

*Supplementary Information*

***In Vitro* Opioid Receptor Affinity and *in Vivo* Behavioral Studies of *Nelumbo nucifera* Flower**

Mallika Kumarihamy<sup>a</sup>, Francisco León<sup>b</sup>, Sara Pettaway<sup>b</sup>, Lisa Wilson<sup>b</sup>, Janet Lambert<sup>b</sup>, Mei Wang<sup>a</sup>, Christopher Hill<sup>a</sup>, Christopher R. McCurdy<sup>b</sup>, Mahmoud ElSohly<sup>a,c</sup>, Stephen J. Cutler<sup>a,b</sup>, Ilias Muhammad<sup>a,\*</sup>

<sup>a</sup>*National Center for Natural Products Research, School of Pharmacy, Research Institute of Pharmaceutical Sciences, University of Mississippi, University, MS 38677, USA*

<sup>b</sup>*Department of BioMolecular Sciences, School of Pharmacy, Research Institute of Pharmaceutical Sciences, University of Mississippi, University, MS 38677, USA*

<sup>c</sup>*Department of Pharmaceutics and Drug Delivery, School of Pharmacy, Research Institute of Pharmaceutical Sciences, University of Mississippi, University, MS 38677, USA*

-----  
\* Corresponding author. Tel.: +1 662 915 1051; fax: +1 662 915 1006.  
E-mail address: [milias@olemiss.edu](mailto:milias@olemiss.edu) (I. Muhammad).

## Table of Content

	Page
Figure 1S. <sup>1</sup> H NMR spectrum of nuciferine (1).	4
Figure 2S. <sup>13</sup> C NMR spectrum of nuciferine (1).	5
Figure 3S. <sup>1</sup> H NMR spectrum of <i>nor</i> -nuciferine (2).	6
Figure 4S. <sup>13</sup> C NMR spectrum of <i>nor</i> -nuciferine (2).	7
Figure 5S. <sup>1</sup> H NMR spectrum of asimilobine (3).	8
Figure 6S. <sup>13</sup> C NMR spectrum of asimilobine (3).	9
Figure 7S. <sup>1</sup> H NMR spectrum of armepavine (4).	10
Figure 8S. <sup>13</sup> C NMR spectrum of armepavine (4).	11
Figure 9S. <sup>1</sup> H NMR spectrum of <i>O</i> -methylcoclaurine (5).	12
Figure 10S. <sup>13</sup> C NMR spectrum of <i>O</i> -methylcoclaurine (5).	13
Figure 11S. <sup>1</sup> H NMR spectrum of <i>N</i> -methylcoclaurine (6).	14
Figure 12S. <sup>13</sup> C NMR spectrum of <i>N</i> -methylcoclaurine (6).	15
Figure 13S. <sup>1</sup> H NMR spectrum of coclaurine (7).	16
Figure 14S. <sup>13</sup> C NMR spectrum of coclaurine (7).	17
Figure 15S. <sup>1</sup> H NMR spectrum of higenamine (8).	18
Figure 16S. <sup>13</sup> C NMR spectrum of higenamine (11).	19
Figure 17S. <sup>1</sup> H NMR spectrum of higenamine derivative 12.	20
Figure 18S. <sup>1</sup> H NMR spectrum of higenamine derivative 13.	21
Figure 19S. UHPLC-UV (280 nm) chromatogram of basic partition of <i>N. nucifera</i> white flower	22
Figure 20S. UHPLC- ESI Positive chromatogram of basic partition of <i>N. nucifera</i> white flower	23
Figure 21S. UHPLC-UV (280 nm) chromatogram of basic partition of <i>N. nucifera</i> pink flower	24
Figure 22S. UHPLC- ESI Positive chromatogram of basic partition of <i>N. nucifera</i> pink flower.	25
Figure 23S. UHPLC-UV (280 nm) chromatogram of acidic partition of <i>N. nucifera</i> white flower	26
Figure 24S. UHPLC- ESI Positive chromatogram of acidic partition of <i>N. nucifera</i> white flower	27
Figure 25S. UHPLC-UV (280 nm) chromatogram of acidic partition of <i>N. nucifera</i> pink flower	28
Figure 26S. UHPLC- ESI Positive chromatogram of acidic partition of <i>N. nucifera</i> pink flower	29

Figure 27S. GC/MS analysis of palmitic acid Me ester (Std)	30
Figure 28S. GC/MS analysis of linolic acid Me ester (Std)	30
Figure 29S. GC/MS analysis of acidic patition (white flower)	30
Figure 30S. GC/MS analysis of acidic patition (pink flower)	30
Figure 31S. GC/MS analysis of fraction G	31
Figure 32S. GC/MS analysis of fraction H	32
Figure 33S. <i>In vivo</i> mouse tetrad assay of acidic partition of <i>N. nucifera</i>	33
Figure 34S. <i>In vivo</i> mouse tetrad assay of basic partition of <i>N. nucifera</i>	34
Figure 35S. <i>In vivo</i> mouse tetrad assay of mixture of compound <b>5-7</b> of <i>N. nucifera</i>	35
Figure 36S. <i>In vivo</i> mouse tetrad assay of coclaurine ( <b>7</b> )	36
Figure 37S. <i>In vivo</i> mouse tetrad assay of nuciferine ( <b>1</b> )	37
Figure 38S. Structure of alkaloids and their analogs from <i>N. nucifera</i>	38

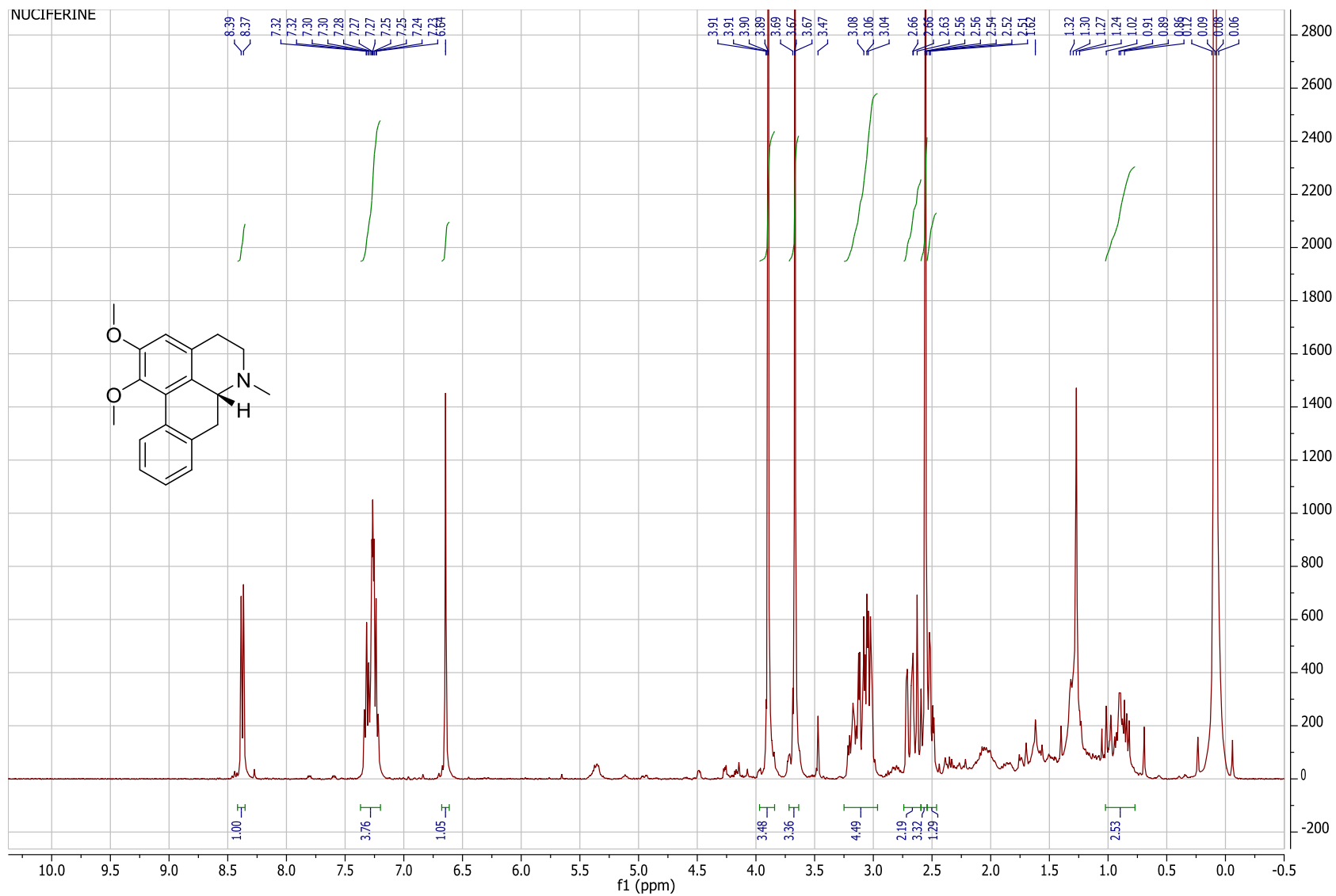
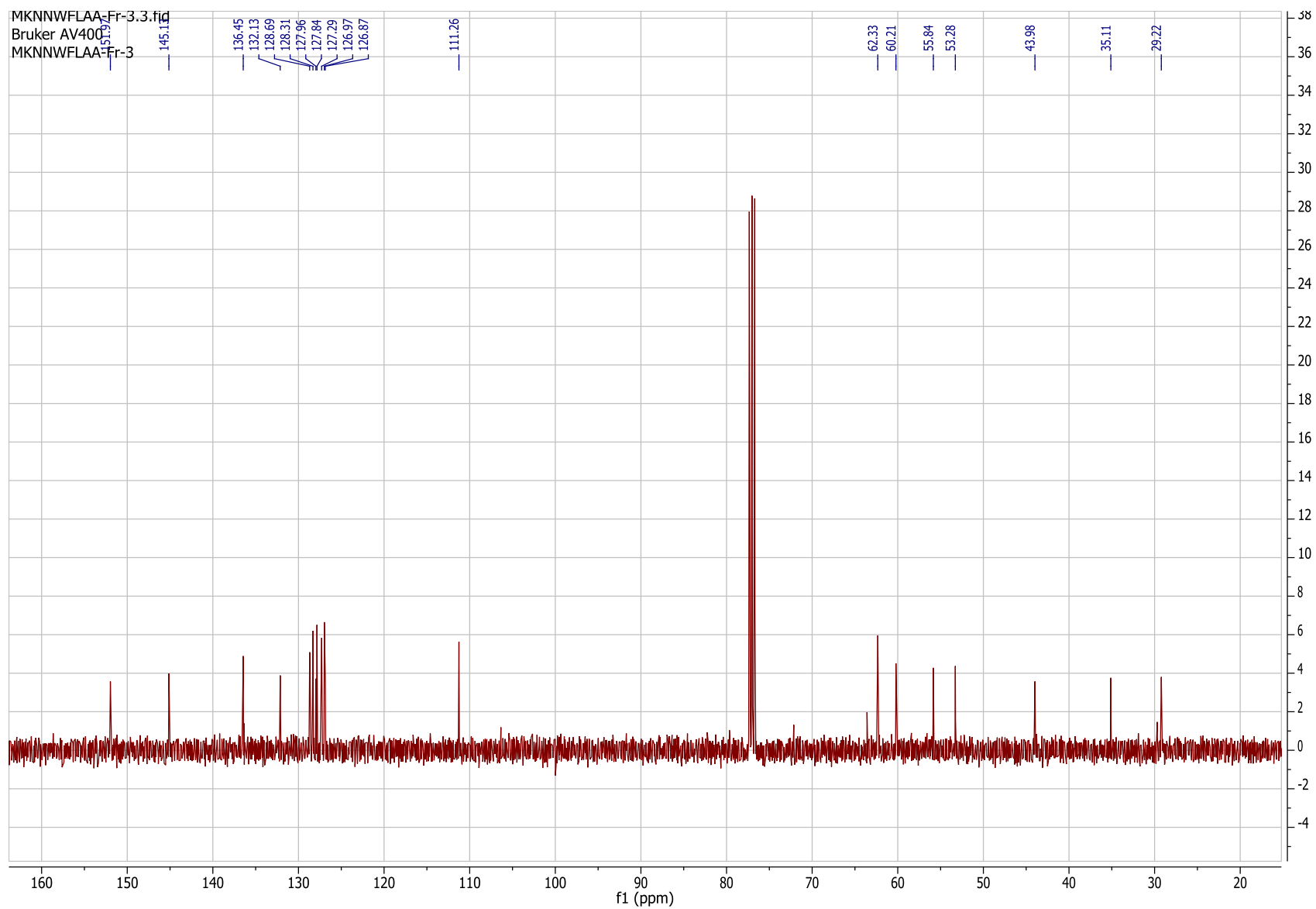


Figure 1S.  $^1\text{H}$  NMR spectrum of nuciferine (1).



**Figure 2S.**  $^{13}\text{C}$  NMR spectrum of nuciferine (**1**).

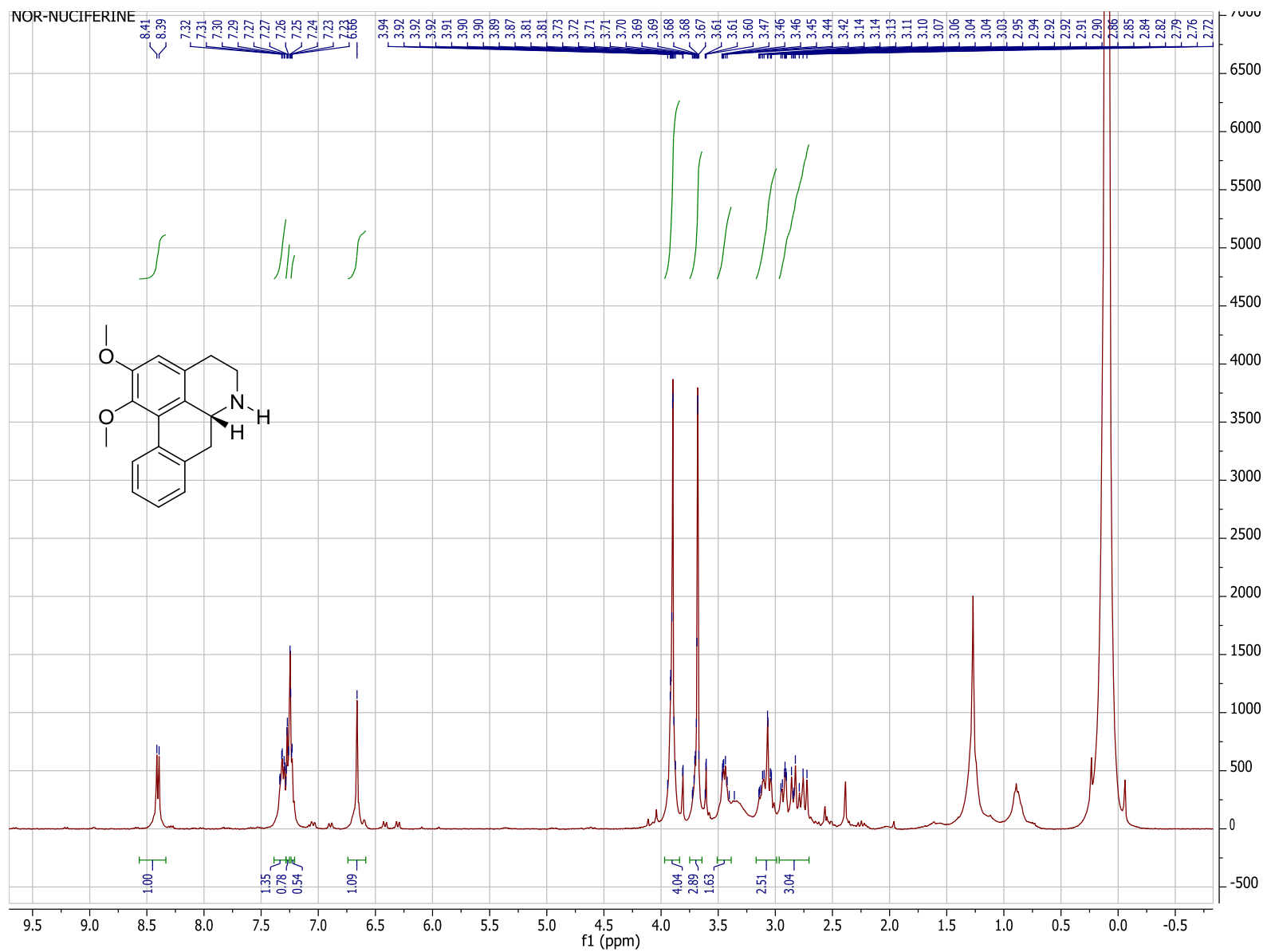
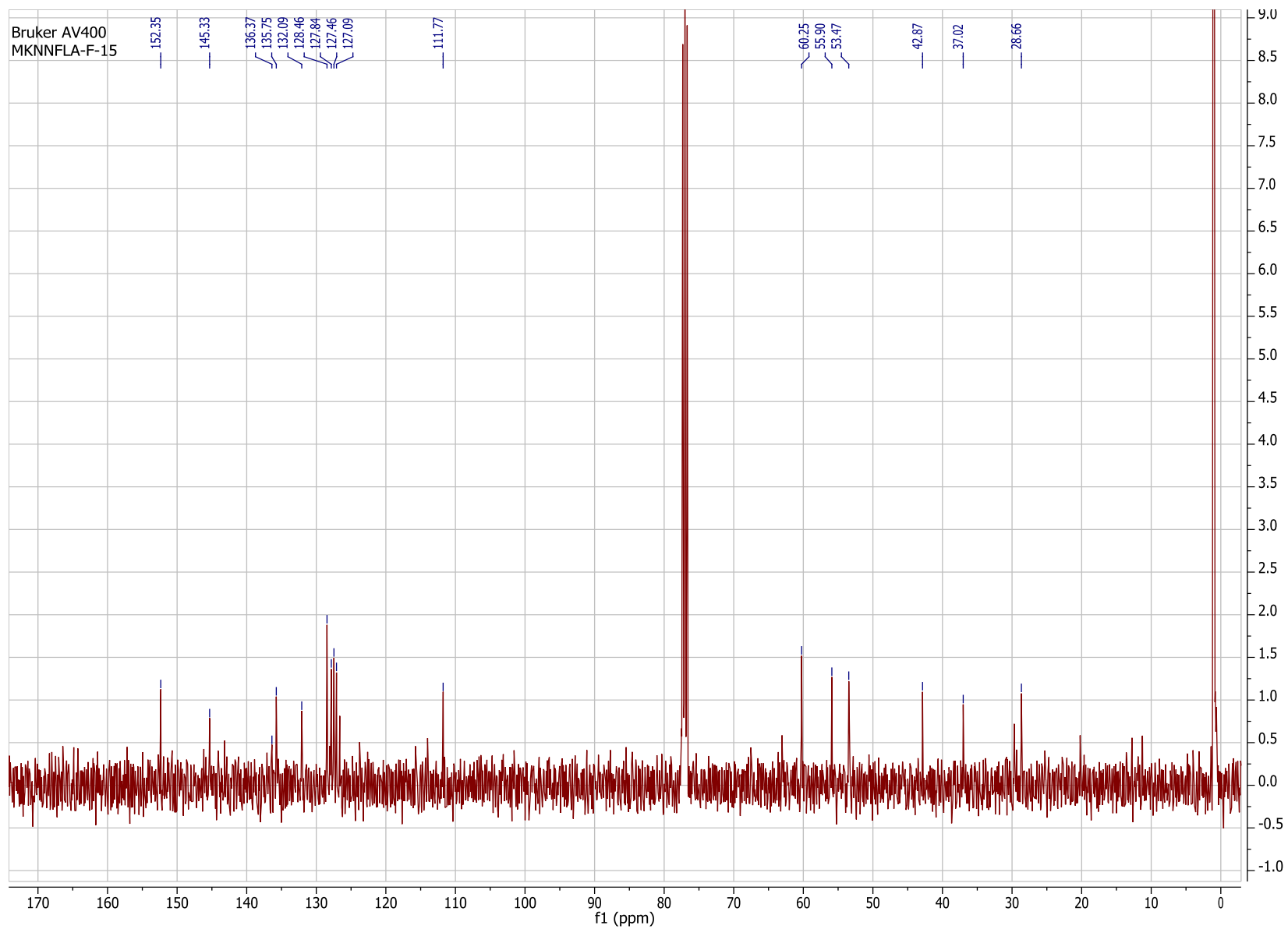


Figure 3S.  $^1\text{H}$  NMR spectrum of nor-nuciferine (2).



**Figure 4S.**  $^{13}\text{C}$  NMR spectrum of nor-nuciferine (**2**).

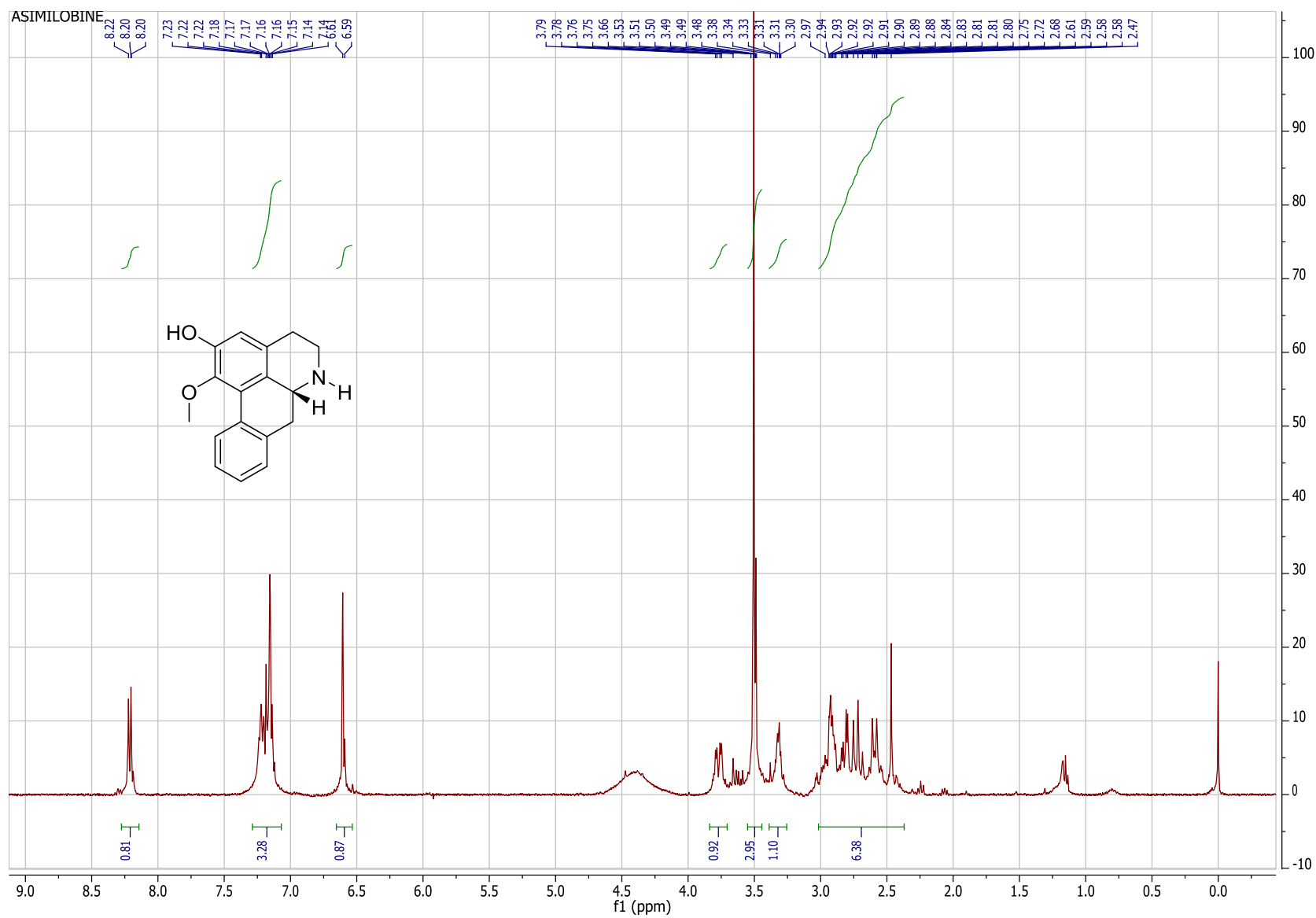
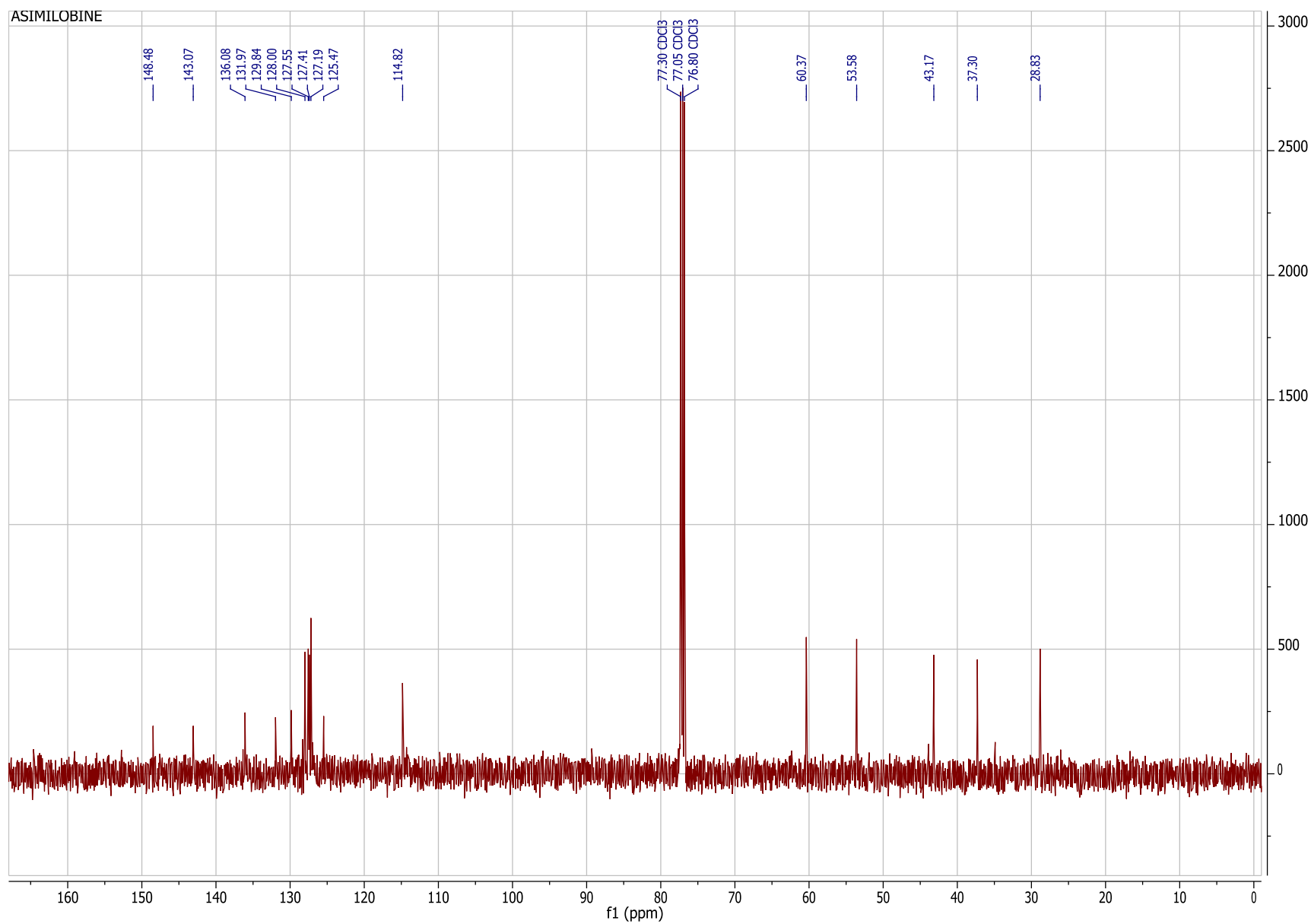


Figure 5S. <sup>1</sup>H NMR spectrum of asimilobine (3).





**Figure 6S.** <sup>13</sup>C NMR spectrum of asimilobine (**3**).

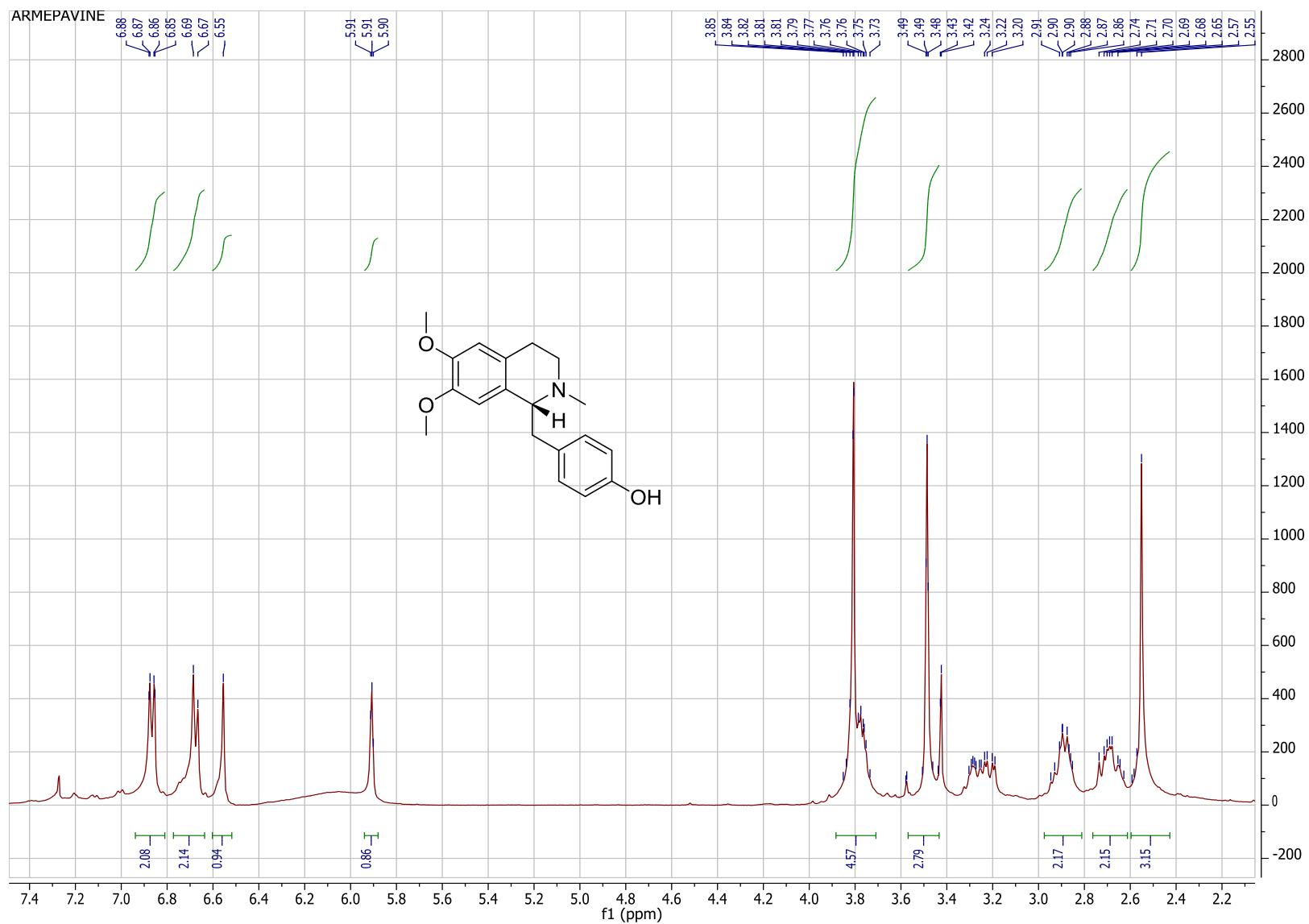
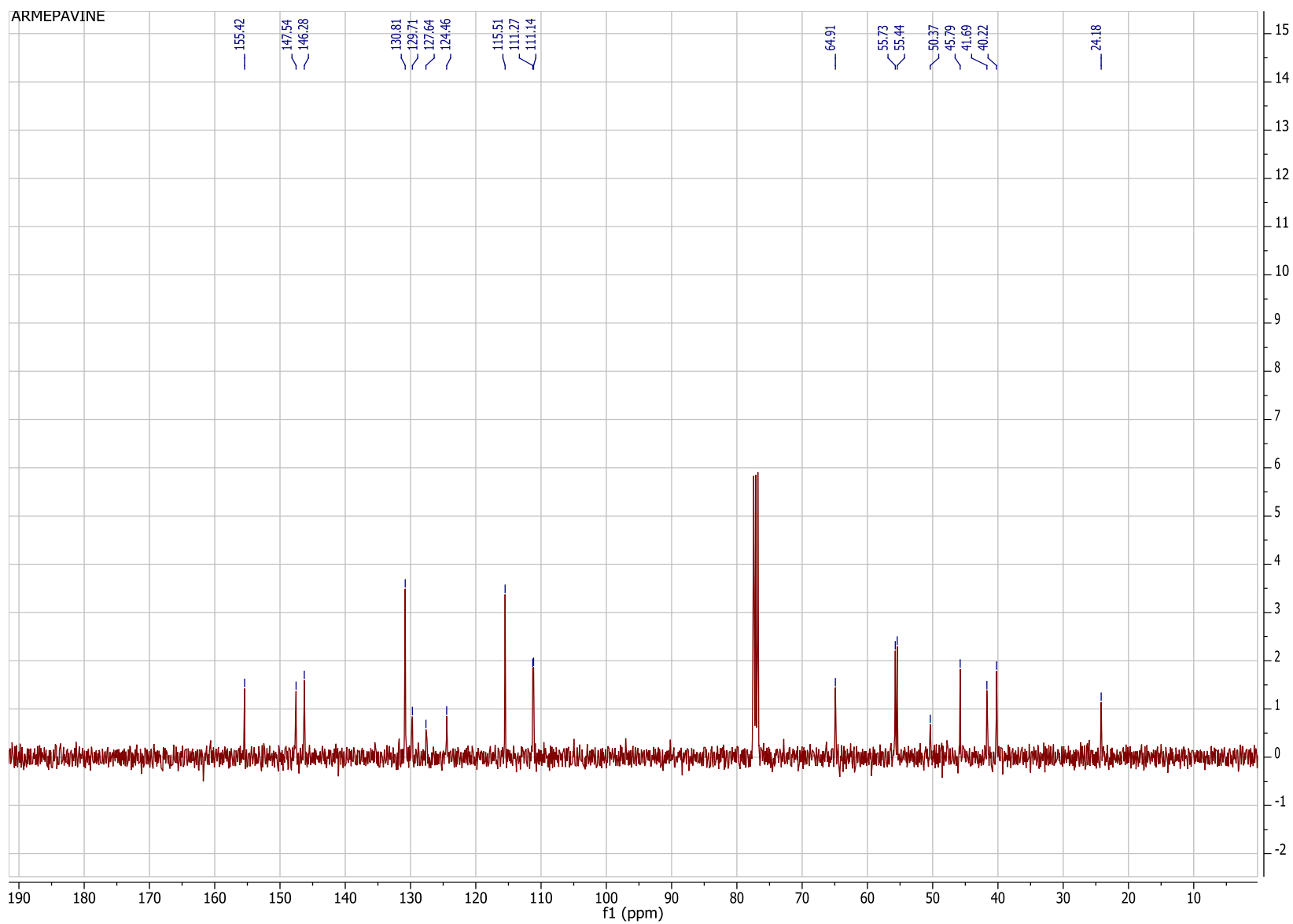


Figure 7S. <sup>1</sup>H NMR spectrum of armapavine (4).



**Figure 8S.**  $^{13}\text{C}$  NMR spectrum of arnepavine (**4**).

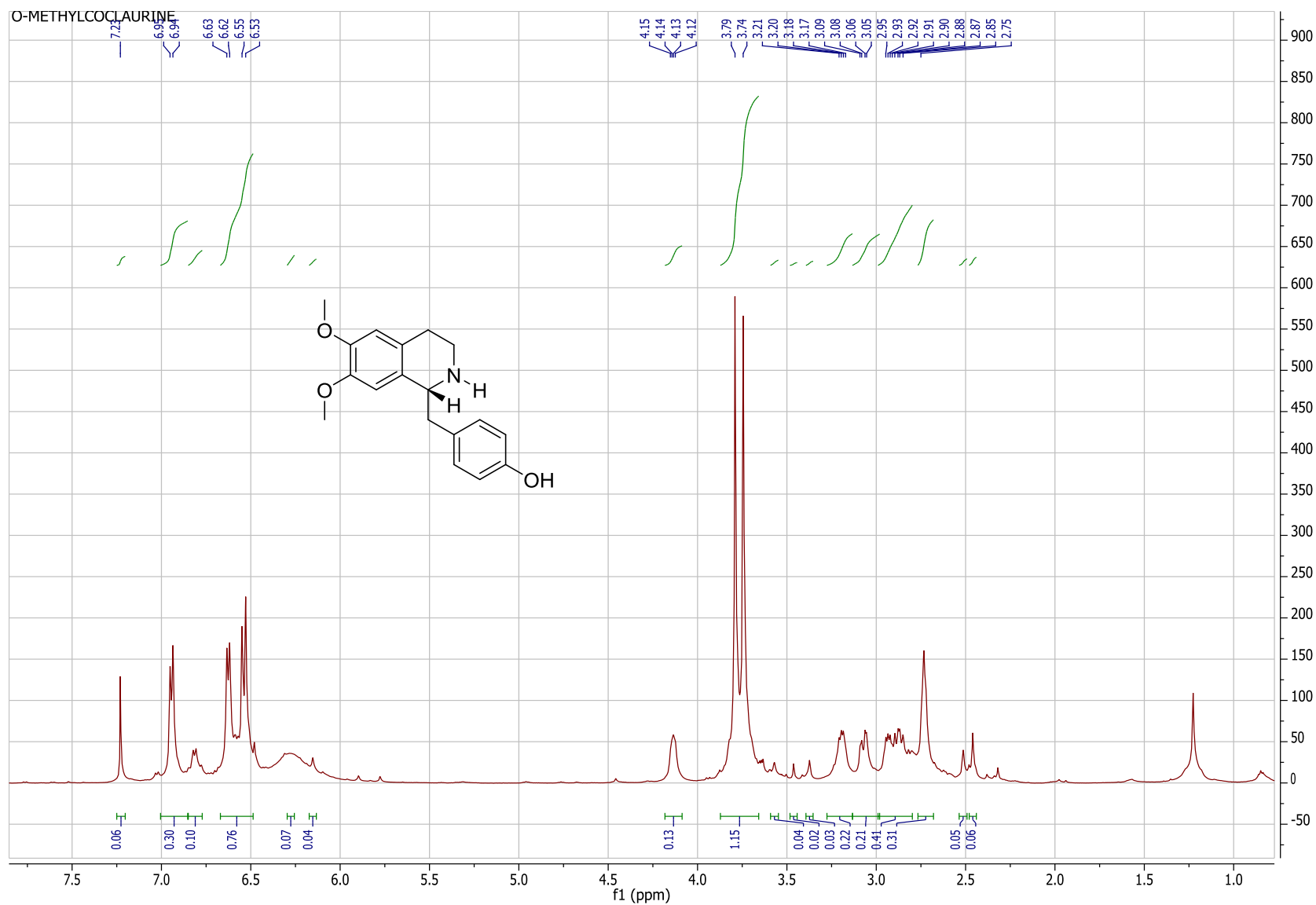
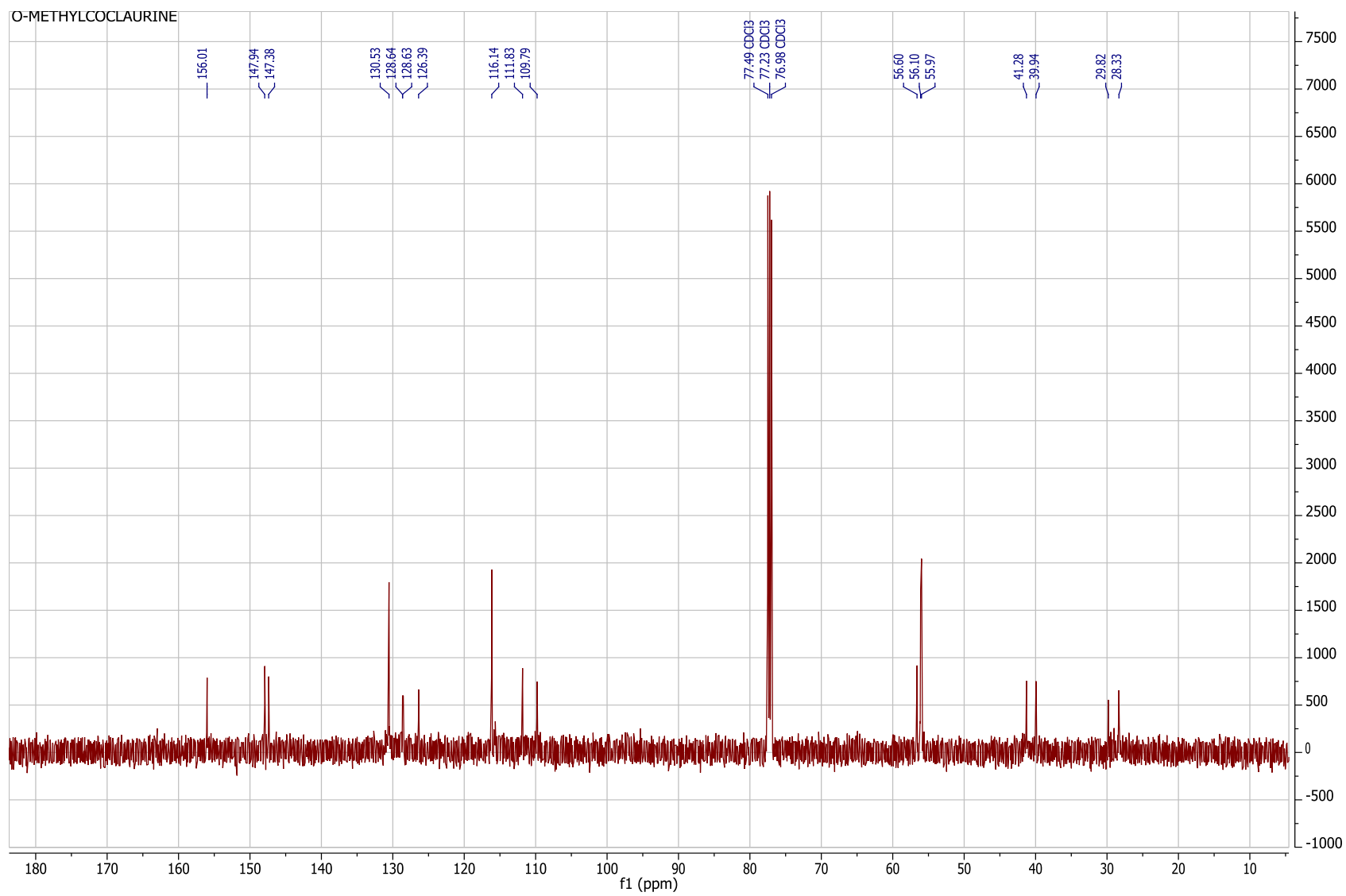


Figure 9S. <sup>1</sup>H NMR spectrum of *O*-methylcoclaurine (5).



**Figure 10S.**  $^{13}\text{C}$  NMR spectrum of *O*-methylcoclaurine (**5**).

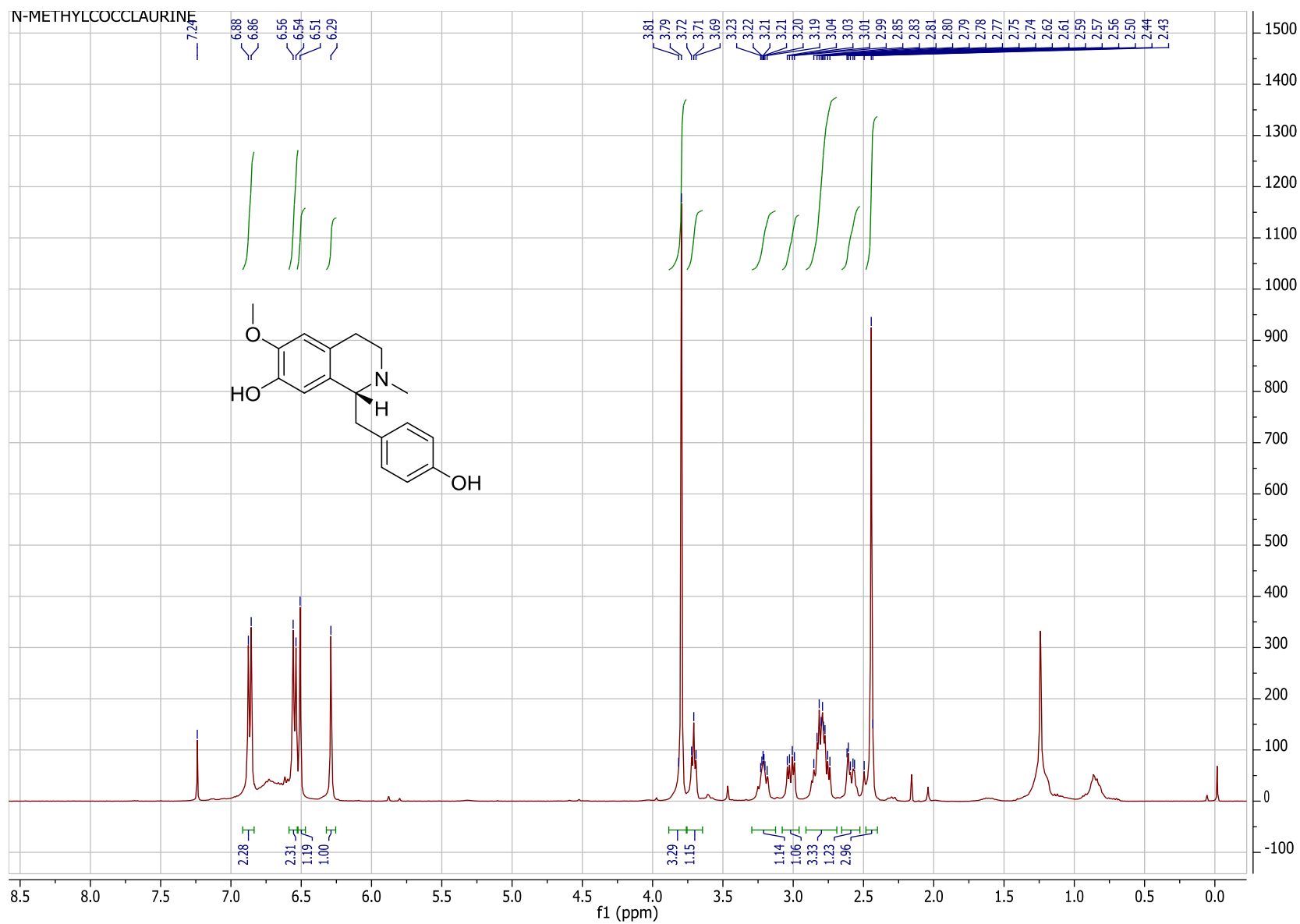


Figure 11S. <sup>1</sup>H NMR spectrum of *N*-methylcocclaurine (6).

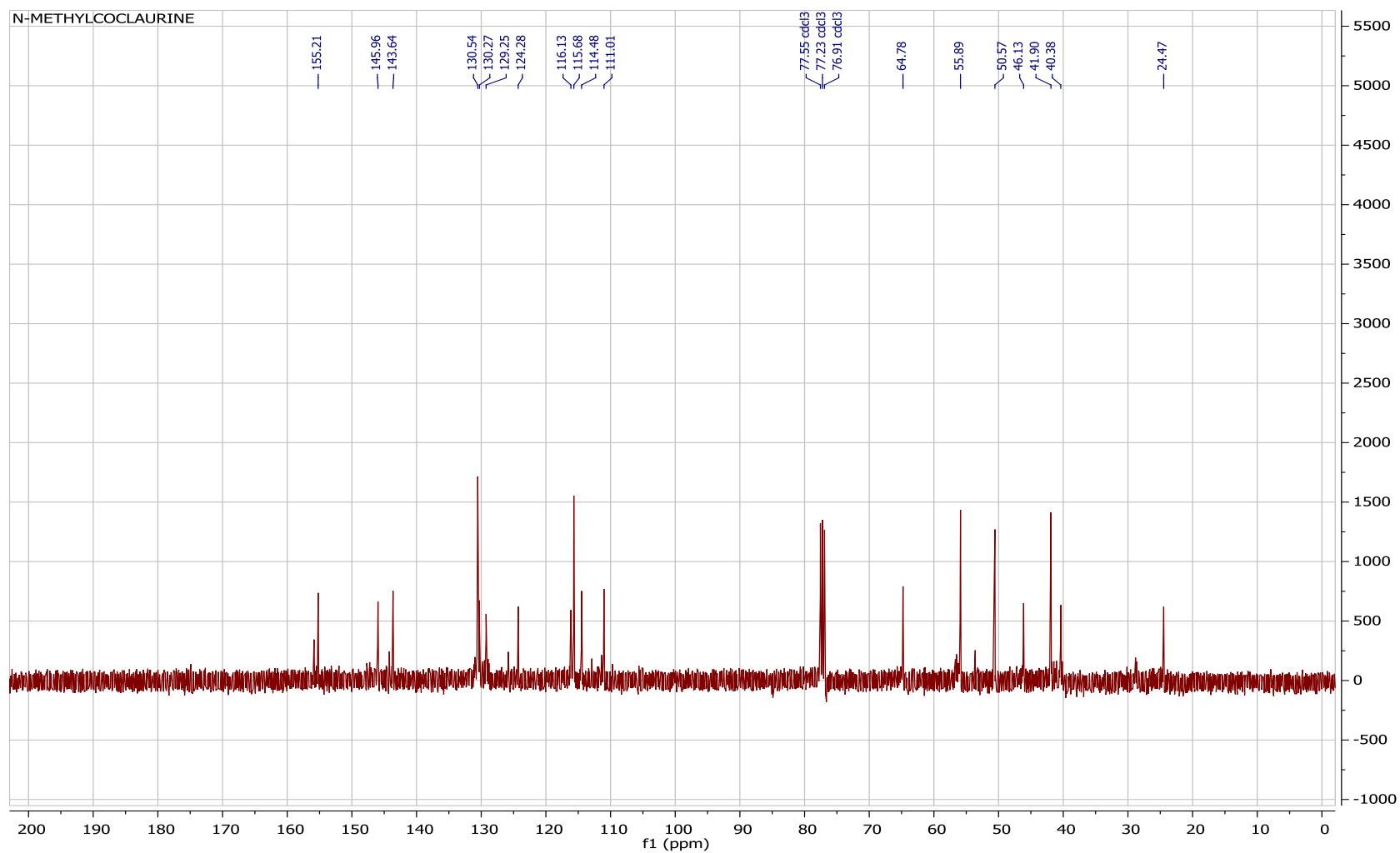


Figure 12S.  $^{13}\text{C}$  NMR spectrum of *N*-methylcoclaurine (6).

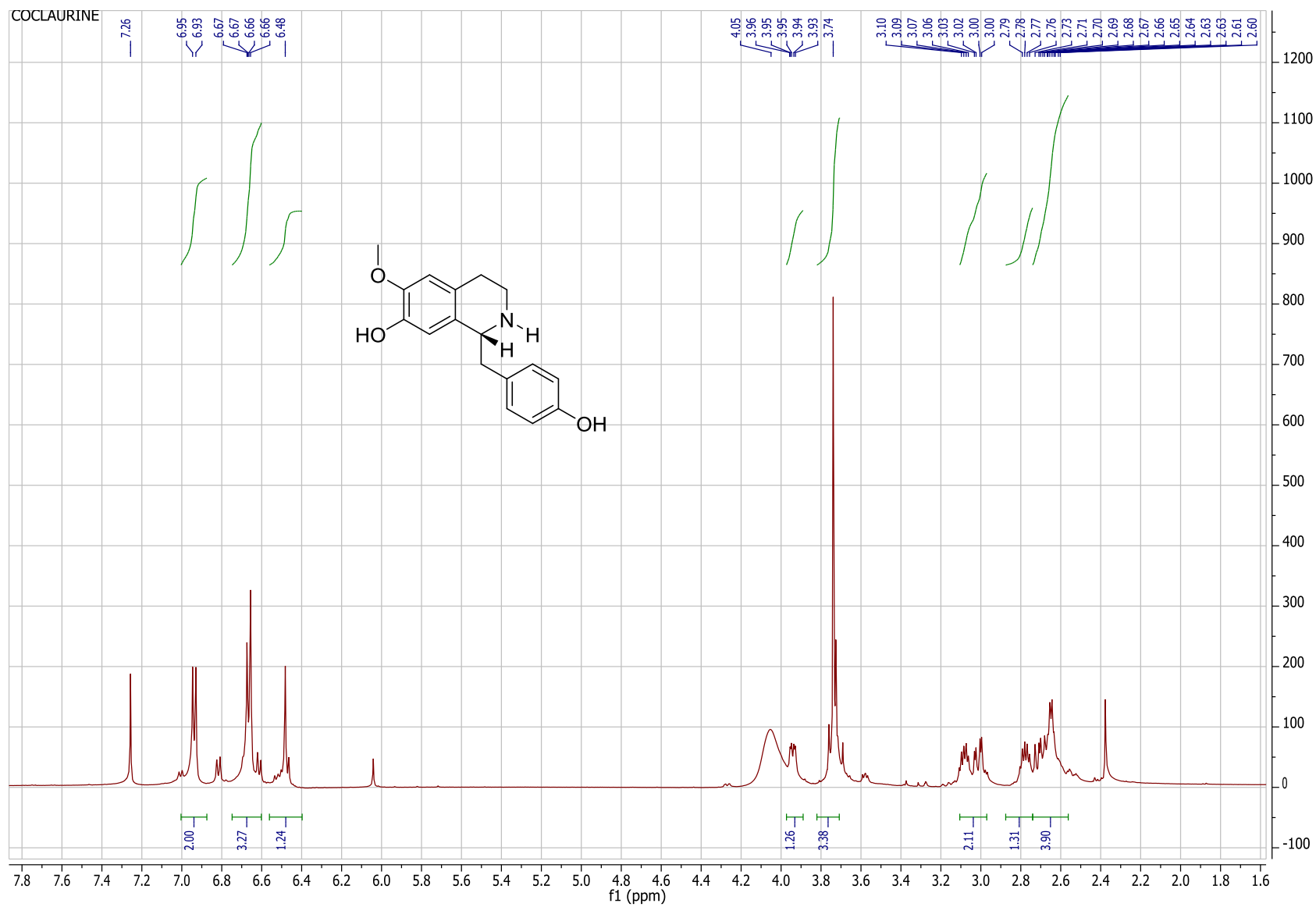
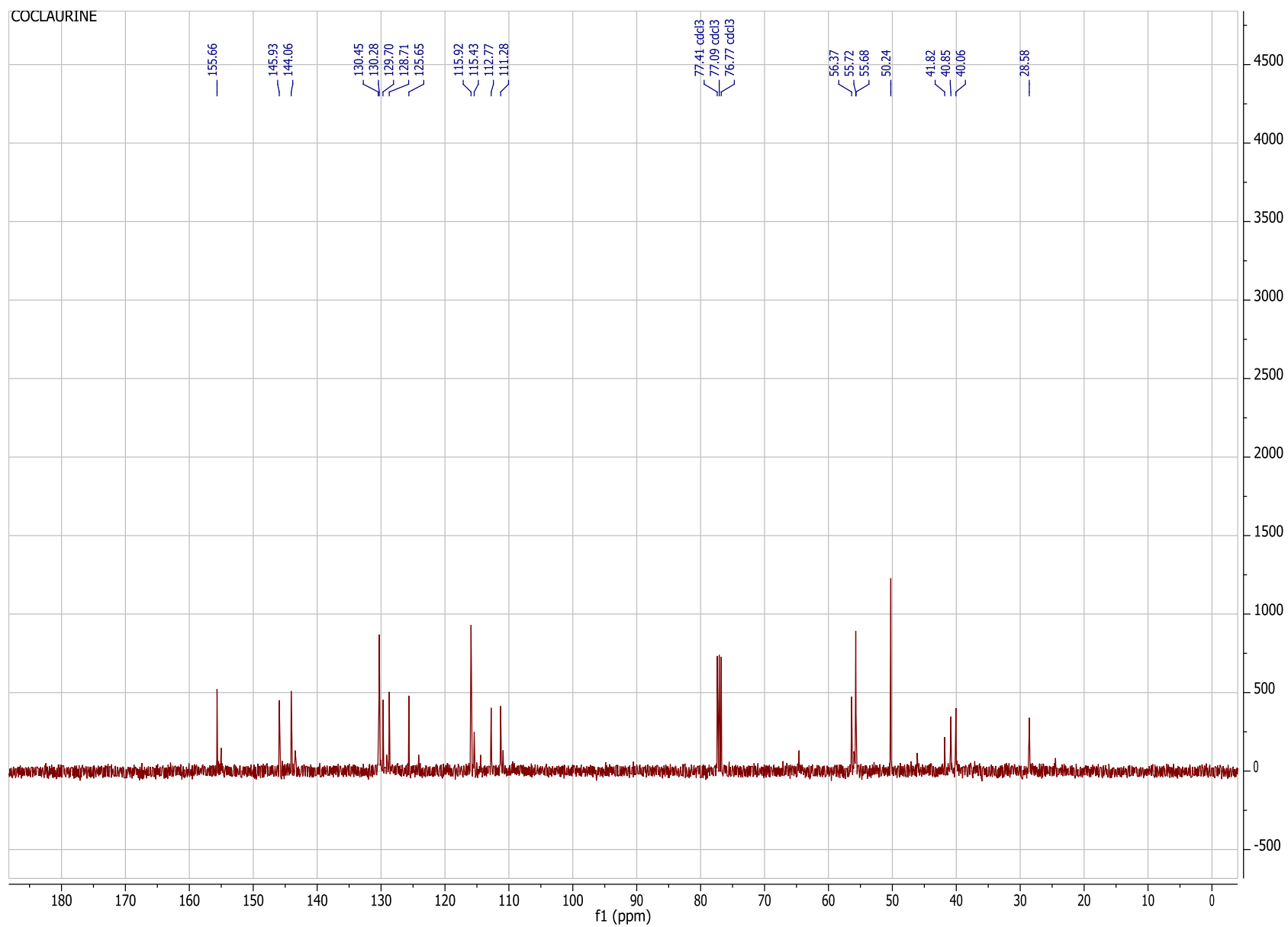


Figure 13S. <sup>1</sup>H NMR spectrum of coclaurine (7).





**Figure 14S.**  $^{13}\text{C}$  NMR spectrum of coclaurine (**7**).

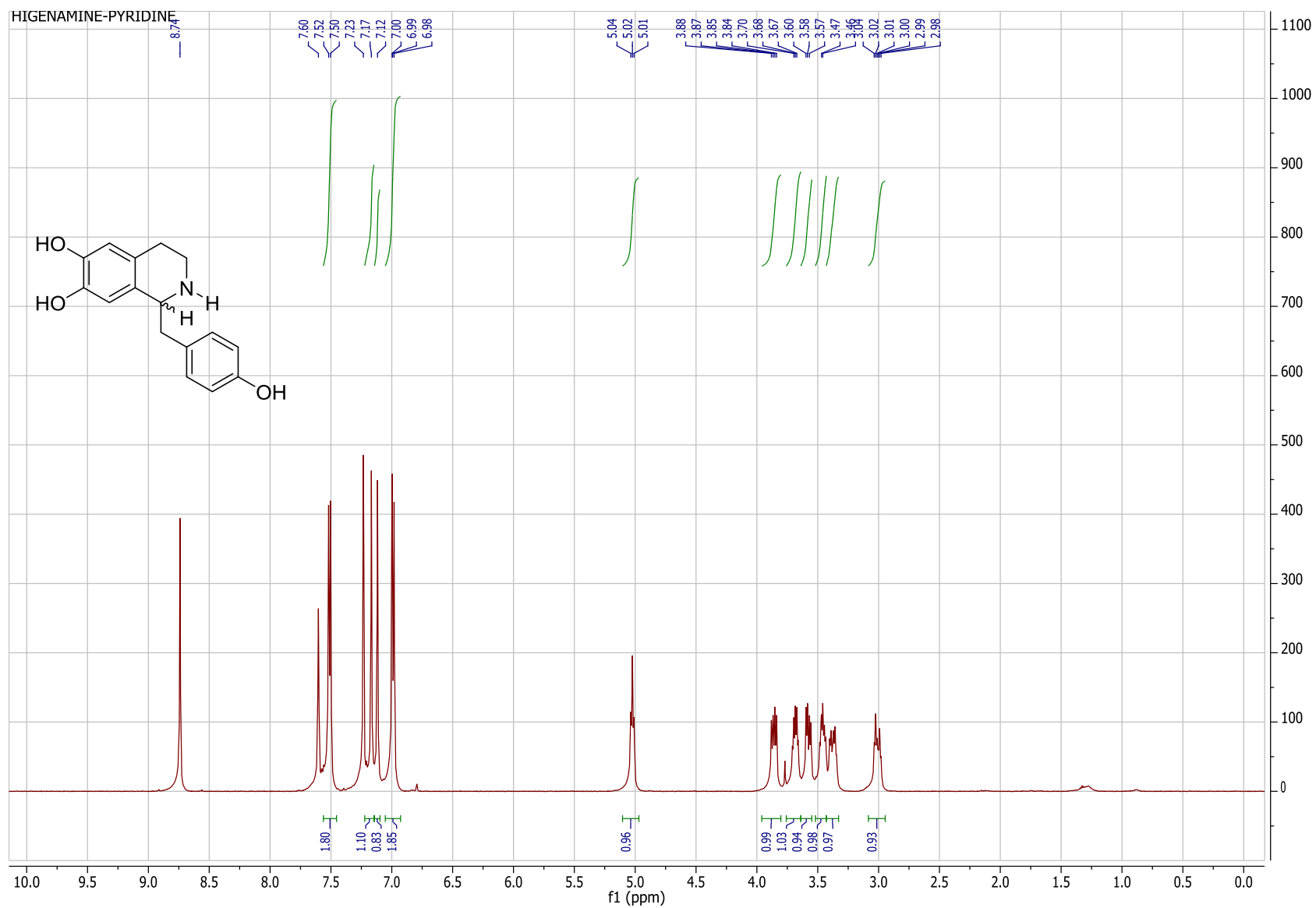
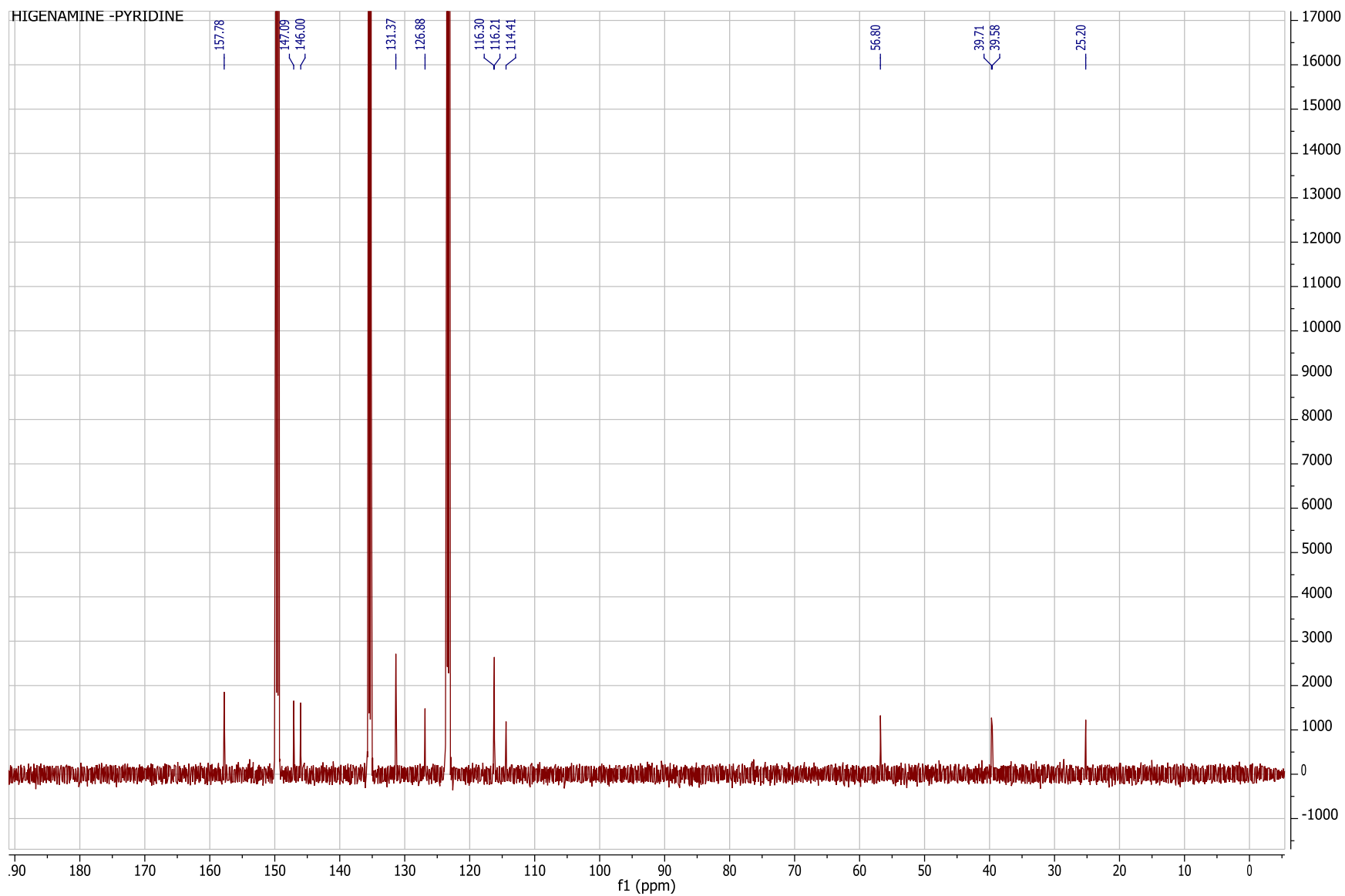
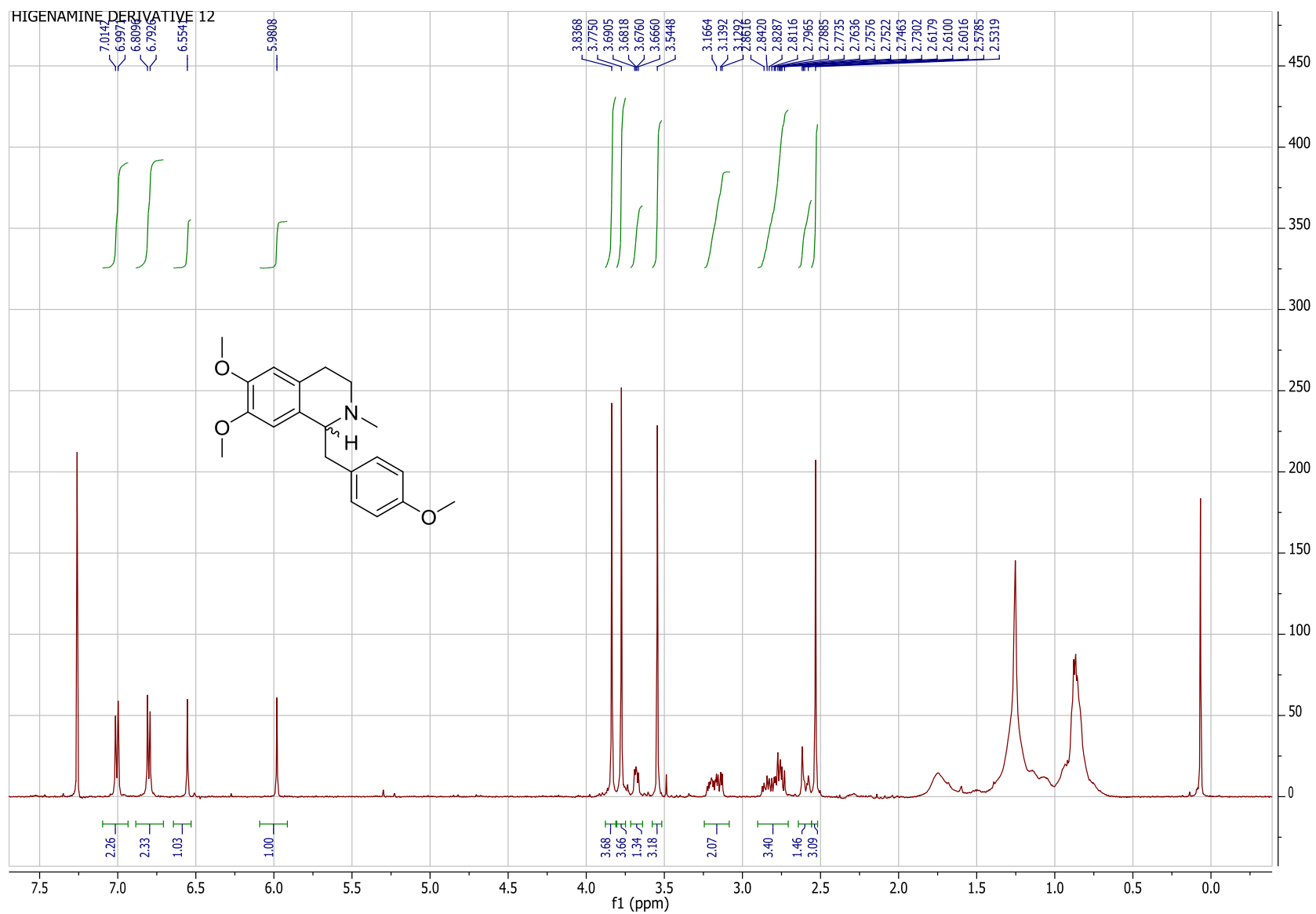


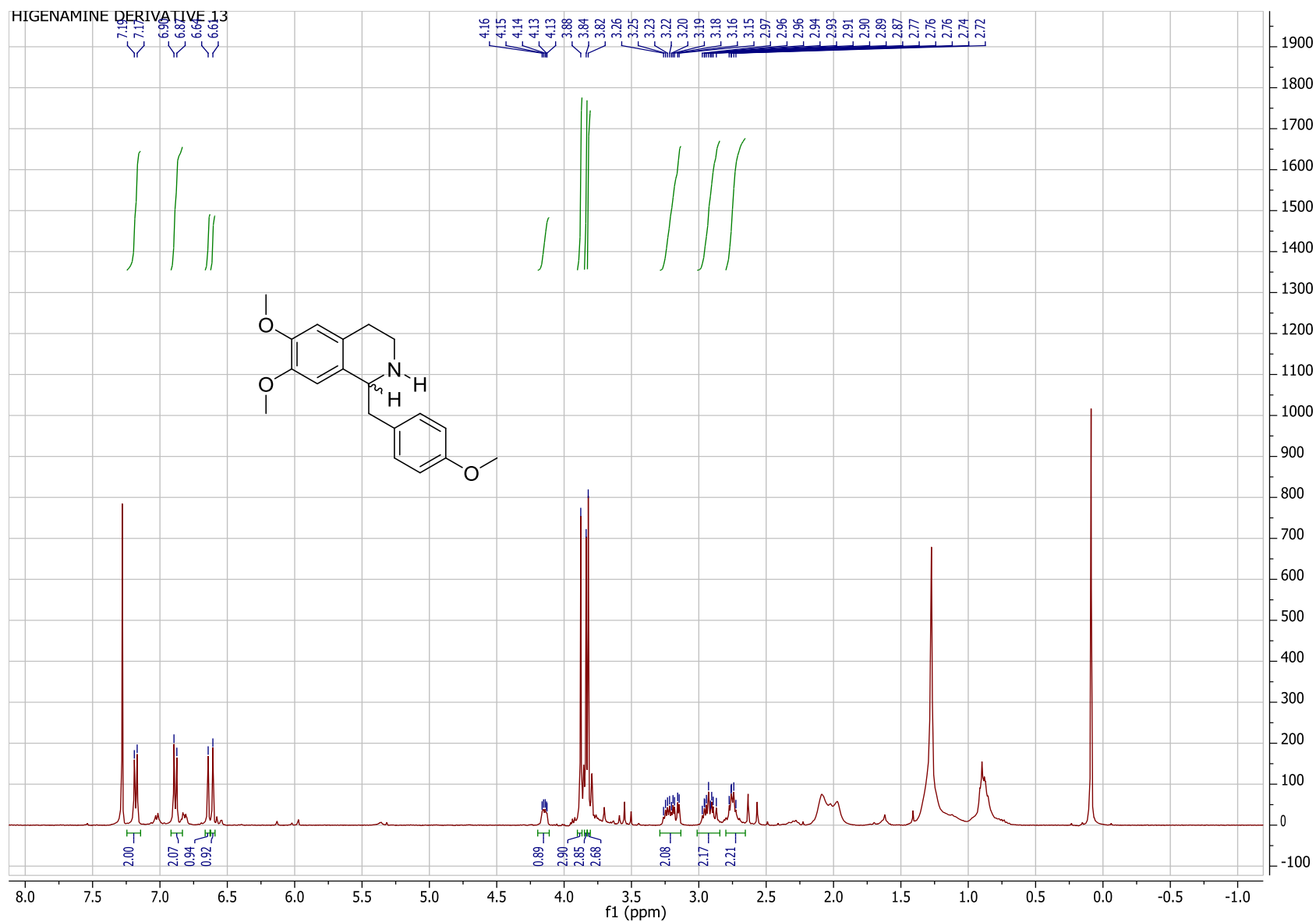
Figure 15S.  $^1\text{H}$  NMR spectrum of ( $\pm$ )-higenamine (**11**).



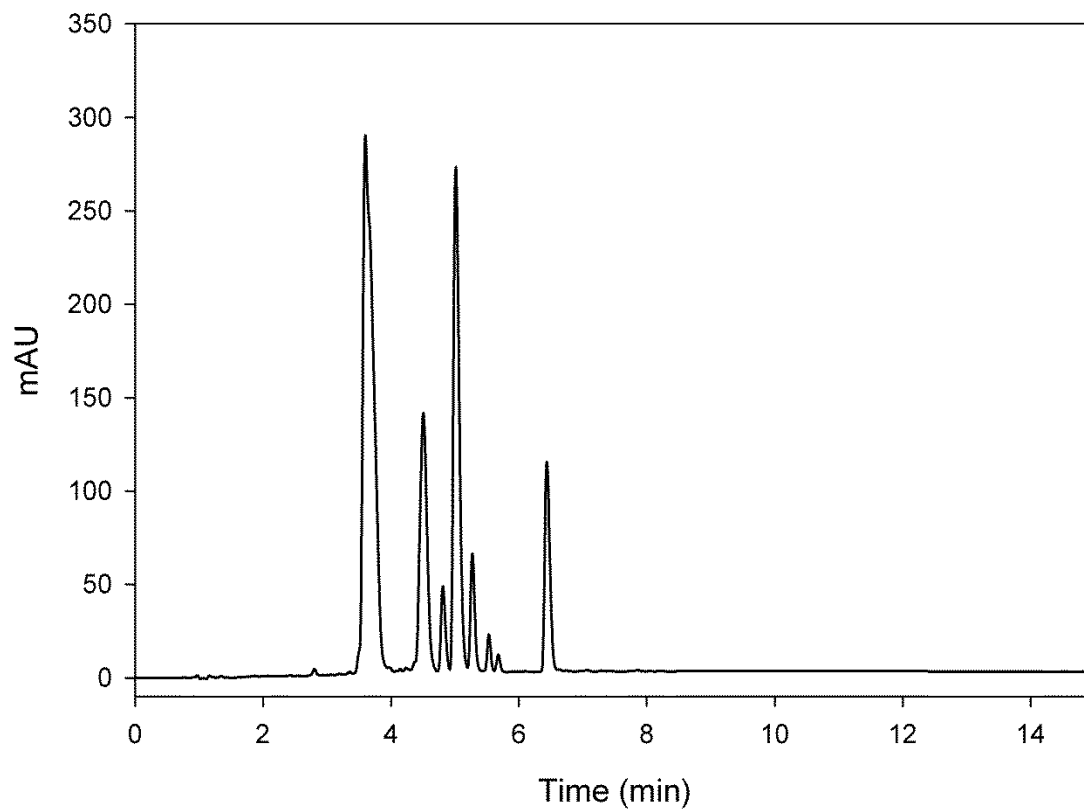
**Figure 16S.**  $^{13}\text{C}$  NMR spectrum of ( $\pm$ )-higenamine (**11**).



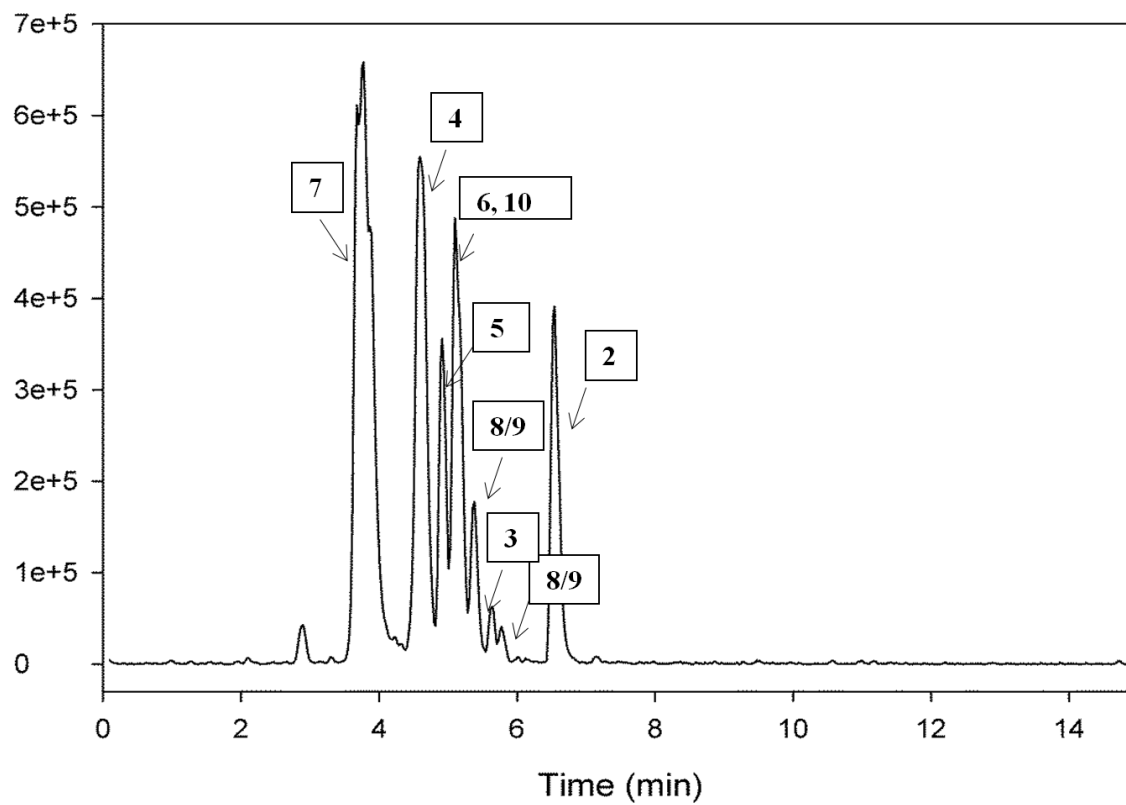
**Figure 17S.**  $^1\text{H}$  NMR spectrum of higenamine derivative **12** (4'-O-methylarmepavine).



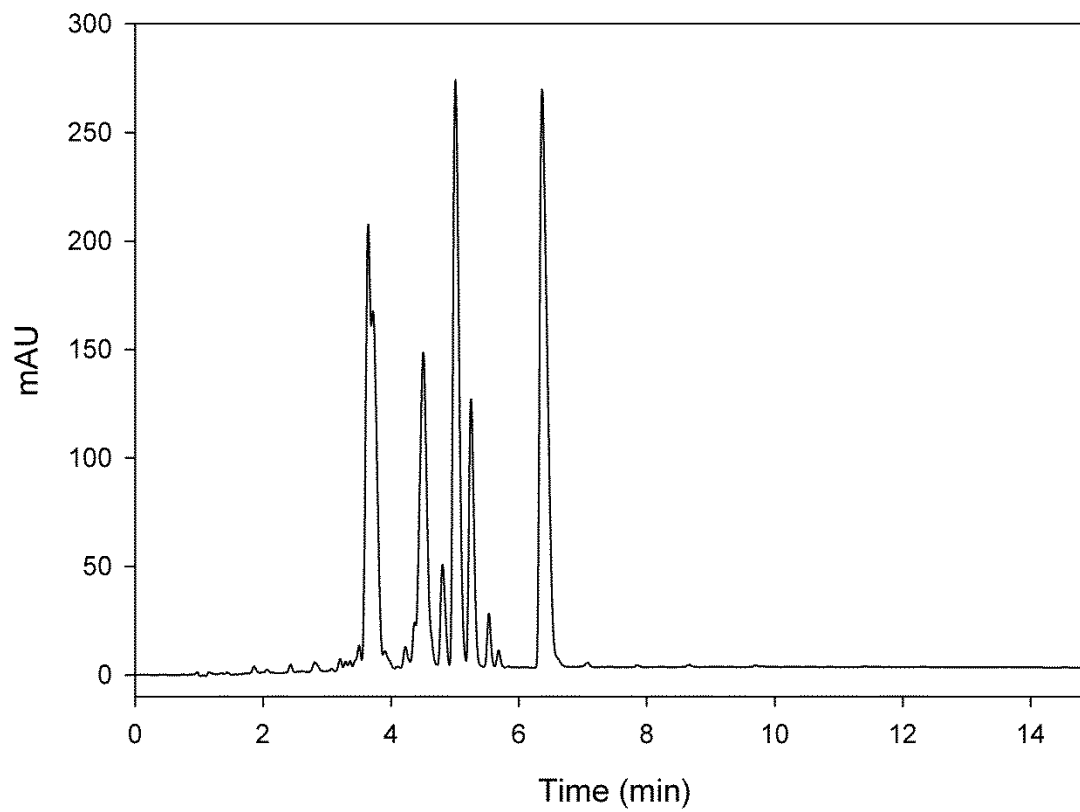
**Figure 18S.**  $^1\text{H}$  NMR spectrum of higenamine derivative **13** (4',7-di-*O*-methylarmepavine).



**Figure 19S.** UHPLC-UV (280 nm) chromatogram of basic partition of *N. nucifera* white flower.

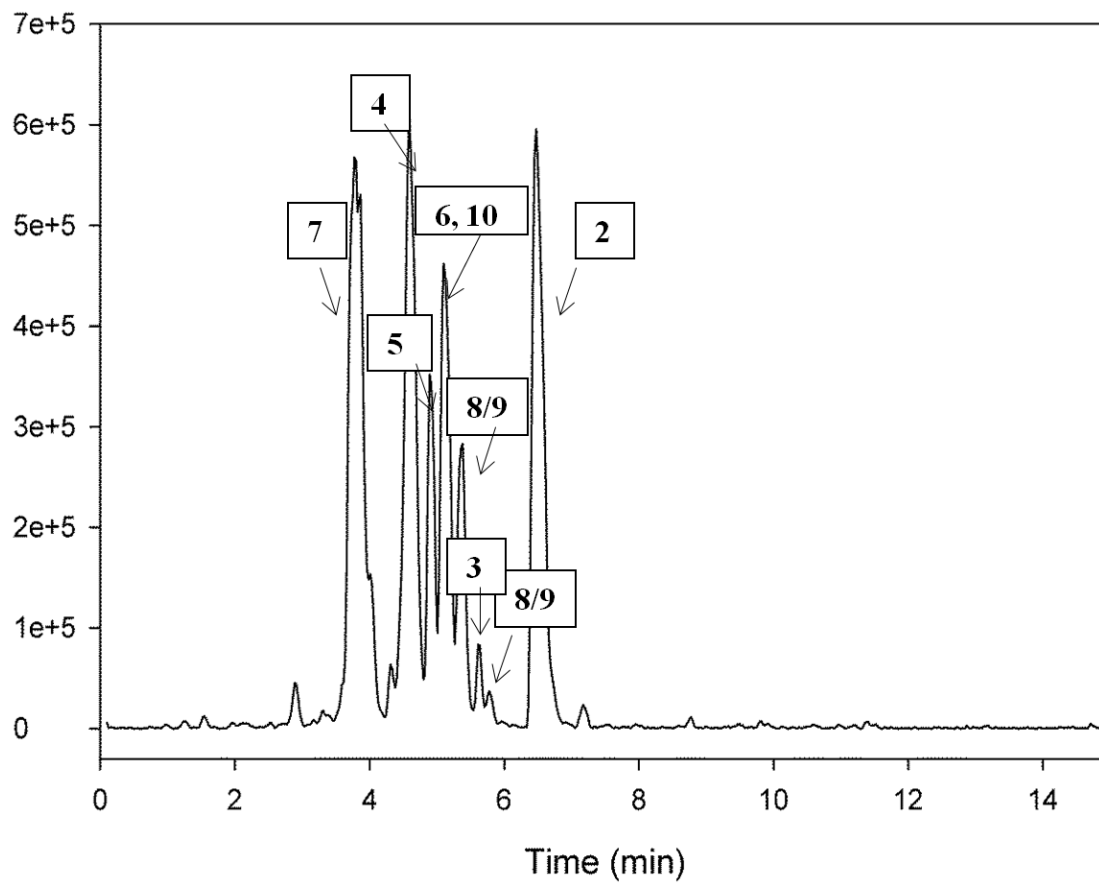


**Figure 20S.** UHPLC- ESI Positive chromatogram of basic partition of *N. nucifera* white flower.

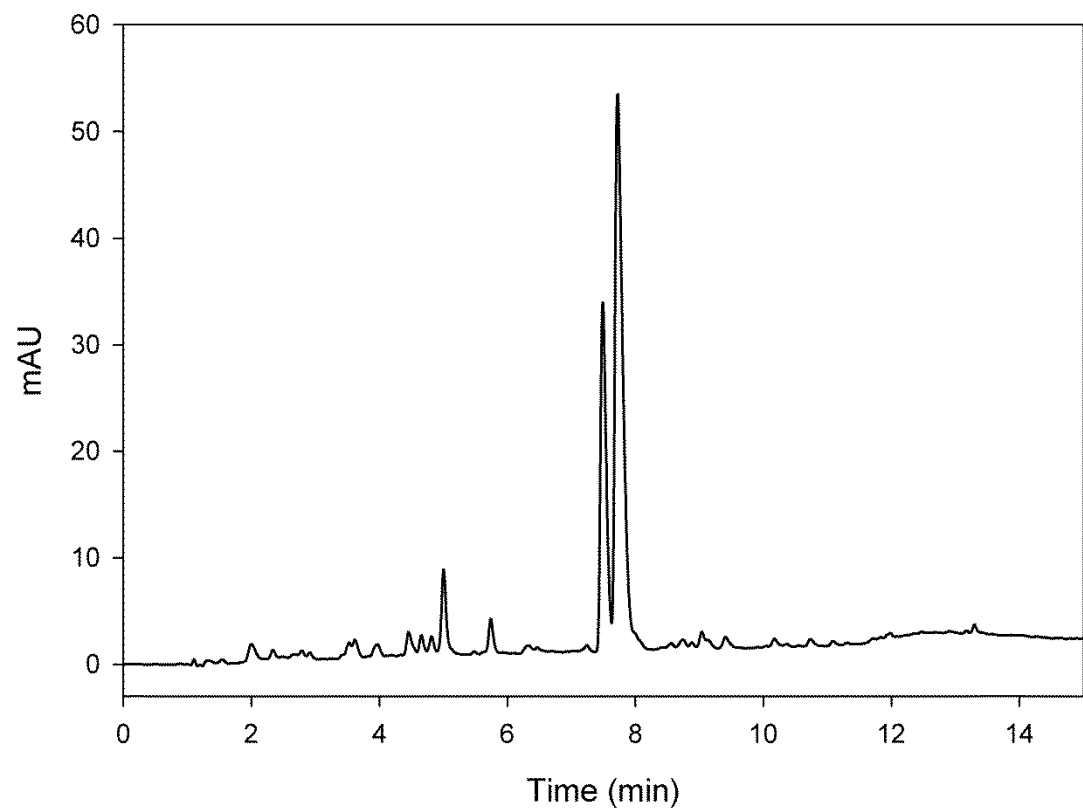


**Figure 21S.** UHPLC-UV (280 nm) chromatogram of basic partition of *N. nucifera* pink flower.

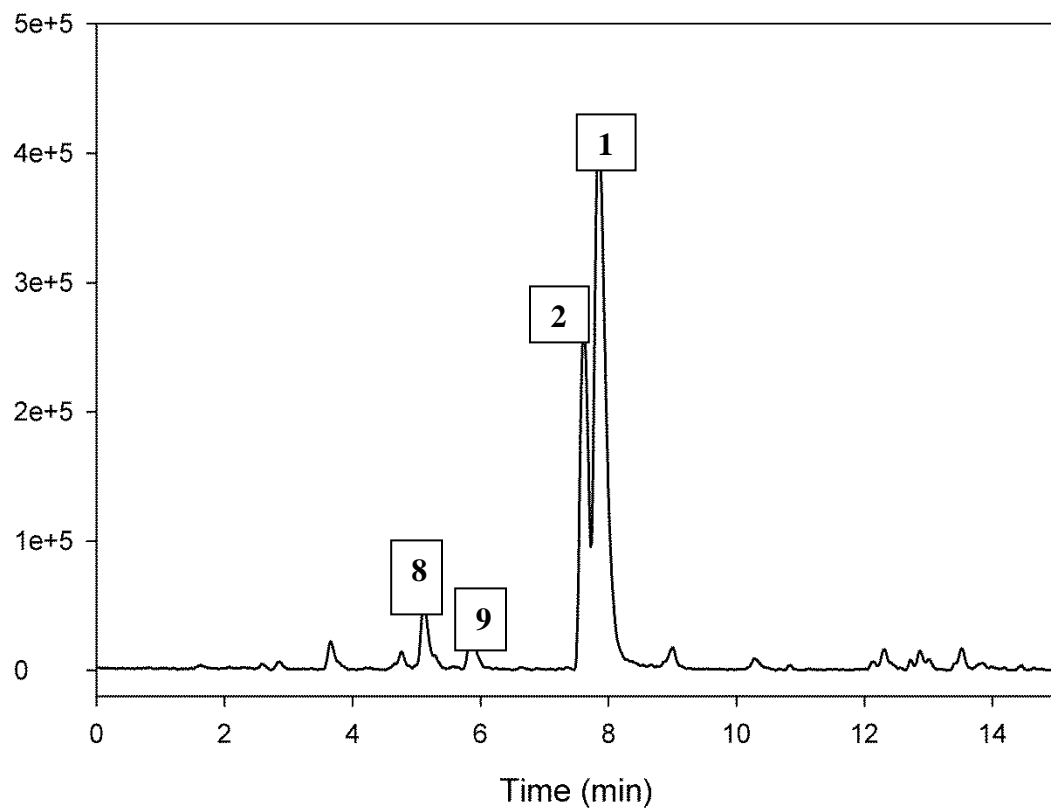




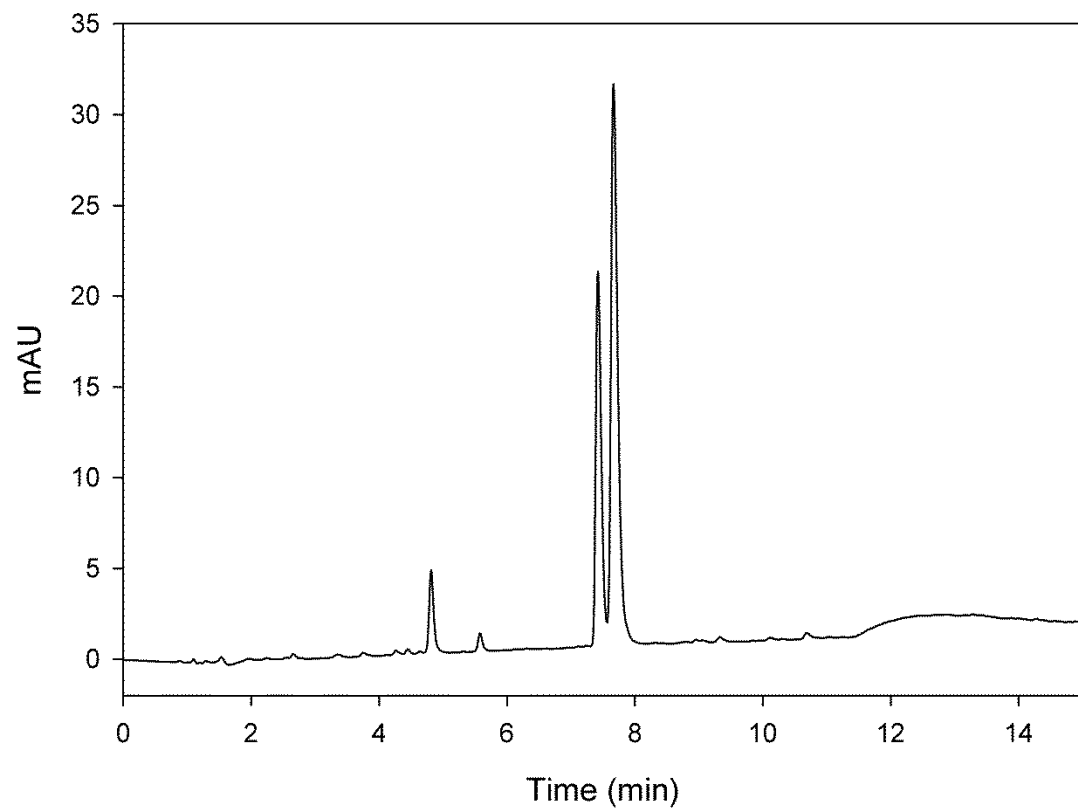
**Figure 22S.** UHPLC- ESI Positive chromatogram of basic partition of *N. nucifera* pink flower.



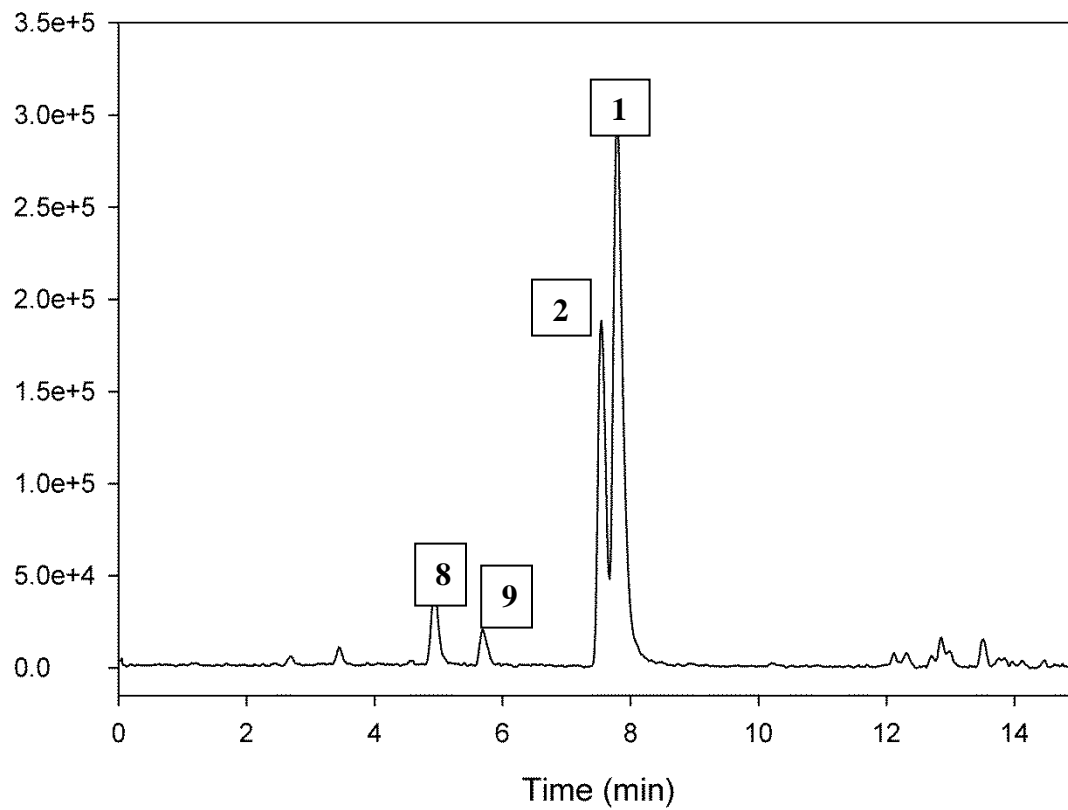
**Figure 23S.** UHPLC-UV (280 nm) chromatogram of acidic partition of *N. nucifera* white flower.



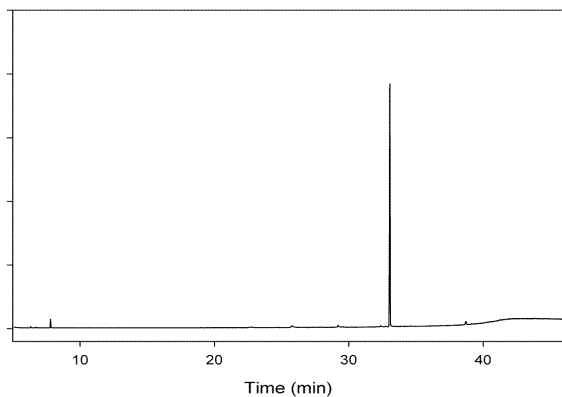
**Figure 24S.** UHPLC- ESI Positive chromatogram of acidic partition of *N. nucifera* white flower.



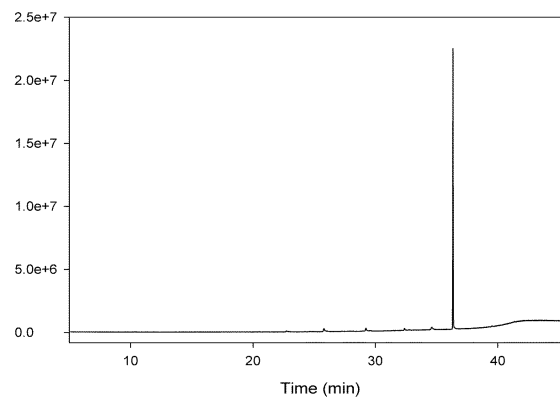
**Figure 25S.** UHPLC-UV (280 nm) chromatogram of acidic partition of *N. nucifera* pink flower.



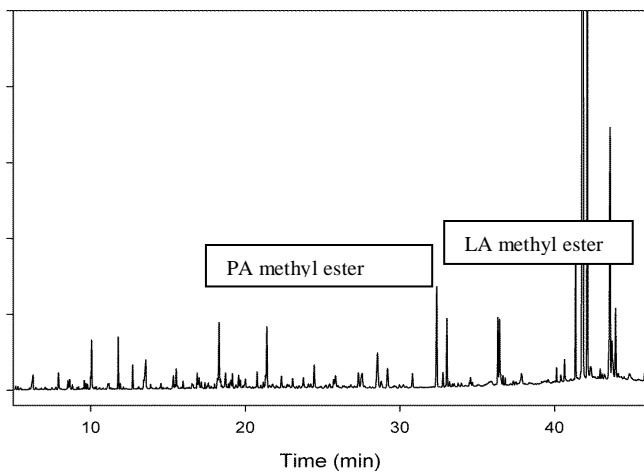
**Figure 26S.** UHPLC- ESI Positive chromatogram of acidic partition of *N. nucifera* pink flower.



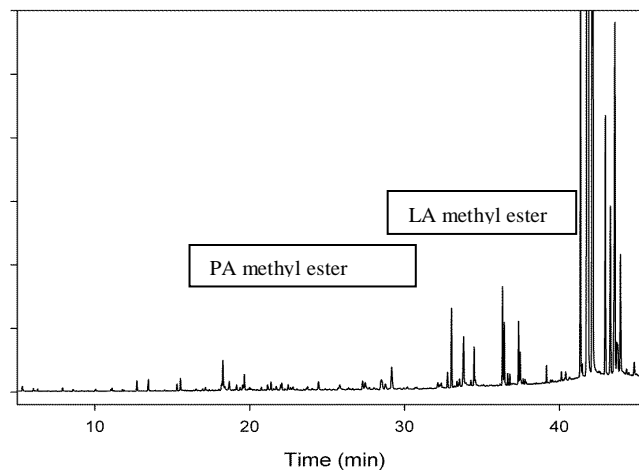
**Figure 27S.** GC/MS analysis of palmitic acid Me ester (Std)



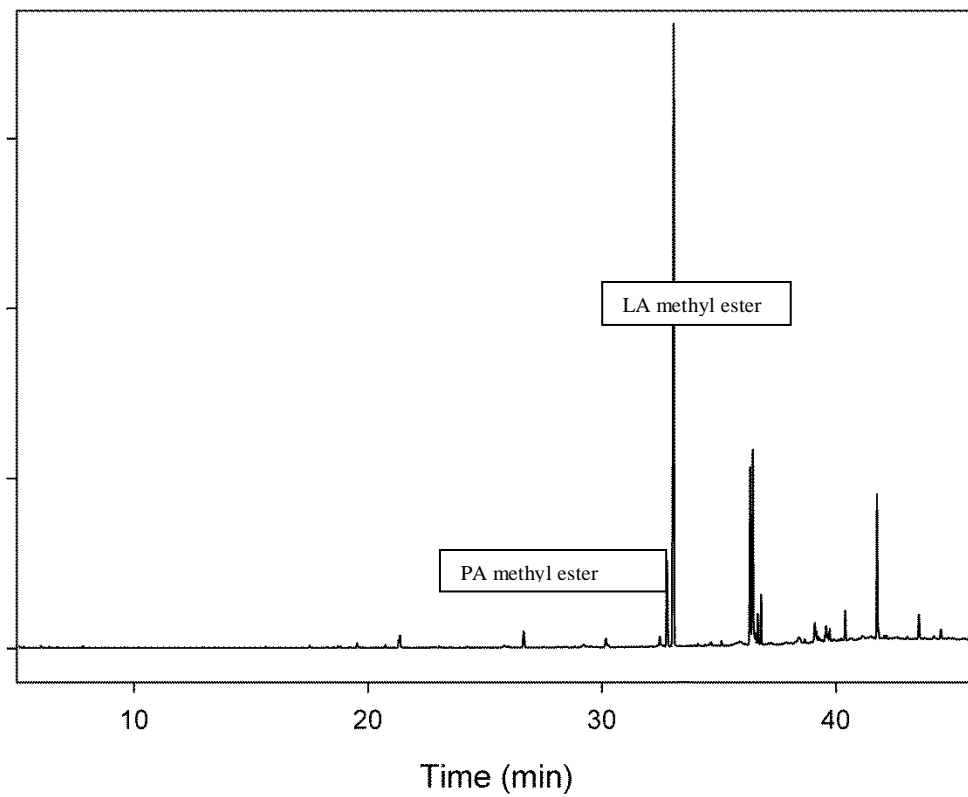
**Figure 28S.** GC/MS analysis of linoleic acid Me ester (Std)



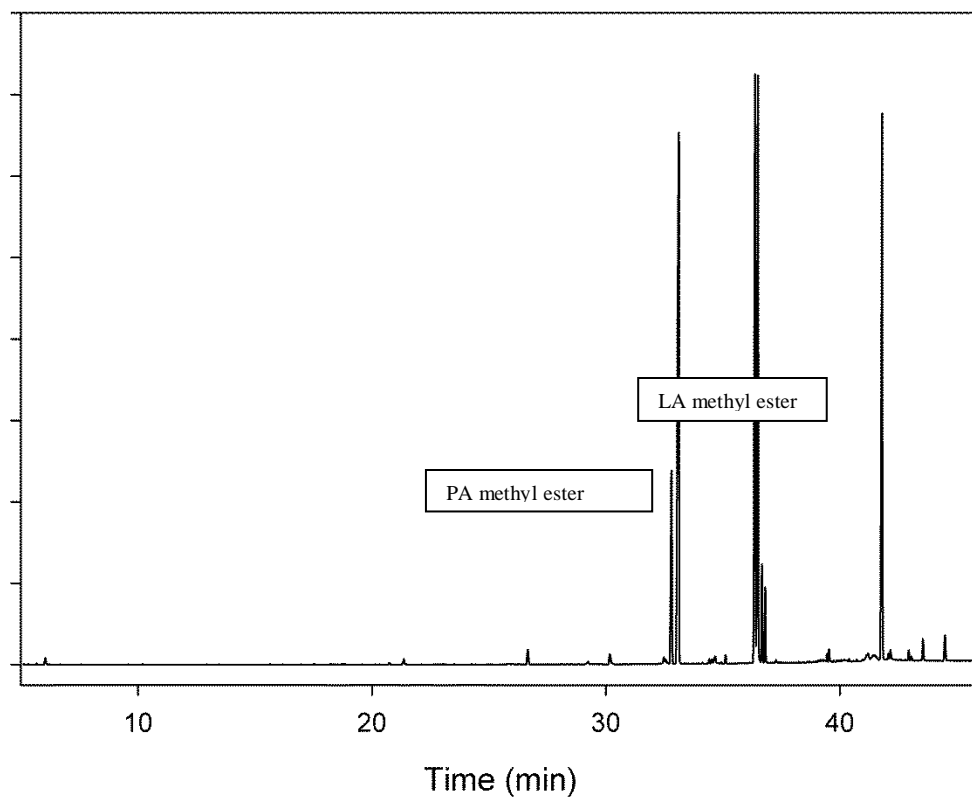
**Figure 29S.** GC/MS analysis of acidic patition (white flower)



**Figure 30S.** GC/MS analysis of acidic patition (pink flower)

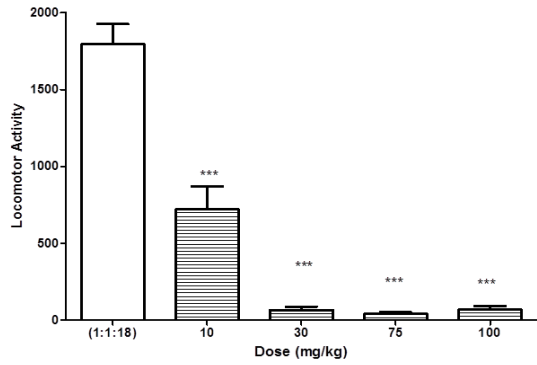


**Figure 31S.** GC/MS analysis of fraction G.

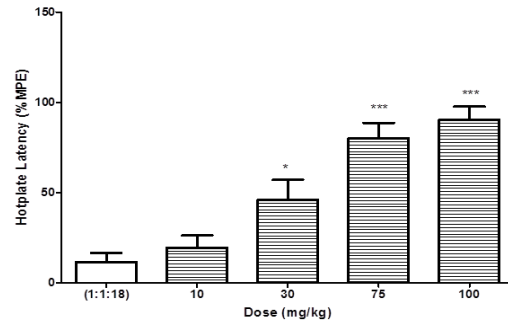


**Figure 32S.** GC/MS analysis of fraction H.

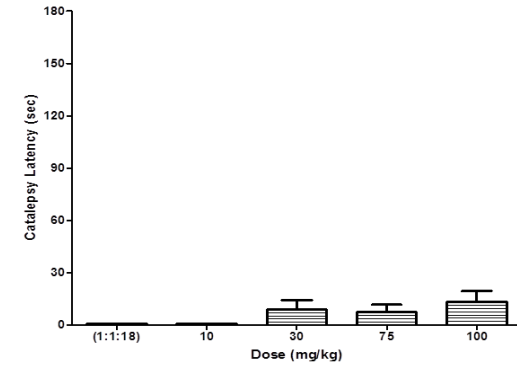




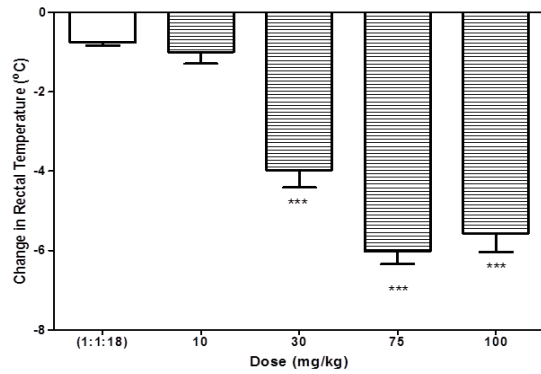
(a). Locomotor activity



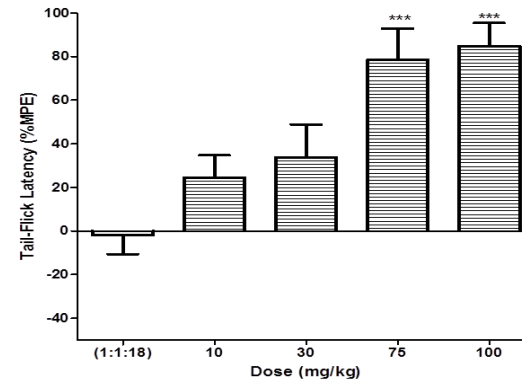
(b). Hotplate latency



(c). Catalepsy latency

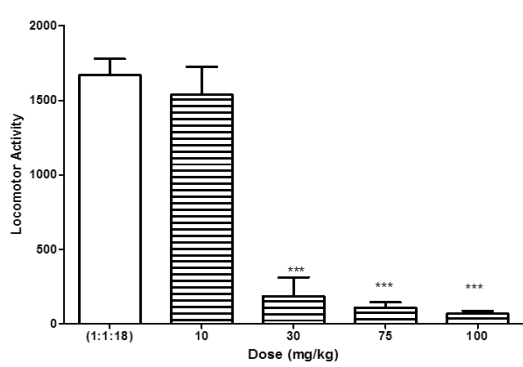


(d). Change in rectal temperature

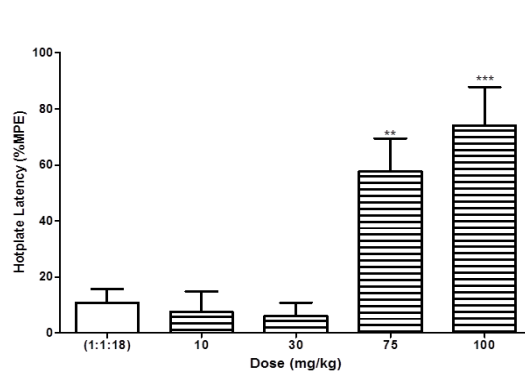


(a). Tail-flick latency

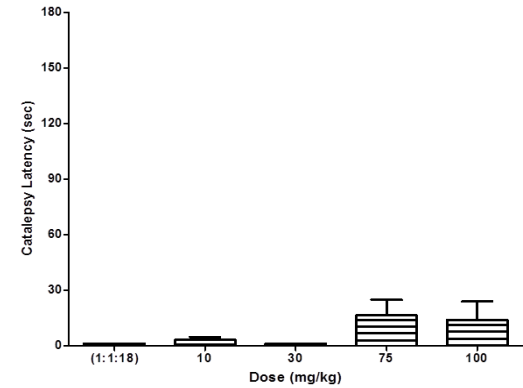
**Figure 33S.** *In vivo* mouse tetrad assay of acidic partition of *N. nucifera*.



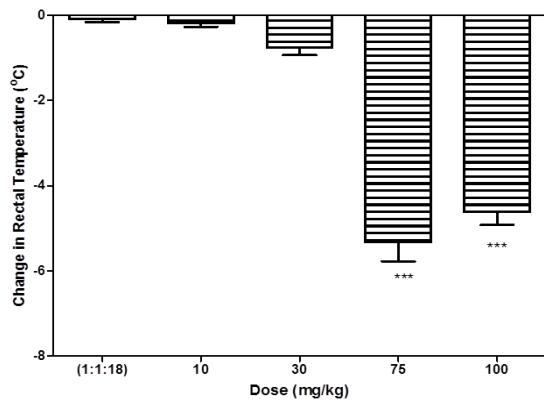
(a). Locomotor activity



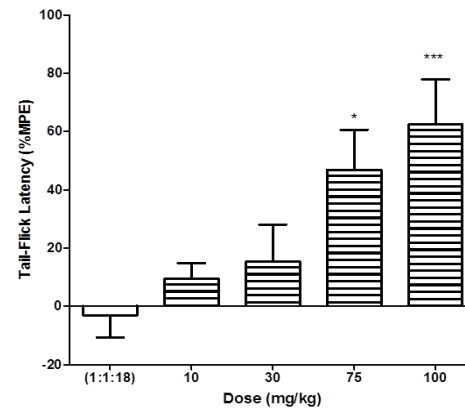
(b). Hotplate latency



(c). Catalepsy latency

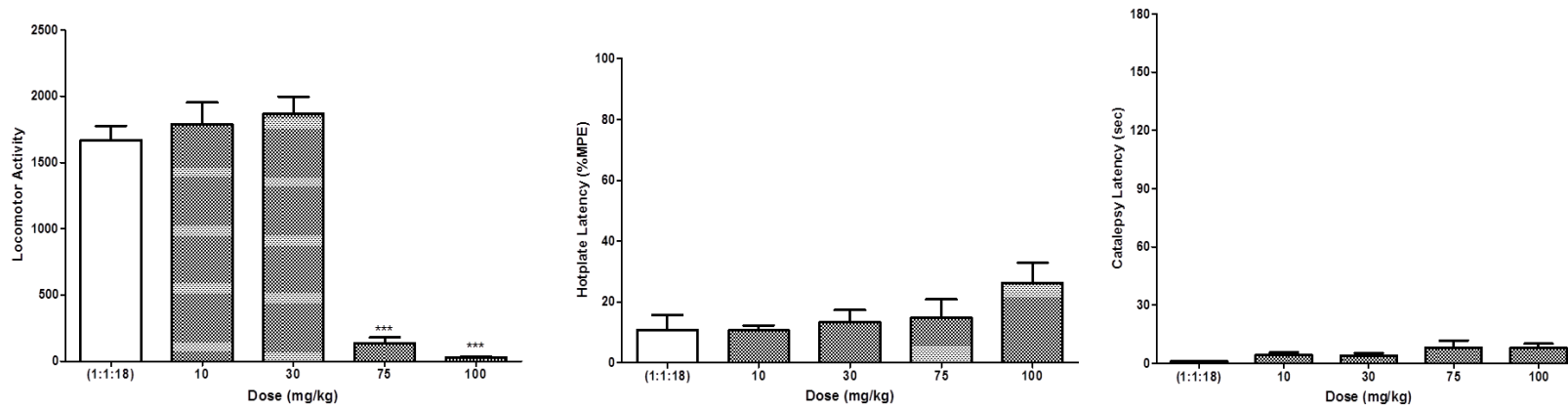


(d). Change in rectal temperature



(e). Tail-flick latency

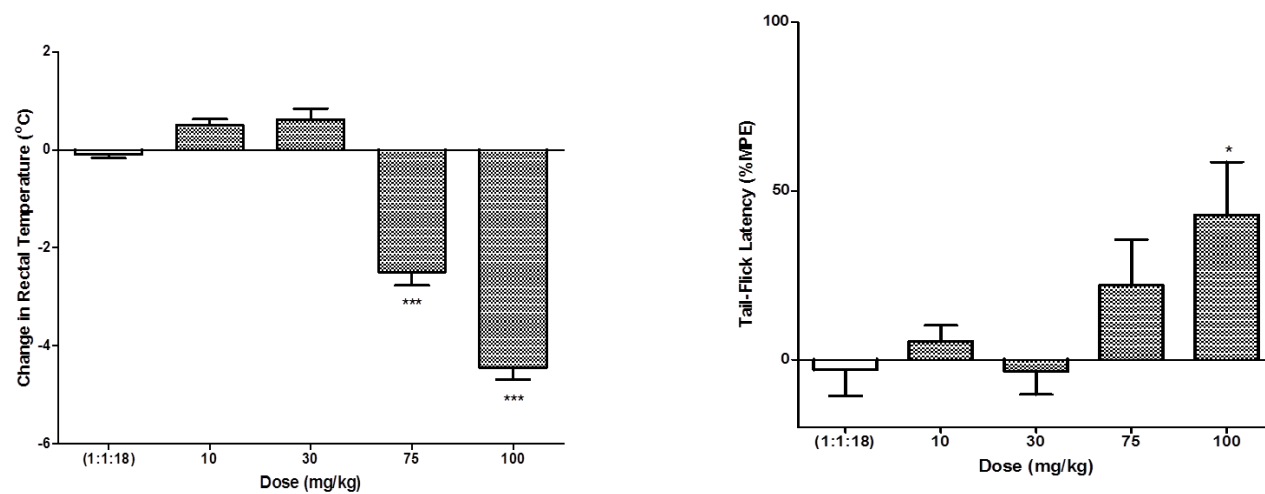
**Figure 34S.** *In vivo* mouse tetrad assay of basic partition of *N. nucifera*.



(a). Locomotor activity

(b). Hotplate latency

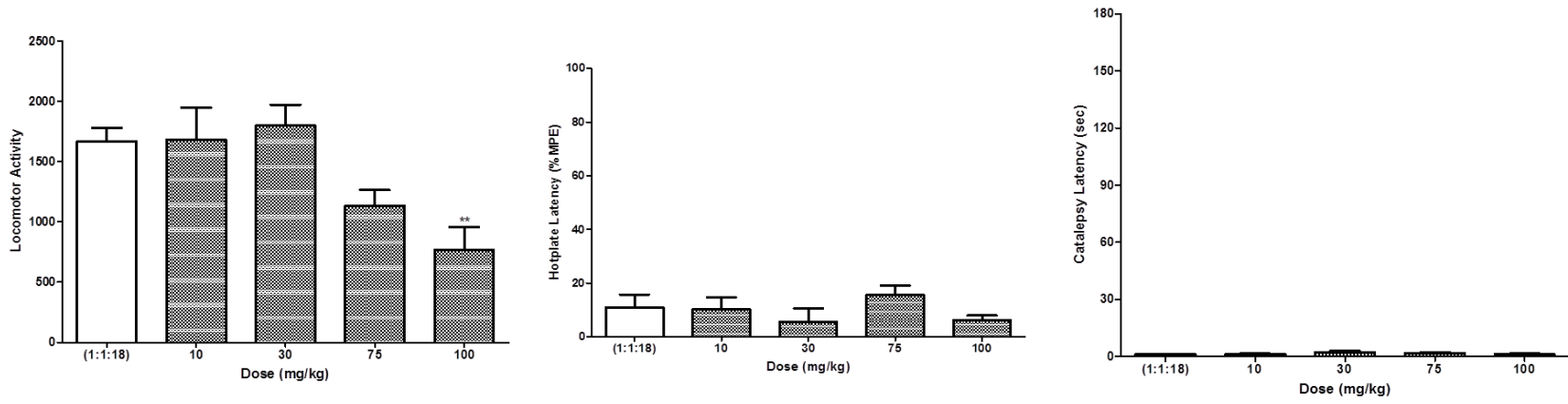
(c). Catalepsy latency



(d). Change in rectal temperature

(e). Tail-flick latency

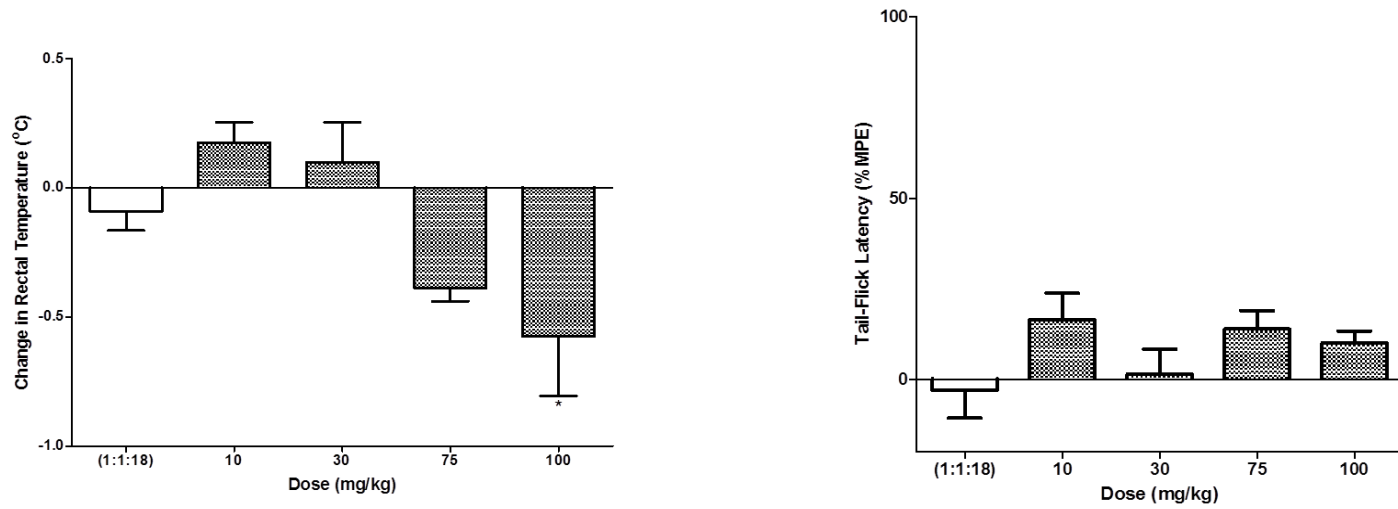
**Figure 35S.** *In vivo* mouse tetrad assay of mixture of compound 5-7 of *N. nucifera*.



(a). Locomotor activity

(b). Hotplate latency

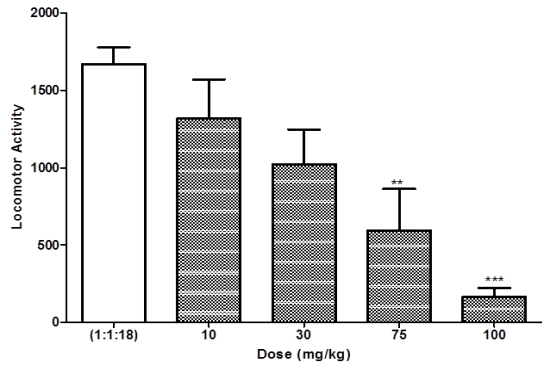
(c). Catalepsy latency



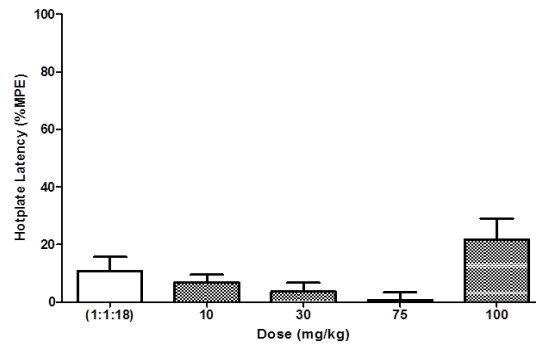
(d). Change in rectal temperature

(e). Tail-flick latency

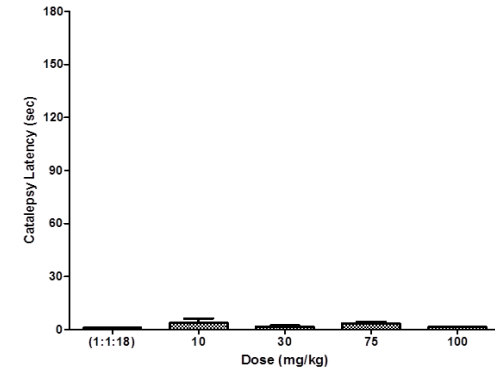
**Figure 36S.** *In vivo* mouse tetrad assay of coclaurine (7).



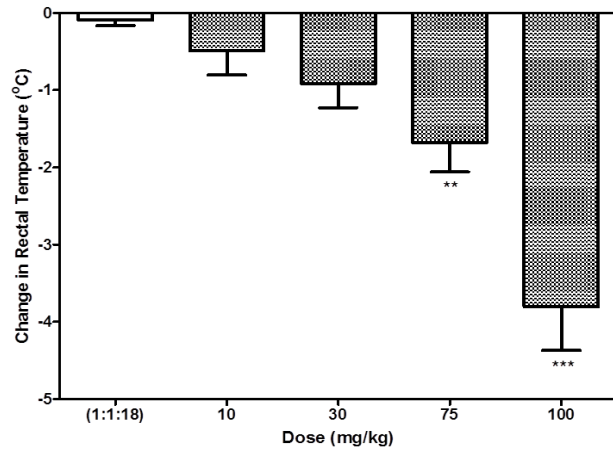
(a). Locomotor activity



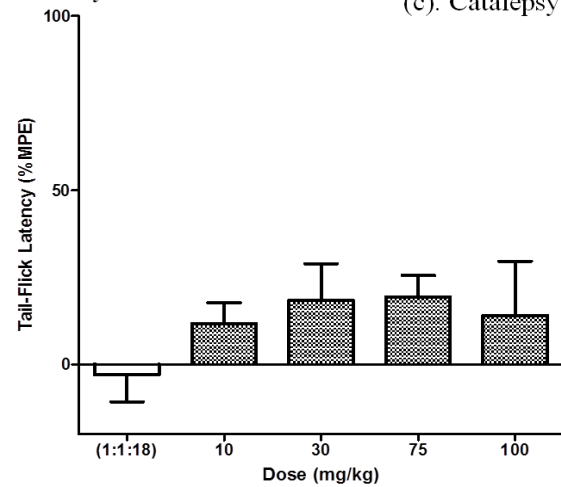
(b). Hotplate latency



(c). Catalepsy latency

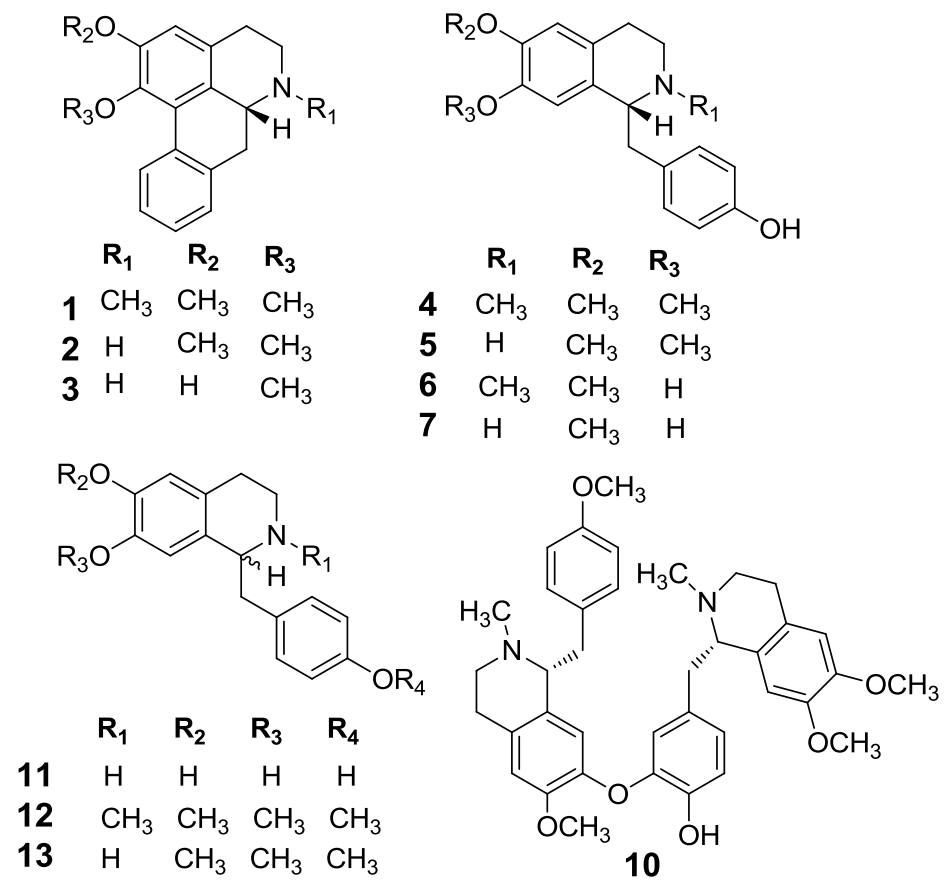


(d). Change in rectal temperature



(e). Tail-flick latency

**Figure 37S.** *In vivo* mouse tetrad assay of nuciferine (**1**).



**Figure 38S.** Structure of alkaloids and their analogs from *N. nucifera*.