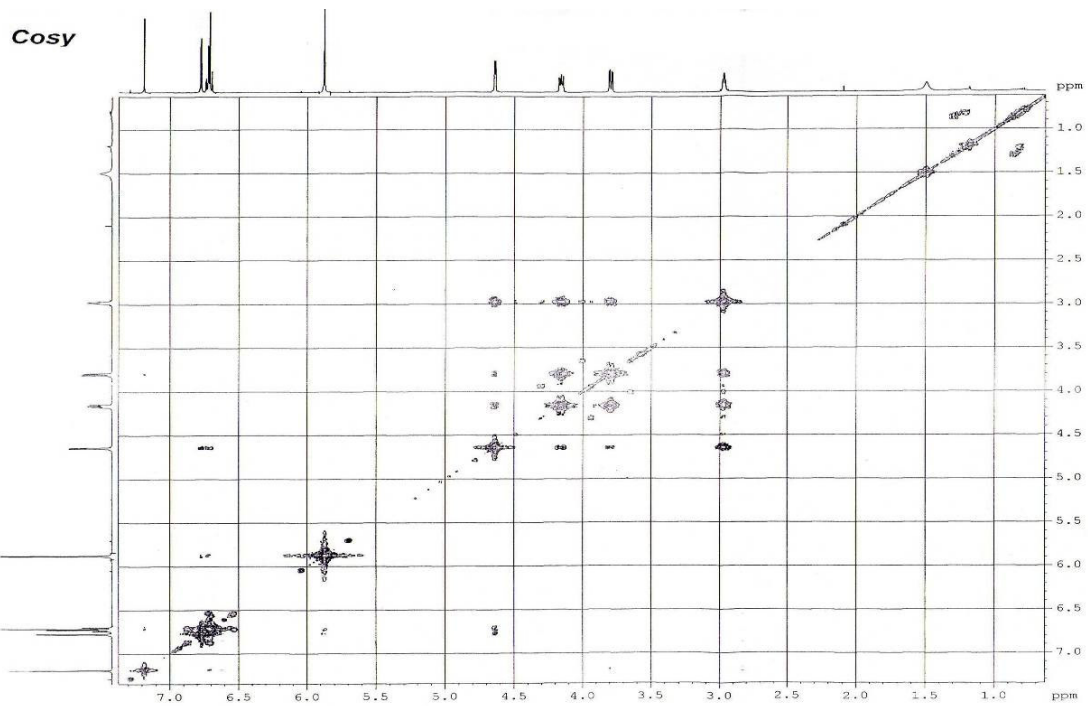
S5: $^1\text{H-NMR}$ Spectrum of Compound **3** ((+)-sesamin) (From 2.90 to 4.90 ppm)



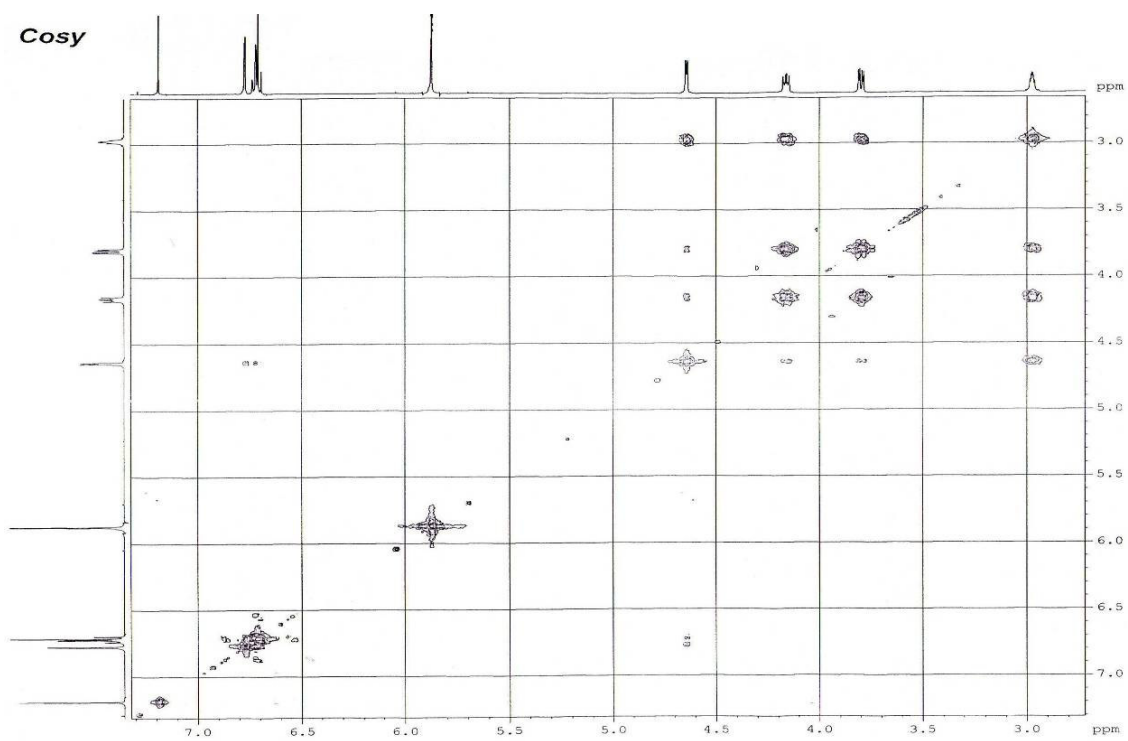
S6: ¹³C-NMR + DEPT (125 MHz, CDCl₃) Spectrum of Compound 3 ((+)-sesamin)



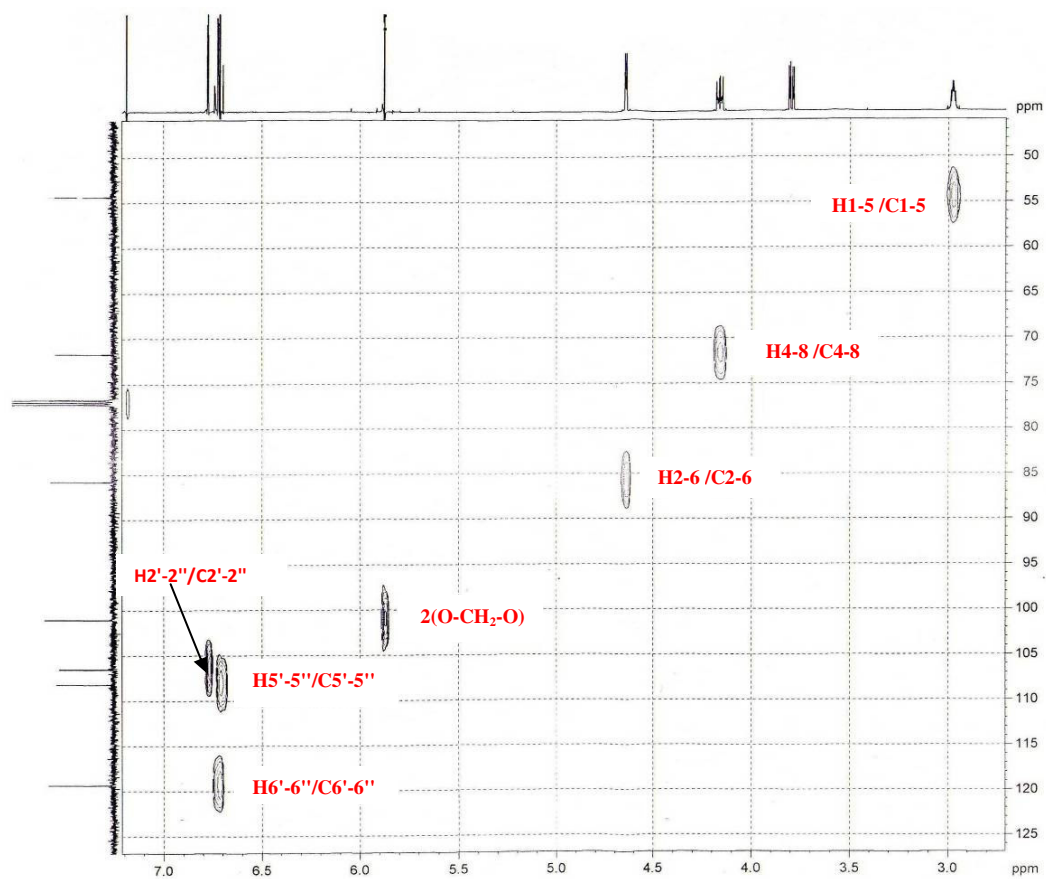
S7: ¹³C-NMR + DEPT Spectrum of Compound **3** ((+)-sesamin) (From 100 to 160 ppm)



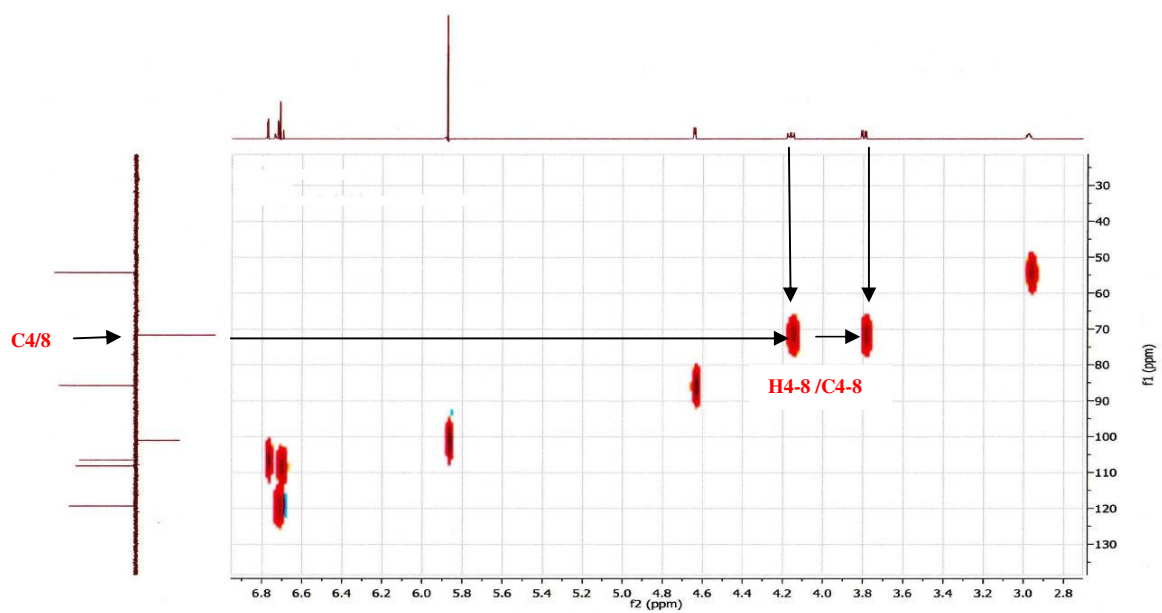
S8: COSY (500 MHz) Spectrum of Compound **3** ((+)-sesamin)



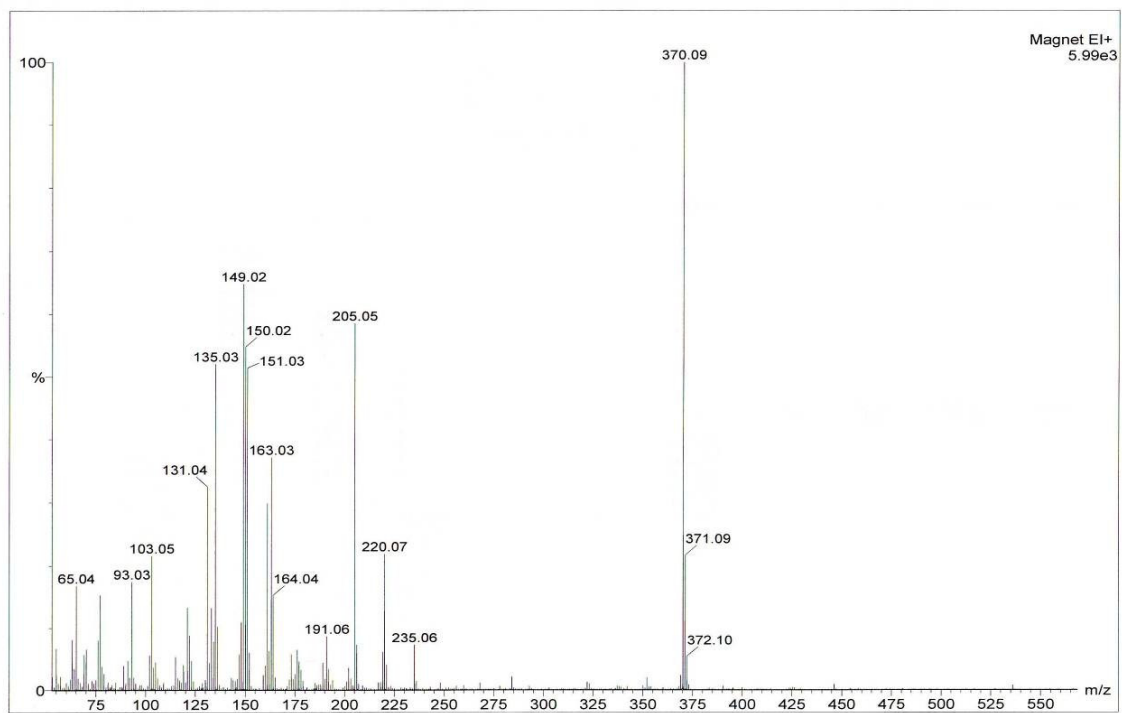
S9: COSY Spectrum of Compound **3** ((+)-sesamin) (From 2.5 to 7.5 ppm)



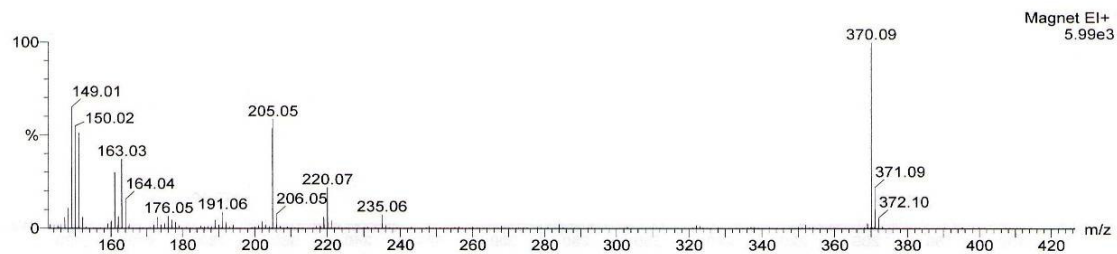
S10: HSQC (500 MHz) Spectrum of Compound **3** ((+)-sesamin) (From 50 to 125 ppm)



S11: HSQC Spectrum of Compound 3 ((+)-sesamin) (From 30 to 130 ppm)

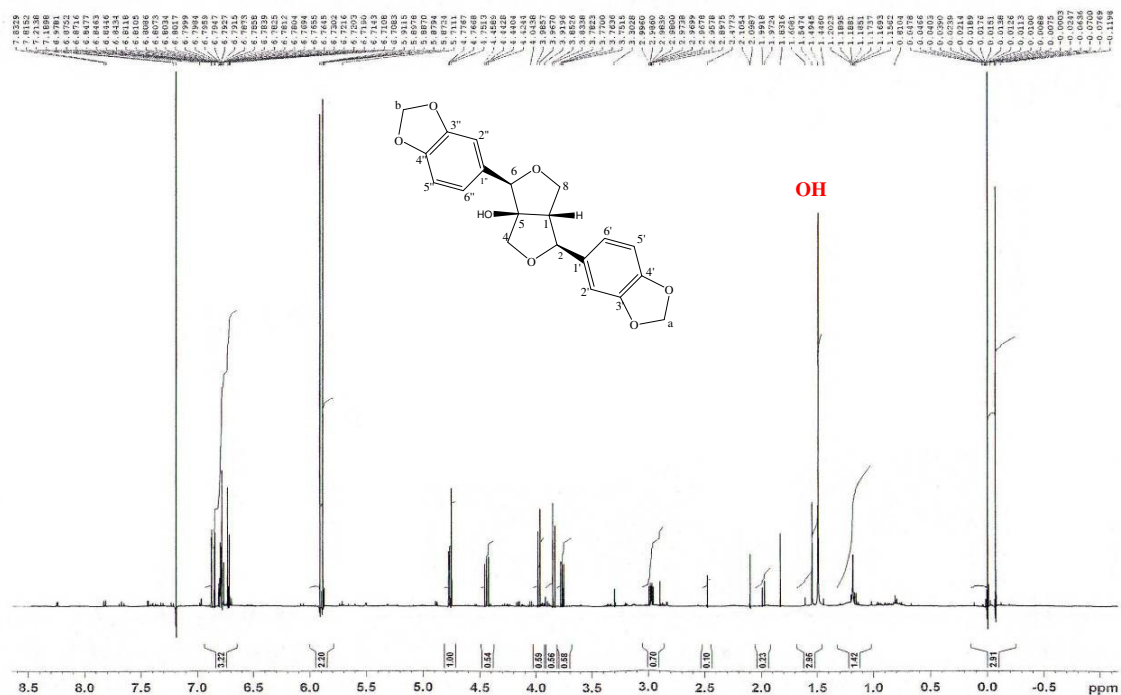


S12: Expansion of ESI-MS Spectrum of Compound **4** ((±)-paulownin)



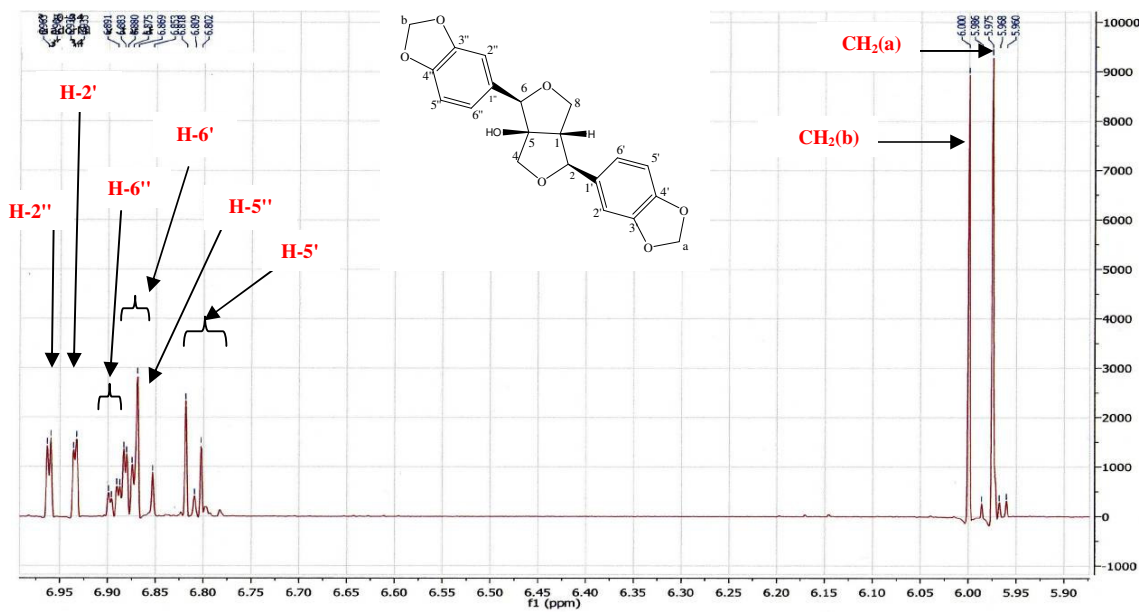
| Mass | RA | Calc. Mass | mDa | PPM | DBE | Formula |
|----------|-------|------------|------|------|------|-------------|
| 370.1256 | 20.18 | 370.1264 | -0.8 | -2.1 | 7.0 | C17 H22 O9 |
| 370.1341 | 7.46 | 370.1358 | -1.7 | -4.5 | 20.0 | C28 H18 O |
| 371.0715 | 9.33 | 371.0708 | 0.7 | 1.8 | 21.5 | C26 H11 O3 |
| 371.0758 | 12.77 | 371.0767 | -0.9 | -2.4 | 12.5 | C19 H15 O8 |
| 371.0843 | 17.98 | 371.0826 | 1.7 | 4.7 | 3.5 | C12 H19 O13 |
| | | 371.0861 | -1.8 | -4.8 | 25.5 | C30 H11 |
| 371.0929 | 21.59 | 371.0919 | 1.0 | 2.6 | 16.5 | C23 H15 O5 |
| 371.0971 | 19.62 | 371.0978 | -0.7 | -1.9 | 7.5 | C16 H19 O10 |
| 371.1057 | 16.76 | 371.1072 | -1.5 | -4.1 | 20.5 | C27 H15 O2 |
| 371.1142 | 12.09 | 371.1131 | 1.1 | 3.0 | 11.5 | C20 H19 O7 |
| 371.1184 | 8.60 | 371.1190 | -0.6 | -1.5 | 2.5 | C13 H23 O12 |
| 370.1044 | 80.28 | 370.1053 | -0.9 | -2.3 | 12.0 | C20 H18 O7 |
| 370.1128 | 58.38 | 370.1111 | 1.7 | 4.5 | 3.0 | C13 H22 O12 |
| 370.1214 | 29.33 | 370.1205 | 0.9 | 2.4 | 16.0 | C24 H18 O4 |

S13:Expansion of HRESI-MS Spectrum of Compound 4 ((±)-paulownin)

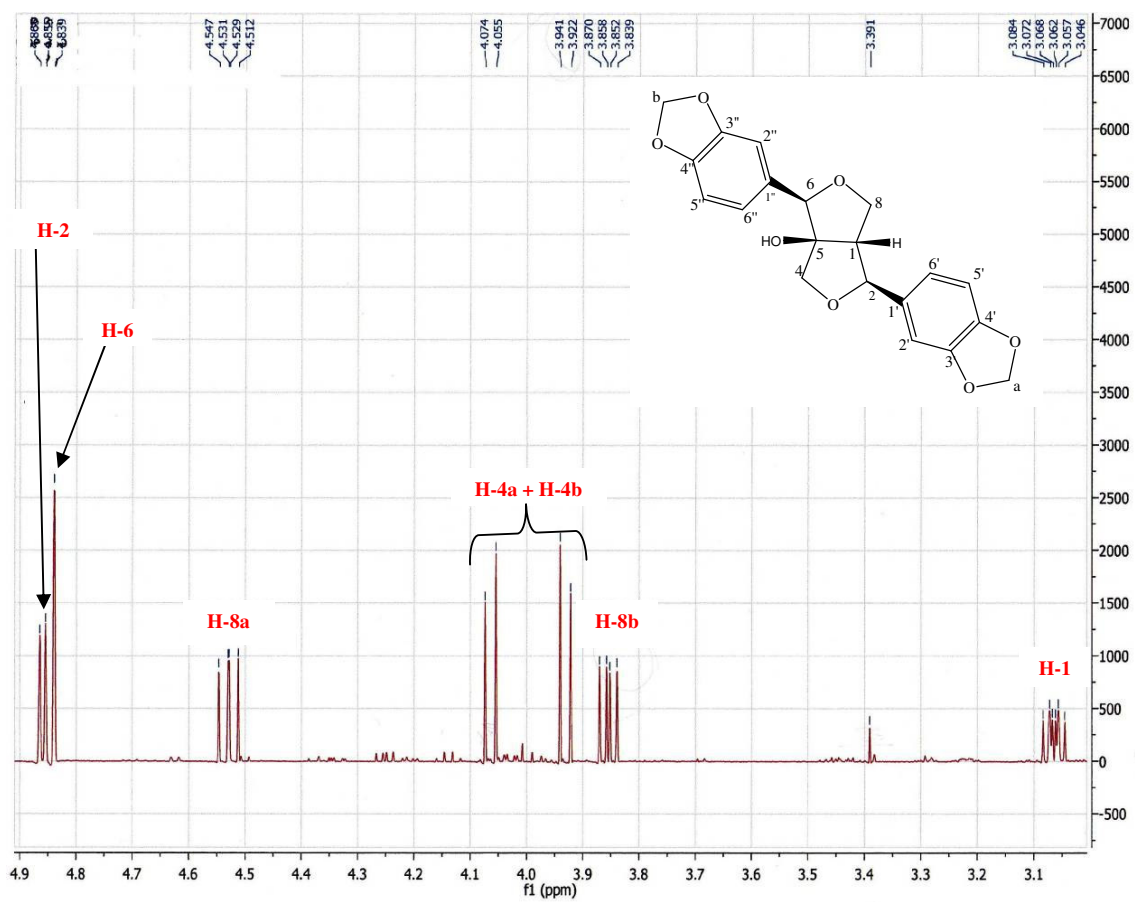


S14: $^1\text{H-NMR}$ (500 MHz, CDCl_3) Spectrum of Compound **4** ((\pm)-paulownin)

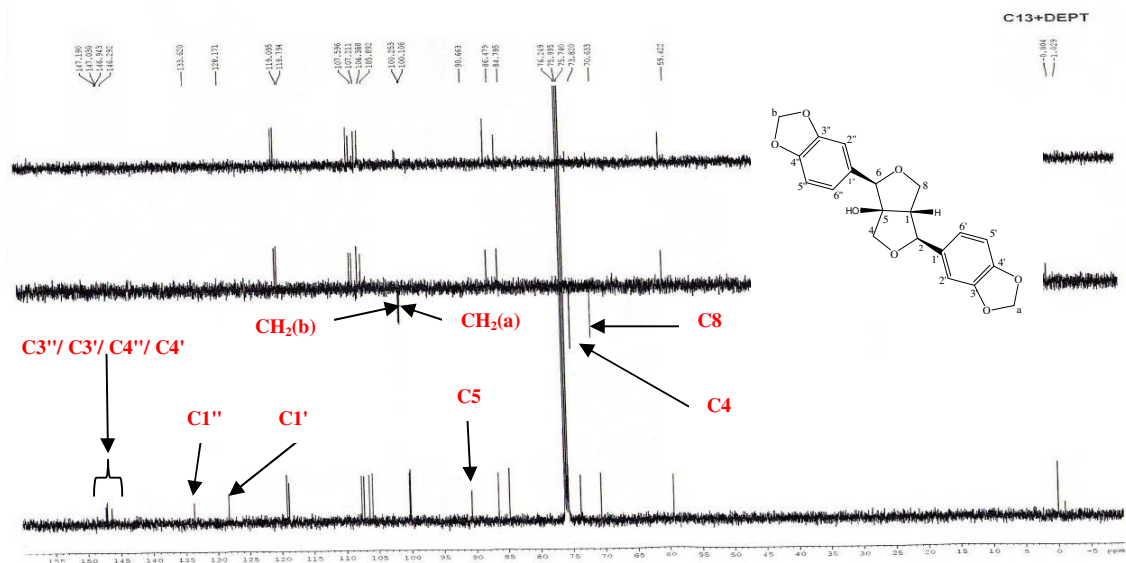
(\pm)-*Paulownin* (**4**): Pink needle crystals, IR (ν_{max} cm^{-1}): 3412 (-OH), 2884. $^1\text{H-NMR}$ (CDCl_3 , 500 MHz), δ : 1.5 (1H, s, OH), 3.06 (1H, ddd, H-1), 3.86 dd (1H, dd, H-8b), 3.93 (1H, d, H-4b), 4.06 (1H, d, H-4a), 4.53 (1H, dd, H-8a), 4.83 (1H, s, H-6), 4.86 (1H, d, H-2), 5.97 (2H, s, CH_2 (a)), 6.00 (2H, s, CH_2 (b)), 6.81 (1H, d, H-5'), 6.86 (1H, d, H-5''), 6.88 (1H, dd, H-6'), 6.89 (1H, dd, H-6''), 6.93 (1H, d, H-2'), 6.96 (1H, d, H-2''). $^{13}\text{C-NMR}$ (CDCl_3 , 125 MHz), δ : 59.4 (C-1), 84.8 (C-2), 73.8 (C-4), 90.7 (C-5), 86.5 (C-6), 70.6 (C-8), 100.1 (CH_2 (a)), 100.2 (CH_2 (b)), 128.1 (C-1'), 133.6 (C-1''), 105.9 (C-2'), 106.4 (C-2''), 147.0 (C-3'), 147.2 (C-3''), 146.3 (C-4'), 146.9 (C-4''), 118.8 (C-5'), 119.0 (C-5''), 107.2 (C-6'), 107.6 (C-6''). EIMS: $m/z = 370$ [M] $^+$ for formula $\text{C}_{20}\text{H}_{18}\text{O}_7$ [5].



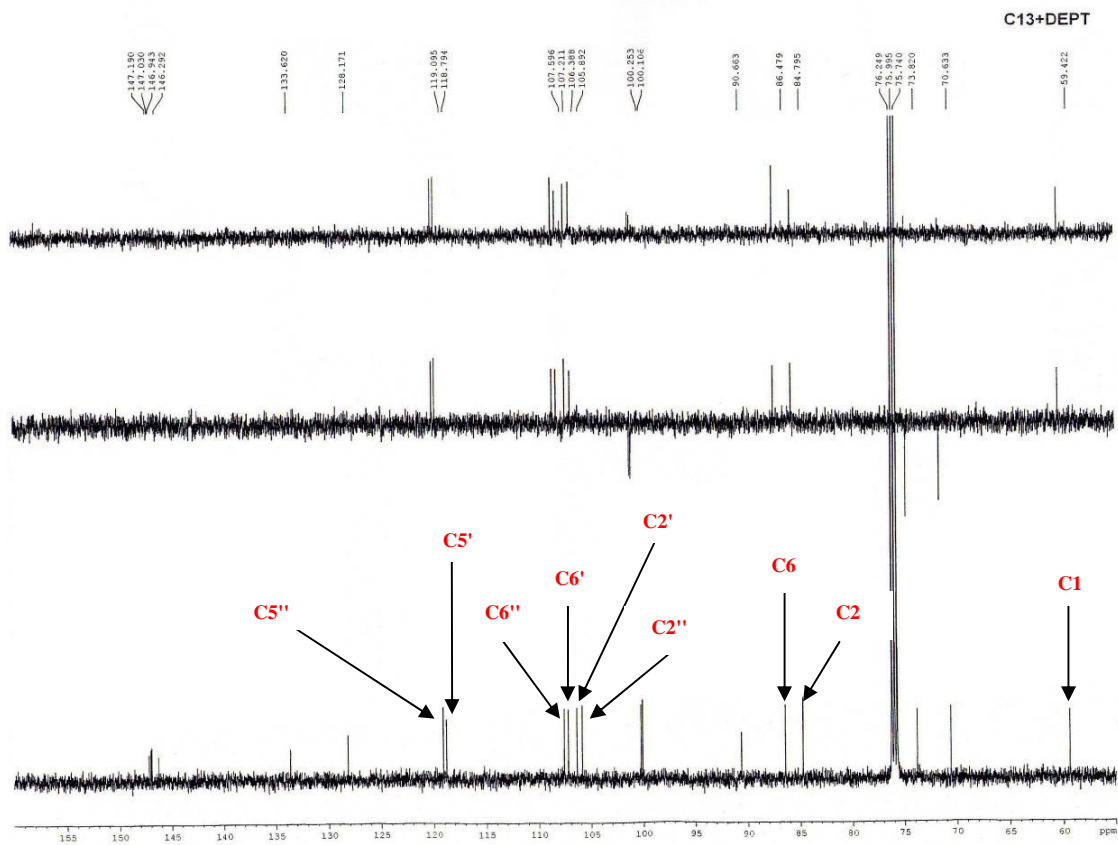
S15: $^1\text{H-NMR}$ Spectrum of Compound 4 ((\pm)-paulownin) (From 5.90 to 7.00 ppm)



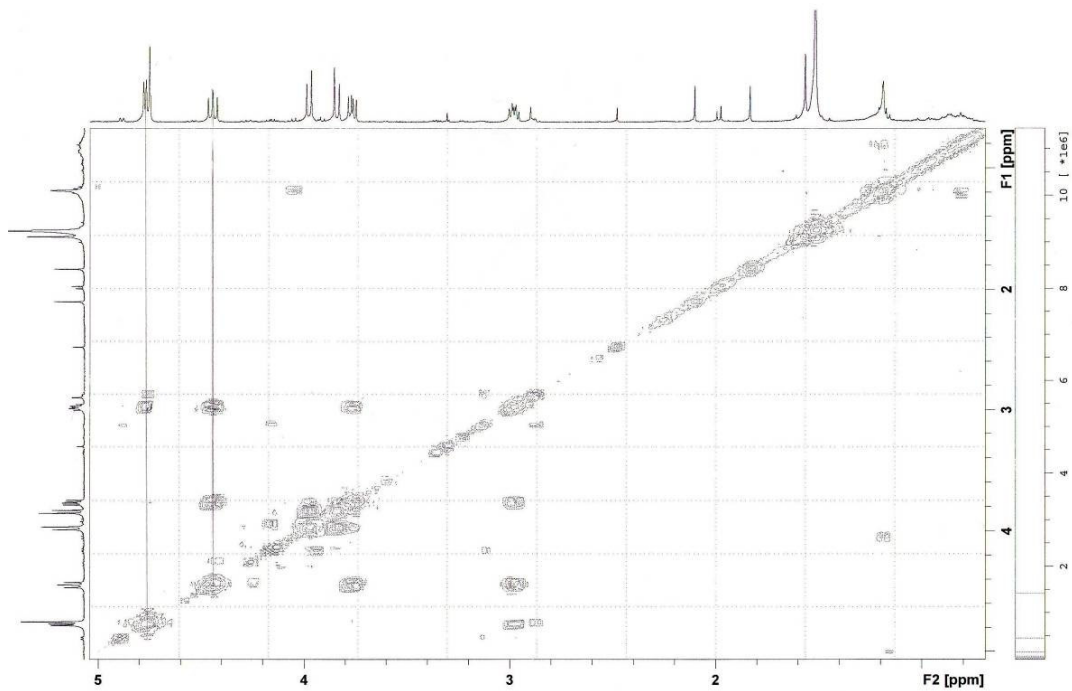
S16: $^1\text{H-NMR}$ Spectrum of Compound 4 ((\pm)-paulownin) (From 3.00 to 4.90 ppm)



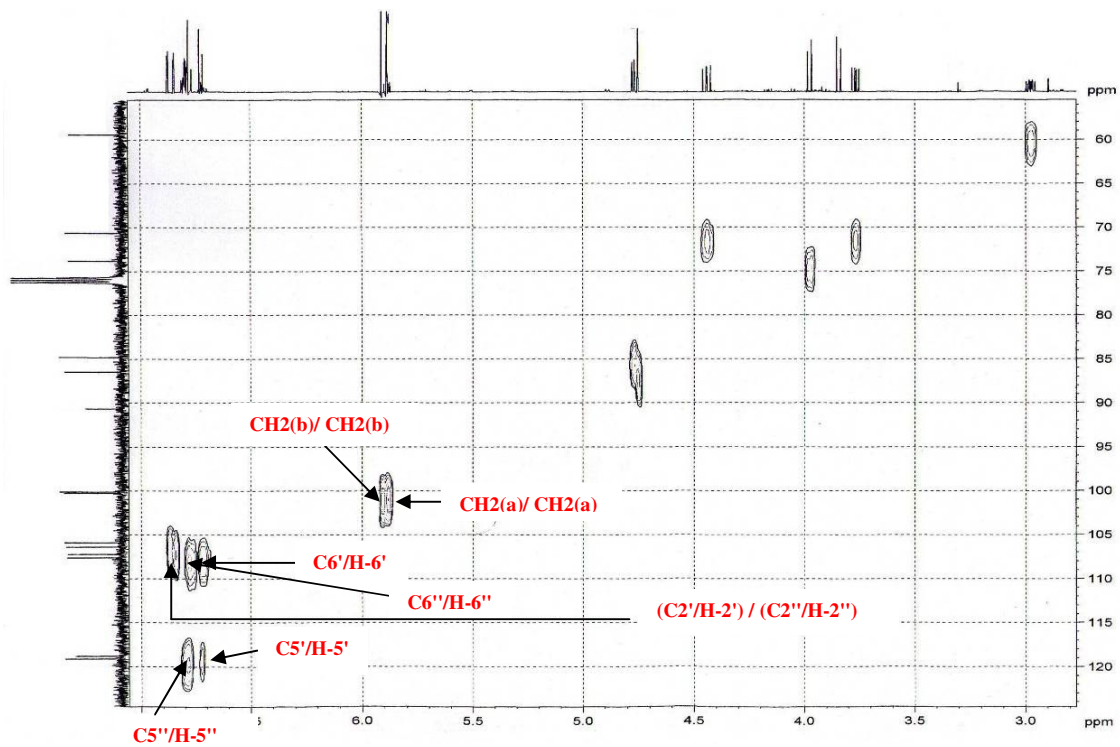
S17: ^{13}C -NMR + DEPT (125 MHz, CDCl_3) Spectrum of Compound **4** ((±)-paulownin)



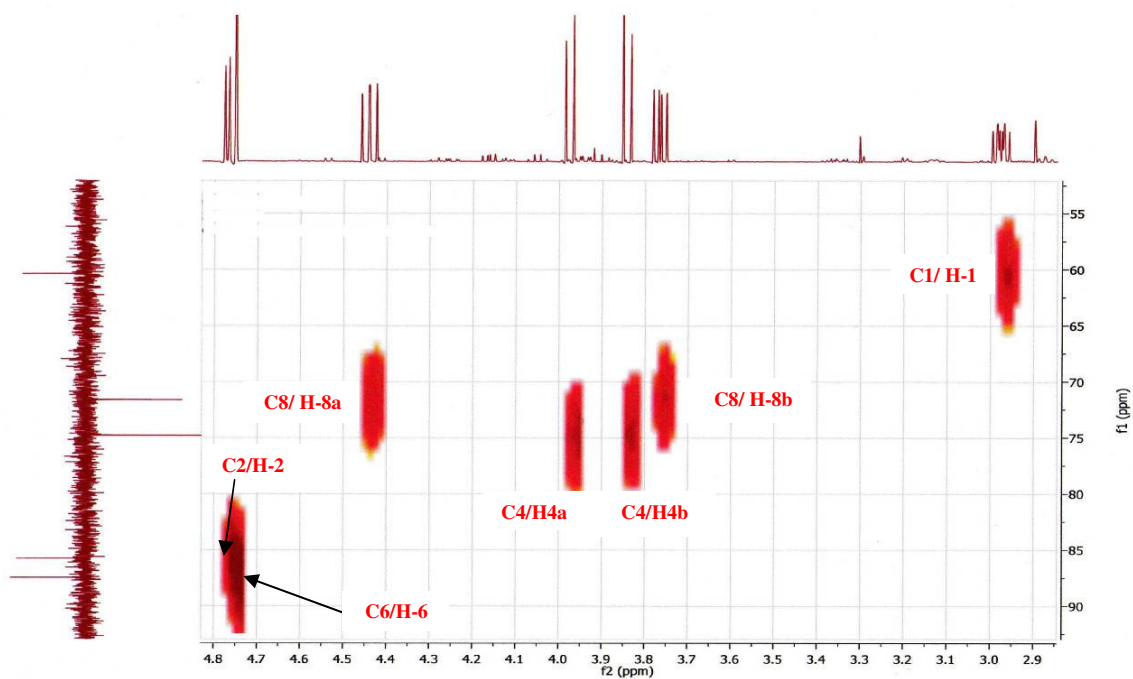
S18: ^{13}C -NMR + DEPT Spectrum of Compound **4** ((\pm)-paulownin) (From 50 to 160 ppm)



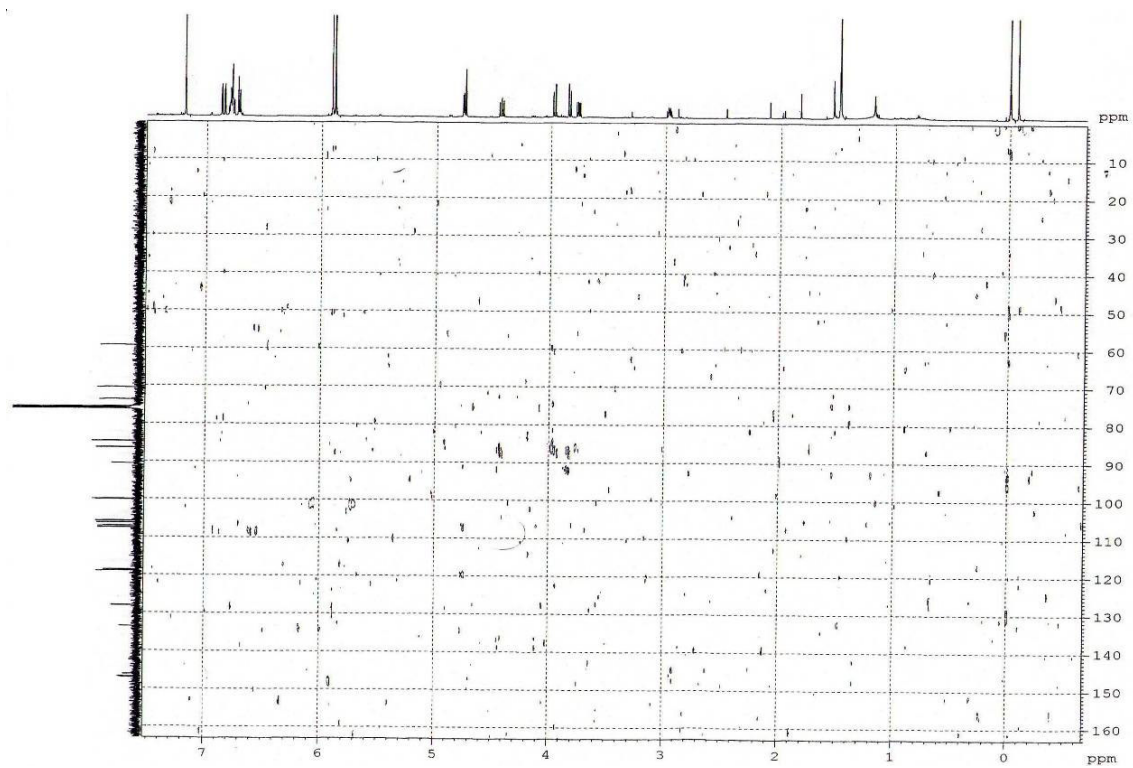
S19: COSY (400 MHz) Spectrum of Compound **4** ((±)-paulownin)



S20: HSQC (500 MHz) Spectrum of Compound 4 ((±)-paulownin) (From 60 to 120 ppm)

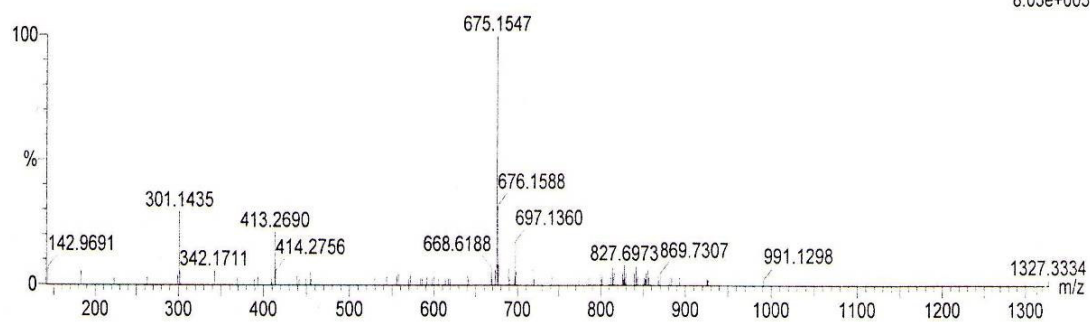


S21: HSQC Spectrum of Compound **4** ((±)-paulownin) (From 55 to 90 ppm)



S22: HMBC (500 MHz) Spectrum of Compound **4** ((±)-paulownin) (From 55 to 90 ppm)

1: TOF MS ES+
8.03e+003

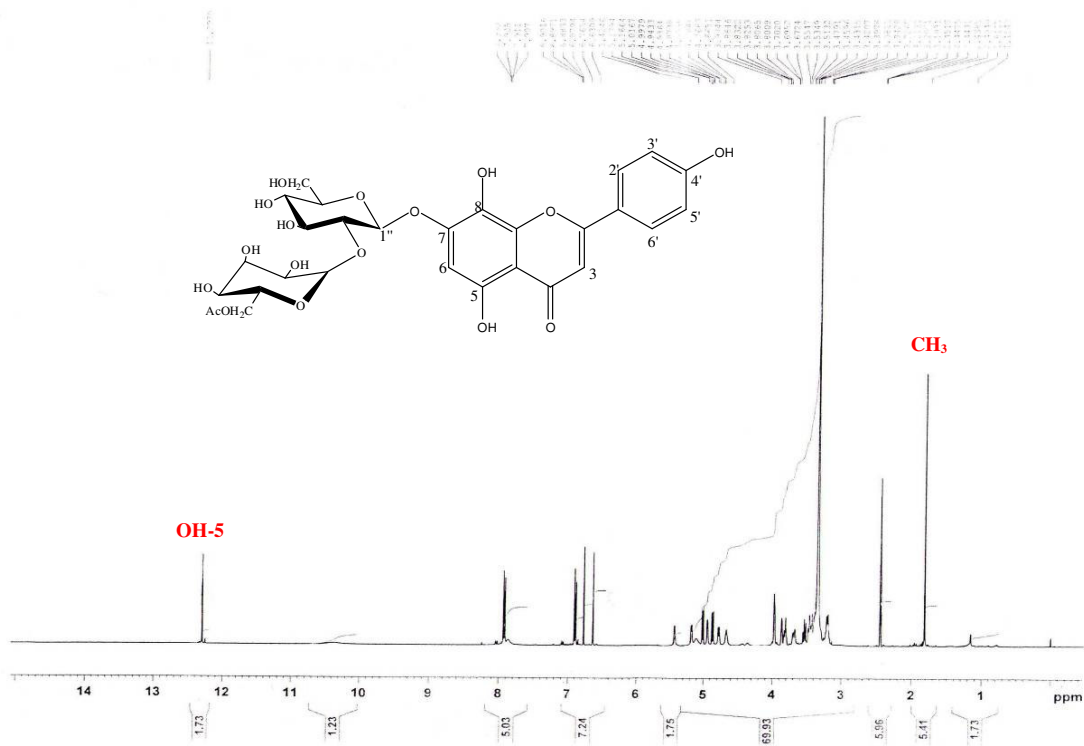


Minimum: 8.00
Maximum: 100.00

| Mass | RA | Calc. Mass | mDa | PPM | DBE | i-FIT | Formula |
|----------|--------|------------|------|------|------|-------|----------------|
| 301.1435 | 29.20 | 301.1440 | -0.5 | -1.7 | 8.5 | n/a | C18 H21 O4 |
| 413.2690 | 21.07 | 413.2692 | -0.2 | -0.5 | 8.5 | n/a | C26 H37 O4 |
| 675.1547 | 100.00 | 675.1537 | 1.0 | 1.5 | 13.5 | 3.2 | C29 H32 O17 Na |

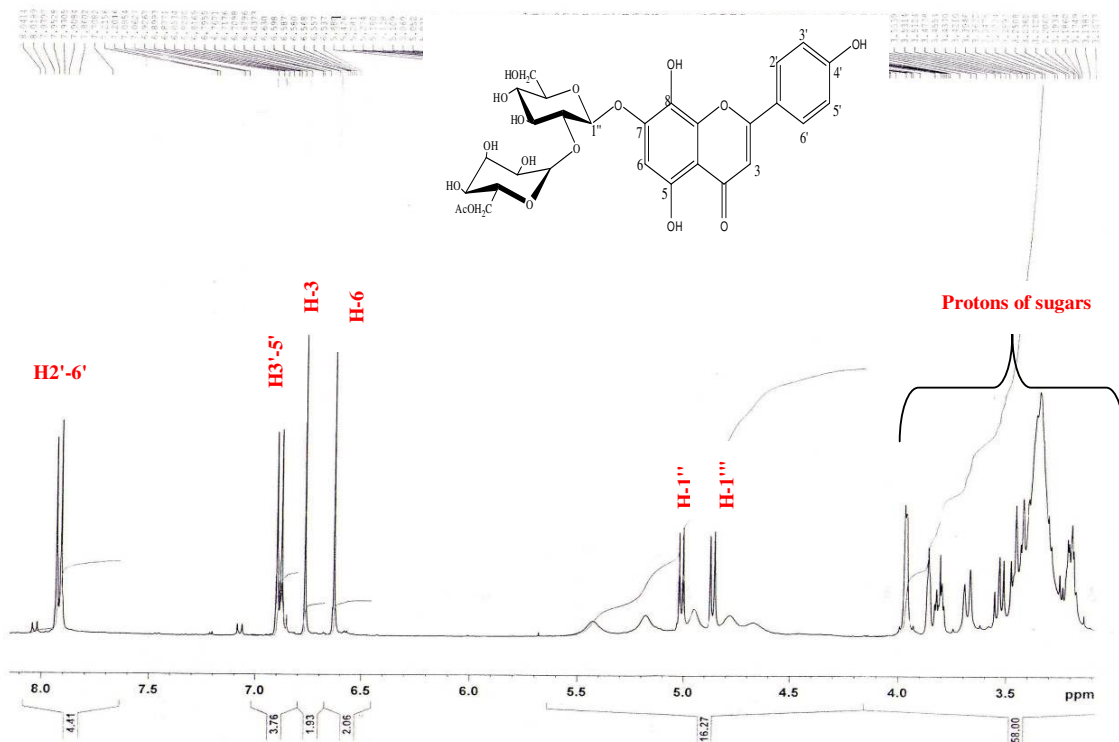
6

S23: HRESIMS Spectrum of Compound **5** (Isoscutellarein-7-*O*-(2"-*O*-6'''-*O*-acetyl- β -D-allopyranosyl- β -D-glucopyranoside)

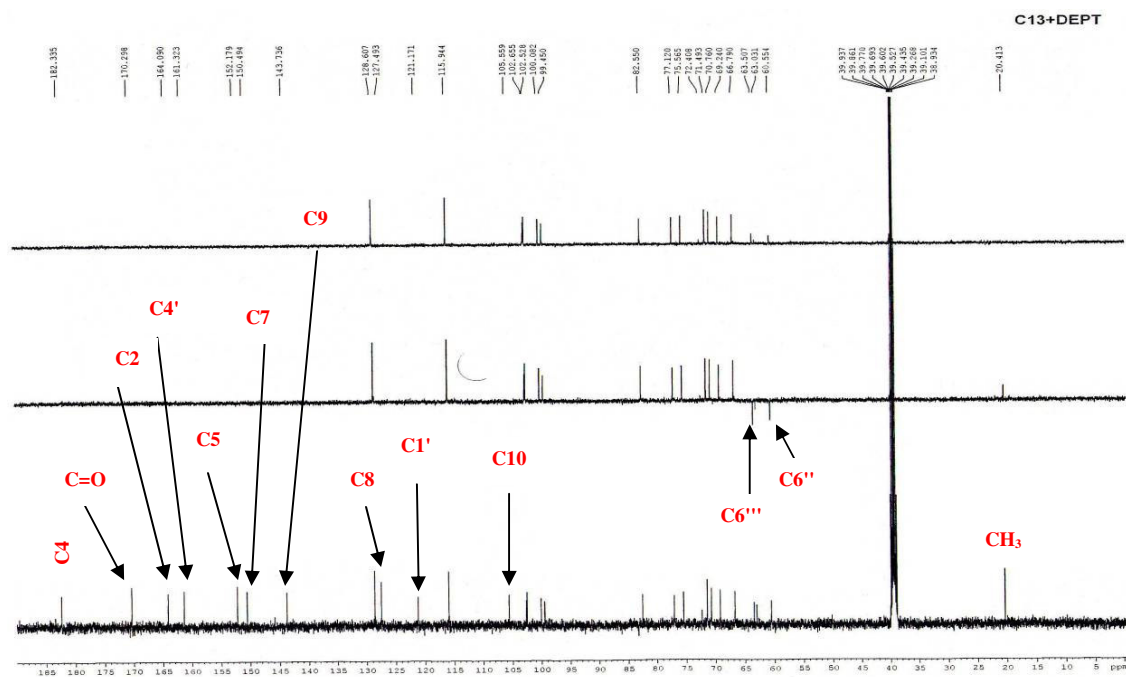


S24: $^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$) Spectrum of Compound **5** (Isoscutellarein-7-*O*-(2''-*O*-6'''-*O*-acetyl- β -D-allopyranosyl- β -D-glucopyranoside)

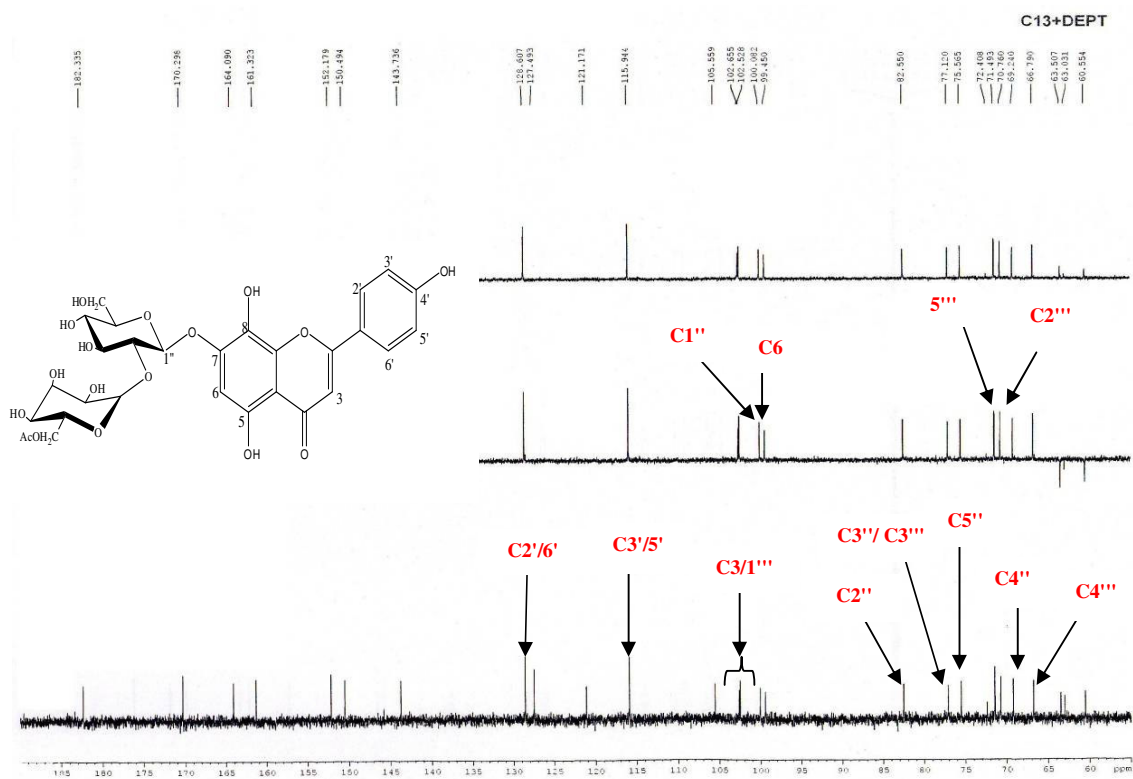
Isoscutellarein 7-O-(2''-O-6'''-O-acetyl- β -D-allopyranosyl- β -D-glucoside (5): Yellow powder. UV (λ_{max} , MeOH, nm): 308, 326, 277; +NaOH: 384 and 275; +NaOAc: 309, 388 and 276; + H_3BO_3 : 309, 327 and 276; + AlCl_3 : 322, 345, 421 and 283; +HCl: 322, 343, 420 and 283. $^1\text{H-NMR}$ ($\text{DMSO-}d_6$, 400 MHz), δ : 1.81 (3H, s, CH_3), 4.87 (1H, d, H-1'''), 5.01 (1H, d, H-1''), 6.63 (1H, s, H-6), 6.76 (1H, s, H-3), 6.89 (2H, d, H-3'/H-5'), 7.95 (2H, d, H-2'/H-6'), 12.29 (s, OH). $^{13}\text{C-NMR}$ ($\text{DMSO-}d_6$, 125 MHz), δ : 164.1 (C-2), 102.6 (C-3), 182.3 (C-4), 152.2 (C-5), 99.4 (C-6), 150.5 (C-7), 127.5 (C-8), 143.7 (C-9), 105.5 (C-10), 121.1 (C-1'), 128.6 (C-2'/C-6'), 115.9 (C-3'/C-5'), 161.3 (C-4'), 100.0 (C-1''), 82.5 (C-2''), 77.1 (C-3''), 69.2 (C-4''), 75.5 (C-5''), 60.5 (C-6''), 102.5 (C-1'''), 70.7 (C-2'''), 77.1 (C-3'''), 66.8 (C-4'''), 71.5 (C-5'''), 63.5 (C-6'''), 20.4 (OCH_3), 170.3 (C=O), HRESIMS: m/z 675.1547 (calc. for $\text{C}_{29}\text{H}_{32}\text{O}_{17}$ Na) [6,7].



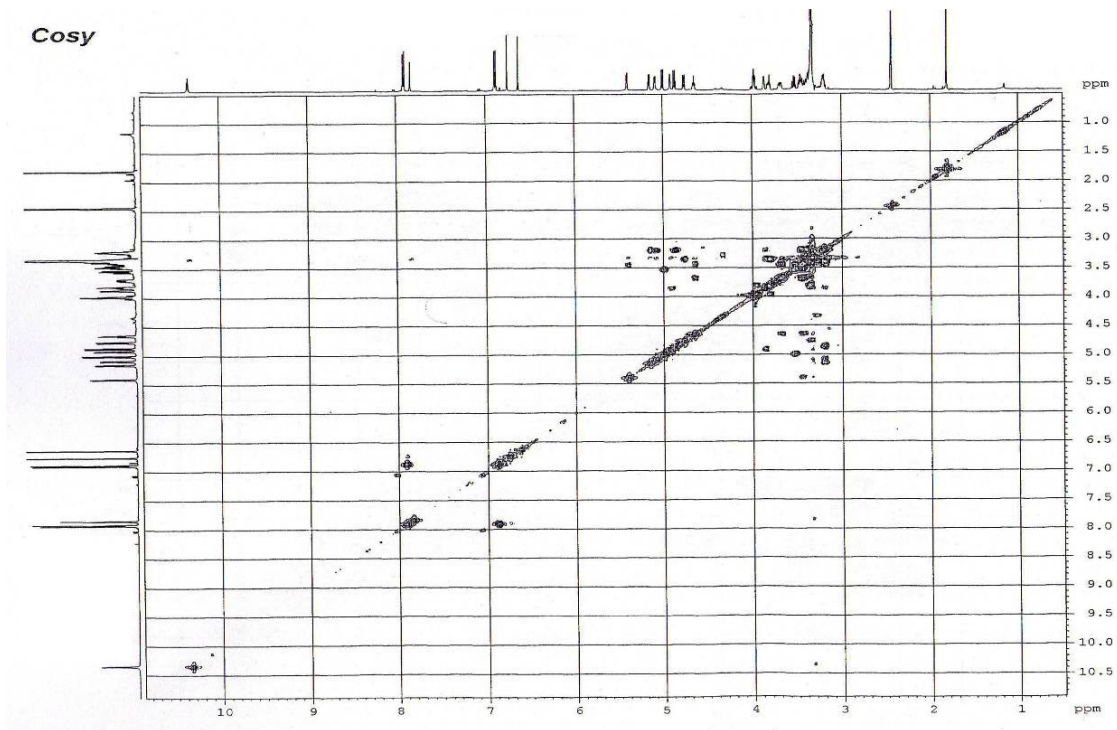
S25: $^1\text{H-NMR}$ Spectrum of Compound **5** (From 3.00 to 8.00 ppm)



S26: ^{13}C -NMR + DEPT (125 MHz, DMSO-*d*₆) Spectrum of Compound 5

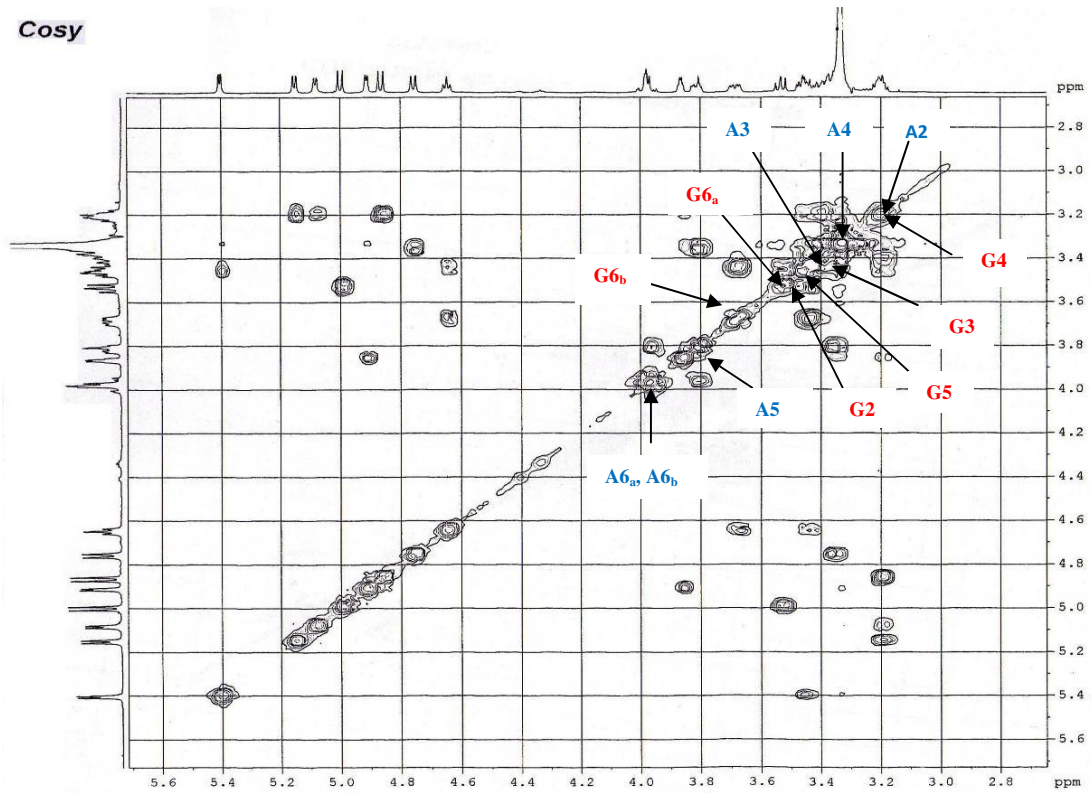


S27: ^{13}C -NMR + DEPT Spectrum of Compound **5** (From 55 to 185 ppm)

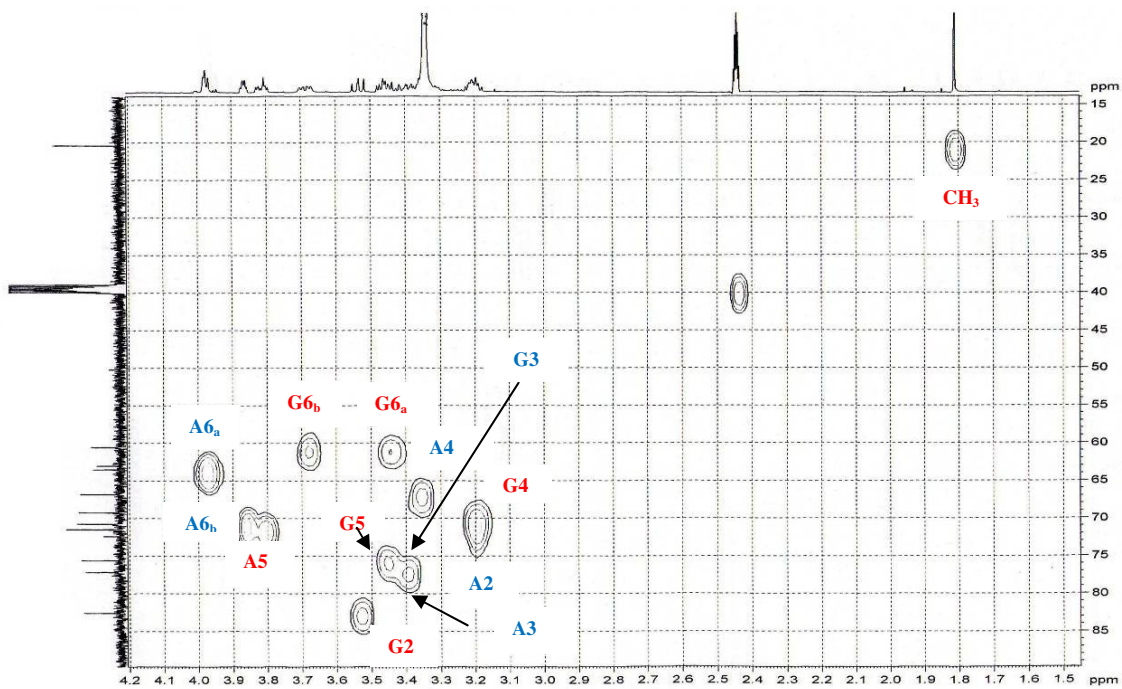


S28: COSY (500 MHz) Spectrum of Compound **5**

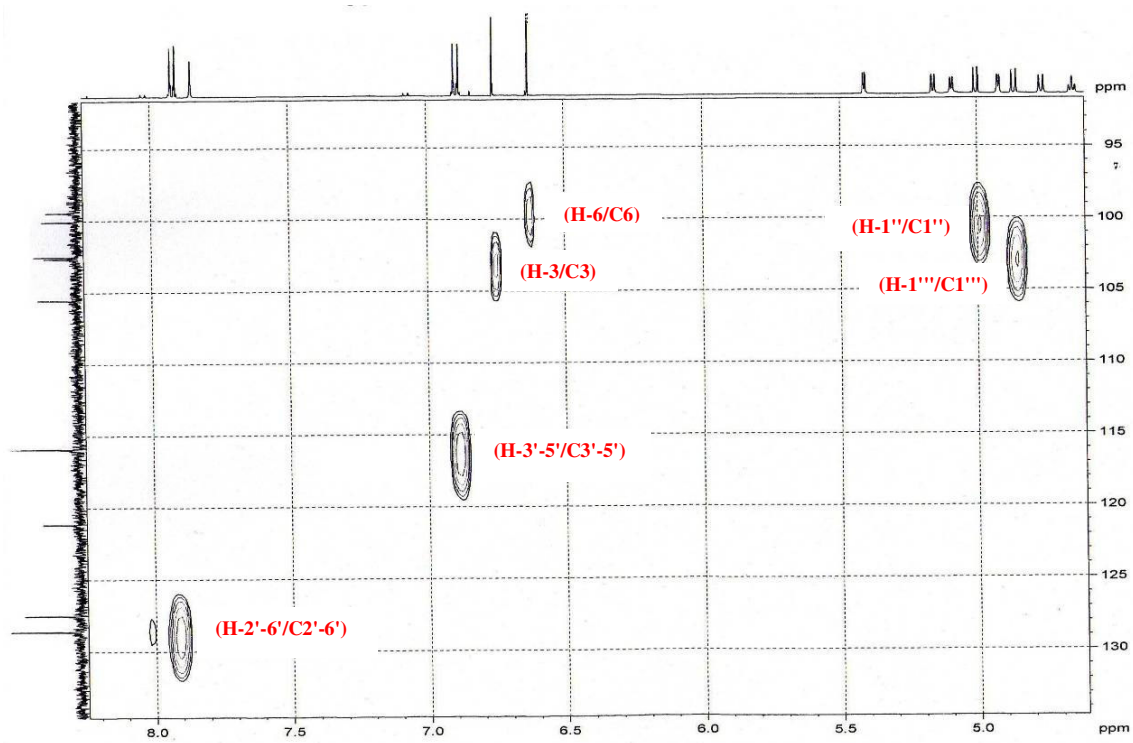
Cosy



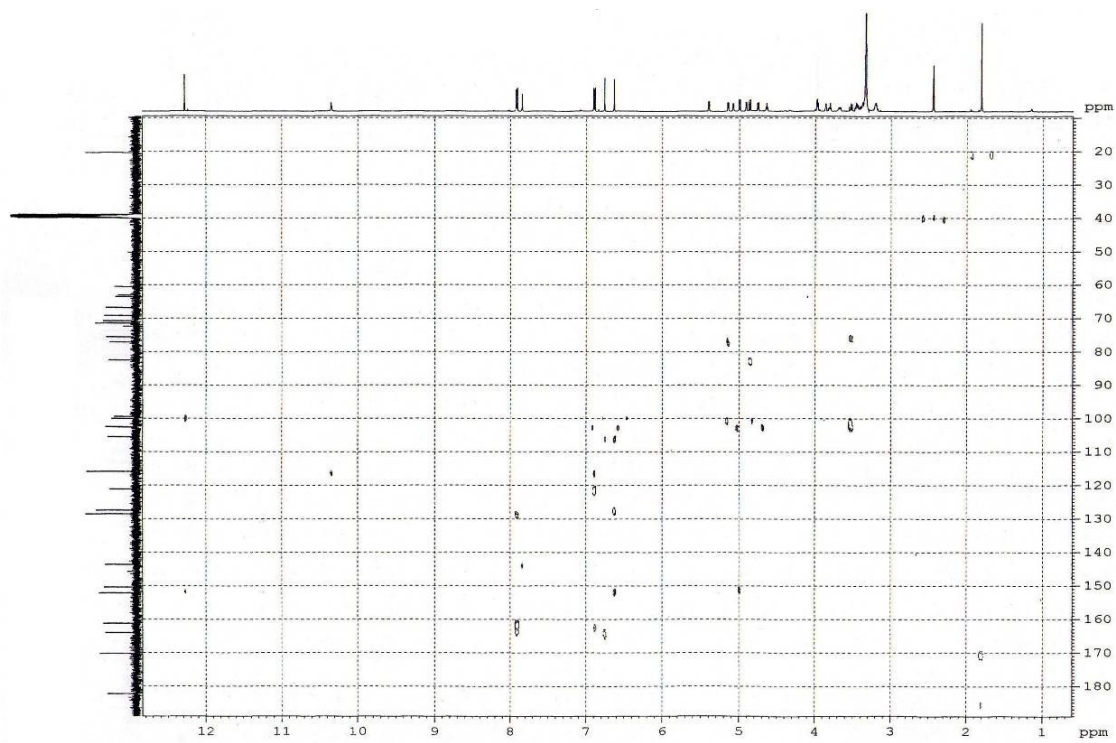
S29: COSY Spectrum of Compound 5 (From 2.8 to 5.6 ppm)



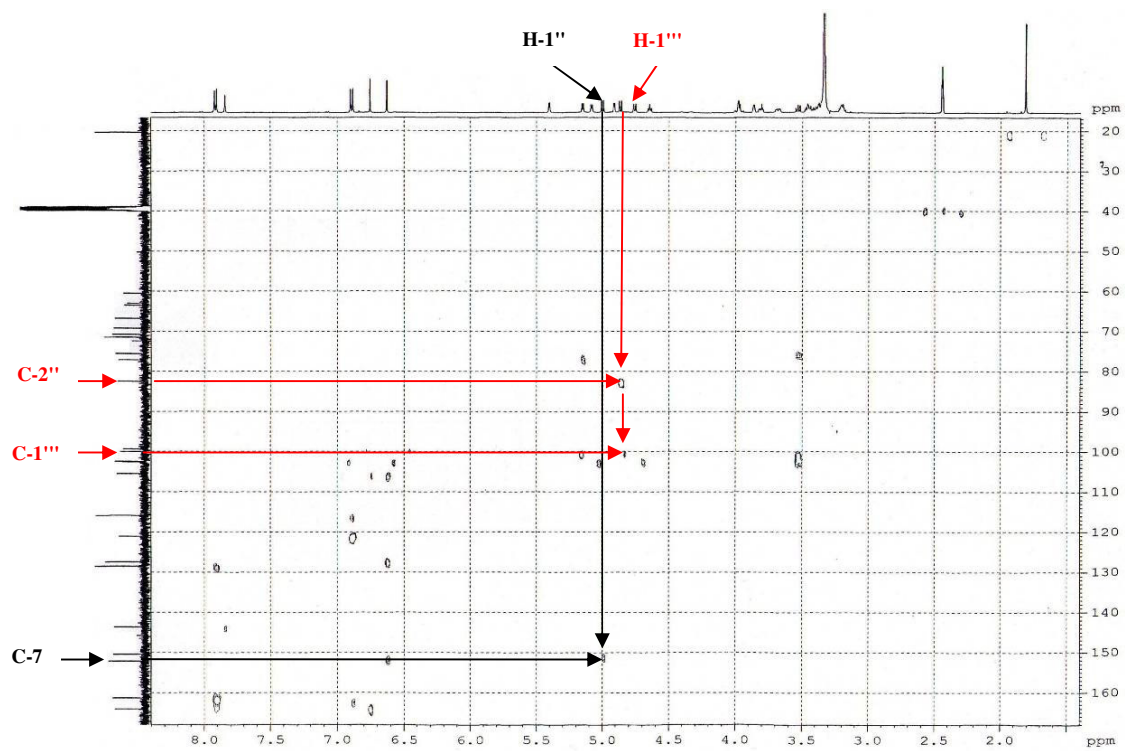
S30: HSQC Spectrum of Compound 5 (From 15 to 85 ppm)



S31: HSQC Spectrum of Compound **5** (From 95 to 130 ppm)



S32: HMBC (500 MHz) Spectrum of Compound 5



S33: HMBC Spectrum of Compound 5 (From 20 to 160 ppm)