

# Synthesis of Dihydrocoumarin derivatives from Methyl *trans*-Cinnamate And Evaluation of their Bioactivity as Potent anticancer Agents

Teni Ernawati<sup>1</sup>, Harpolia<sup>2,3</sup>, Suzana<sup>3</sup>, Dila Fairusi<sup>4</sup> and Muhammad Hanafi<sup>1</sup>

<sup>1</sup>Research Center for Chemistry- Indonesian Institute of Sciences (LIPI)  
Kawasan Puspiptek Serpong, Tangerang Selatan 15314, Indonesia  
Email: teni001 {at} lipi.go.id

<sup>2</sup>Jurusan Farmasi Politeknik Kesehatan Kementerian Kesehatan  
Percetakan Negara No 23, Jakarta Pusat, Indonesia

<sup>3</sup>Jurusan Farmasi Universitas Pancasila  
Srengseng Sawah, Jagakarsa, Jakarta selatan 12640, Indonesia

<sup>4</sup>FMIPA Universitas Indonesia  
Depok, Indonesia

---

**ABSTRACT**— *Synthesis of dihydrocoumarin derivatives from methyl trans-cinnamate is described. The reaction can be carried out as a simple one pot reaction using acid catalyst. Firstly, methyl trans-cinnamate was converted into cinnamic acid via hydrolysis reaction with alkaline. Secondly, esterification reaction of cinnamic acid was carried out by using an acid catalyst p-toluensulfonic acid with phenol, o-cresol, p-cresol which lead to the production of 4-phenylchroman-2-one, 8-methyl -4-phenylchroman-2-one, and 6-methyl-4-phenylchroman-2-one.*

**Keywords**— dihydrocoumarin derivatives, methyl *trans*-cinnmate, cinnamic acid, 4-phenylchroman-2-one, 8-methyl -4-phenylchroman-2-one, and 6-methyl-4-phenylchroman-2-one.

---

## 1. INTRODUCTION

Methyl *trans*-cinnamate is basically a kind of essential oil which is contained in a lot of quantities in a plant from *Alpinia malaccensis* species of Zingiberaceae families, which is one of the most important groups of medicinal plants<sup>1</sup>. Methyl *trans*-cinnamate has been successfully isolated from *A. malaccensis* with a high yield and good purity<sup>2</sup>. Research in the field of organic synthesis of methyl *trans*-cinnamate derivative also has evolved. Cinnamic acid is an analog derivative of methyl *trans*-cinnamate, which is included in pathway derivative of shikimic acid<sup>3</sup>. Cinnamic acid and its natural derivatives are known for its use in the treatment of cancer for several centuries. Ginsenoside Rg1 from ginseng, cinnamic acid from Xuanshen, and tanshinon IIA from Danshen (RCT) is the traditional treatment for keeping someone from aging and maintaining the chemical balance for the whole the body to prevent from any disease. Exploring further, RCT is given into osteosarcoma cell (MG-63) which is a histologic form of the most common of bone-cancer<sup>4</sup>. Other derivatives of cinnamic acid compounds are caffeic acid, cinnamide, cinnamoyl ester, cinnamic acid, and hydrazide. These derivatives of cinnamic acid compounds, both natural and synthetic one, have been tested as anti-cancer agents. Cinnamic acid and some compounds of its derivatives have been examined as good inhibitor against activity of AKR1C3. AKR1C3 is cancer cell which forms with the existence of hormones such as prostate cancer, breast cancer, and endometrial cancers. Cinnamic acid and 3,4,5-trimetoksisinamat acid (IC<sub>50</sub> = 50 μM) are good inhibitors against AKR1C3 (IC<sub>50</sub> = 50 μM). In addition, caffeic acid has a potential as low cytotoxic *in vitro* who fight the cells myeloid leukemia (HL-60) and also potentially as a chemopreventive agent for fight against skin cancer<sup>5</sup>. Combination of cinnamic acid compounds and guanlylhydrazone also has been synthesized. The resulted compounds of the synthesis has a high activity for fighting the Mycobacterium tuberculosis H37Rv which is a causal agent of tuberculosis (TB)<sup>6</sup>.

Bairwa et al. (2010) and Li et al. (2011) reported the synthesis of cinnamic acid with thionyl chloride producing halide acid<sup>6</sup>. This halide acid can be reacted with alcohol so that become ester cinnamate (cinnamoyl ester). Cinnamate ester is a group of anticancer agent. Some cinnamate ester which was isolated from Netherlands propolis, benzyl caffeate, phenethyl caffeate, and cinnamoyl caffeate were found to be potent an antiproliferative agents toward colon 26-L5 carcinoma with EC<sub>50</sub> values of 0.288, 1.76, and 0.114 μg/mL respectively. Phenethyl caffeate (Caffeic Acid Phenethyl Ester, CAPE) has several biology activities such as antioxidants, anti-inflammation, and inhibition of tumor growth<sup>5</sup>. One way of manufacturing of coumarin is with hydroarylation of cinnamic acid and phenol by using strong acid<sup>7</sup>. Coumarin

derivatives have long been known to have broad biological activities such as anti-inflammatory, antioxidant, anti-aging, and anticancer. Esculetin is potentially the best among other coumarins as a radical catcher on antioxidant testing<sup>8</sup>. 7-isopentenyl-oxy-coumarin is one of the active compounds from *Heracleum lanatum* Michx. It is to be potent as an against tumor prevention<sup>9</sup>. Calanon (derivate of coumarin compound) from *Callophyllum* sp. has activities as anticancer against HeLa cervical cancer cells with value of IC<sub>50</sub> 22.887 μM<sup>10</sup>. Coumarin is also active towards cytotoxic testing of HeLa cervical cancer cells with value of IC<sub>50</sub> 54.2 μM<sup>11</sup>.

The purpose of this research is to study and synthesize dihydrocoumarin derivatives and also to test the inhibitory activity of the synthesized compounds on the growth of P388 Leukemia cells and HeLa cells. Synthesized compounds of dihydrocoumarin derivatives were 4-phenylchroman-2-one, 8-methyl-4-phenylchroman-2-one, and 6-methyl-4-phenylchroman-2-one. Here, we report synthesis of dihydrocoumarin derivatives using acid catalyst in the absence of solvent at 170°C.

## 2. MATERIALS AND METHODS

### 2.1 Chemicals and Materials

The materials used in this study were methyl *trans*-cinnamate compounds isolated from galangal (*A. malaccensis*) through the steam distillation process to produce galangal oil, then recrystallized, filtration and drying so as to obtain crystals of methyl cinnamate, cinnamic acid obtained from hydrolysis of methyl *trans* cinnamate in alkaline condition, sodium hydroxide (NaOH), thionyl chloride (SOCl<sub>2</sub>), phenol, *p*-cresol, *o*-cresol, *p*-toluene sulfonic acid monohydrate (*p*-TSOH), organic solvents (methanol, ethanol, butanol, ethyl acetate, dichloromethane, and *n*-hexane), hydrochloric acid (HCl), sodium sulfate anhydrous (Na<sub>2</sub>SO<sub>4</sub>), distilled water, sea water, shrimp *Artemia salina*, dimethyl sulfoxide (DMSO), filter paper, silica gel, and thin layer chromatography plate (TLC) GF<sub>254</sub>.

### 2.2 Instruments

The equipments used in this study were spherical flask, column chromatography, thermometer, hot plate, stirrer, rotary evaporators, oil bath, UV light, pipette Eppendorf, UV-Vis spectrophotometer, and <sup>1</sup>H-NMR spectrometer (500 MHz) and <sup>13</sup>C-NMR (125 MHz), LC-MS, GC-MS as well as other chemical glassware.

### 2.3 Method

#### 2.3.1 Synthesis of dihydrocoumarin derivatives

Into a round flask 30 mL was added cinnamic acid, *p*-TSOH acid, and phenol/*o*-cresol/*p*-cresol with equivalent mole ratio of 1: 1.2: 1.2, respectively. The reaction mixture was heated with an oil bath to a temperature of 170 °C for 3 hours. This reaction takes place without the use of solvents. The reaction mixture was diluted with ethyl acetate and neutralized using 0.5 M NaOH. The reaction mixture was extracted with ethyl acetate several times. Ethyl acetate phases were dried with anhydrous Na<sub>2</sub>SO<sub>4</sub>, evaporated, and purified by column chromatography with eluent *n*-hexane: ethyl acetate 10:1, v/v<sup>10</sup>. Products were weighed and identified by <sup>1</sup>H NMR, <sup>13</sup>C NMR.

#### 2.3.2 Toxicity Test in BSLT<sup>13,14</sup>

##### 2.3.2.1 Shrimp larvae hatching

Approximately 50-100 mg of eggs hatched shrimp in the place rectangular (2 x 10 cm) equipped with 2 mm diameter perforated barrier and had been filled with sea water, covered with aluminum foil on the part that contains the eggs, and left for 48 hours under lights. The eggs would hatch and the larvae of shrimps to be tested were taken with a pipette on the shiny side.

##### 2.3.2.2 Preparation of the solution to be tested

Pure compounds to be tested were made with concentrations of 2000, 1000, 200, 20 ppm in seawater. Insoluble compounds in seawater, added 10 mL DMSO.

##### 2.3.2.3 Toxicity tests

Shrimp larvae which live as much as 10-15 tails inserted into the test vial containing 100 mL seawater. Added to the sample solution to be tested each 100 mL, so the final concentration to be 10, 100, 500, 1000 ppm. Solution was stirred until homogeneous, for each concentration performed three repetitions. Controls carried out without the addition of the sample, and then allowed to stand for 24 hours. Dead and live larvae were counted amount. Furthermore the mortality rate is calculated by comparing the number of dead larvae divided by the total number of larvae. Then made the graph between log concentrations and % mortality in order to obtain the linear regression equation  $y = ax + b$ . LC<sub>50</sub> values obtained by inserting the value of  $y = 50$ , the obtained concentrations of test compound that caused 50% mortality of larvae, called LC<sub>50</sub>.

##### 2.3.2.4 Cytotoxicity test by MTT method<sup>15,16</sup>

Test compounds were made as much as 1000 ppm stock solution with dimethylsulfoxide. Stock solution was then diluted with media Dulbecco's Modified Eagle Media (DMEM) to test against HeLa cells (ATCC CCL2) and P388 cells to obtain the variation of the concentration of the test solution. Concentrations of test solutions used were 100, 50, and 10 ppm. Cells were grown in DMEM medium and distributed into 96 well plate with the number 2000 cells / well (100μl) were then incubated for 24 hours at 37 °C in a 5% CO<sub>2</sub> incubator. Test solution in various concentrations of each added to the HeLa cells/P388, and incubated again for 24 hours. Each test compound tested three times repetition. 3-(4,5-dimethylthiazol-2-yl)-(-2,5-diphenyl tetrazolium bromide) (MTT) in sodium hydrogen carbonate (NaHCO<sub>3</sub>) was then added to 10 mL/well at each concentration and further incubated for 4 h at 37 °C to form formazan. Living cells will

convert the MTT into dark-blue formazan. Formazan formed was dissolved in 96% ethanol. Uptake read by ELISA reader at a wavelength of 562 nm.

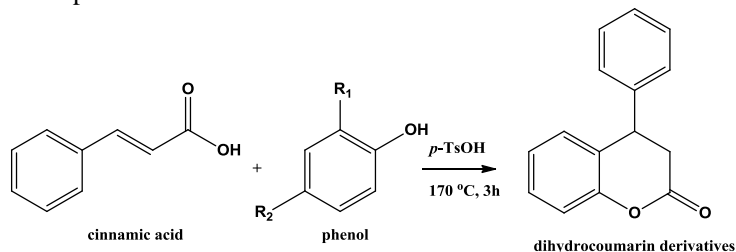
Percent inhibition of proliferation was calculated based on the value of Optical Density (OD) with the formula:

$$\text{Inhibition\%} = \frac{(\text{Mean OD control} - \text{OD sample mean})}{\text{Average OD control}} \times 100 \quad \dots(1)$$

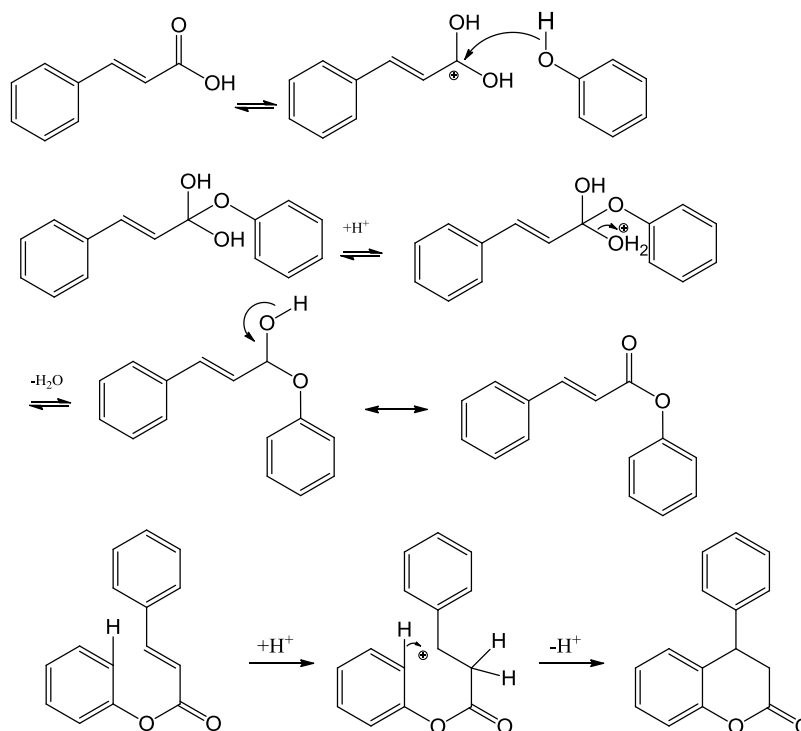
Results of inhibition then plotted with concentration or log concentration and the linear regression equation. 50% cell viability rates included in the linear regression equation obtained in order to obtain IC<sub>50</sub> values.

### 3. RESULTS AND DISCUSSION

Cinnamic acid obtained from hydrolysis of methyl *trans* cinnamate in alkaline condition, in this reaction was produce a product of cinnamic acid in 85 % yield, respectively. The cyclization of cinnamic acid with phenolic compounds in high temperature and the absence of solvent were resulted of the dihydrocoumarin derivatives (Scheme 1). We used cinnamic acid (an electron-rich) as starting material in addition to phenol, *o*-cresol and *p*-cresol which were examined as shown in Table 1. The mixture of starting material and substrate in *p*-toluensulfonic acid was stirred for 3 h at 170°C. Workup (neutralization and extraction) followed by purification (column chromatography on silica gel) gave 4-phenylchroman-2-one, 8-methyl-4-phenylchroman-2-one, and 6-methyl-4-phenylchroman-2-one. The *p*-toluensulfonic acid afforded dihydrocoumarins derivative through inter-molecular reaction type. This reaction is a very simple and useful method. The structures of compounds were elucidated from their <sup>1</sup>H-NMR and <sup>13</sup>C-NMR.



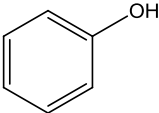
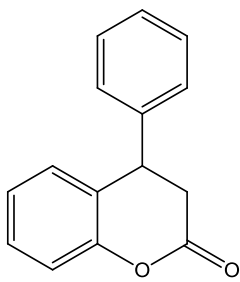
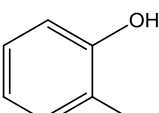
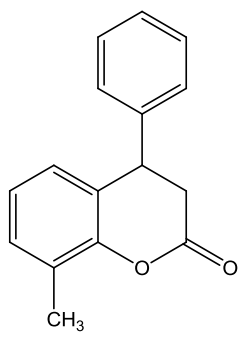
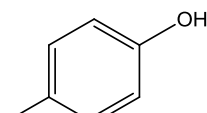
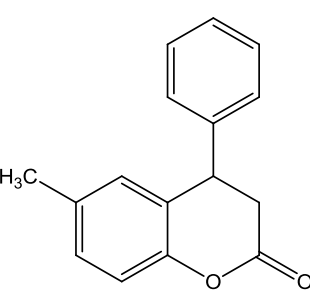
**Scheme 1.** Synthesis of dihydrocoumarin derivatives; 4-phenylchroman-2-one (R<sub>1</sub>=H, R<sub>2</sub>=H), 8- methyl-4-phenylchroman -2-one (R<sub>1</sub>=CH<sub>3</sub>, R<sub>2</sub>=H), 6-methyl-4-phenylchroman-2-one (R<sub>1</sub>=H, R<sub>2</sub>=CH<sub>3</sub>)



**Scheme 2.** Mechanism synthesis reaction of dihydrocoumarin derivatives

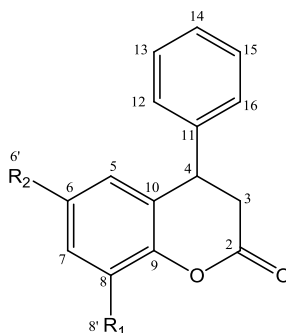
For example; the  $^1\text{H}$  NMR spectrum of 4-Phenylchroman-2-one exhibited two doublets at 3.09 and 3.06 (J=7 Hz; J=10 Hz, 2H), one triplet at 4.37 (1H), one doublet at 6.99 (J=7.8, 1H), two doublet doublets at 7.15 (J=3.3; J=7.8, 2H), one triplet at 7.17 (1H), four doublet doublets at 7.32 (J=2 Hz; 6.5 Hz, 2H) and 7.37 (J=2 Hz; 6.5 Hz, 2H), one triplet at 7.10 (1H). The  $^{13}\text{C}$ -NMR spectrum of 4-Phenylchroman-2-one exhibited carbonyl group at 167.8, cyclic group in lactone ring at 37.2 and at 40.8, aromatic group at 129.0, 124.8, 128.5, 117.3, 151.9 and 125.9 and phenyl group at 140.4, 127.7, 127.8, and 129.3 (Table 2).

**Table 1.** Synthesis Dihydrocoumarin from hydroarylation of cinnamic acids with phenol, *o*-cresol and *p*-cresol

Entry	Substrate	Product	Temperatur (°C)	Melting point (°C)	Time (h)	Yield (%)
1.			125	78	3	56.2
2.			170	60	3	41.6
3.			170	70	3	55.2

4.

The reaction of cinnamic acid as an electron rich substrate with phenol was examined 4-phenylchroman-2-one in 56.2 % yield. Similar reaction of cinnamic acid with *o*-cresol and *p*-cresol gave the corresponding 8-methyl-4-phenylchroman-2-one and 6-methyl-4-phenyl chroman-2-one.



**Figure 1.** Dihydrocoumarin derivatives ; 4-phenylchroman-2-one ( $R_1=H$ ,  $R_2=H$ ), 8-methyl-4-phenylchroman 2-one ( $R_1=CH_3$ ,  $R_2=H$ ), 6-methyl-4-phenylchroman-2-one ( $R_1=H$ ,  $R_2=CH_3$ )

**Table 2.** H-NMR and  $^{13}C$ -NMR Data for Dihydrocoumarin derivatives

Position	Chemical Shift ( $\delta$ , J in Hz)					
	4-Phenylchroman-2-one		8- Methyl-4-phenylchroman 2-one		6- Methyl -4-phenylchroman 2-one	
	$^1H$ -NMR	$^{13}C$ -NMR	$^1H$ -NMR	$^{13}C$ -NMR	$^1H$ -NMR	$^{13}C$ -NMR
2	-	167.8	-	168.0	-	168.1
3	3.09( <i>dd</i> , 1H, J=7; J=10) 3.06 ( <i>dd</i> , 1H, J=7; J=10)	37.2	3,07 ( <i>dd</i> , 1 H, J = 9,7. 16,5) 3,04 ( <i>dd</i> , 1 H , J = 5,8. 16,5)	37.1	3,04 ( <i>dd</i> , 1H, J=16,2; 5,85) 3.03 ( <i>dd</i> , 1H, J=16,2; 6,5 )	37.3
4	4.37 ( <i>t</i> , 1H, J=7)	40.8	4,33 ( <i>t</i> , 1 H, J = 6,5)	40.9	4,28 ( <i>t</i> , 1H, 6,5)	40.9
5	6.99 ( <i>d</i> , 1H, J=7.8)	129.0	6,82 ( <i>d</i> ,1H, J = 7,1)	126.0	6,77 ( <i>d</i> )	127.7
6	7.15 ( <i>dd</i> , 1H, J=3.3; J=7.8)	124.8	7,28 ( <i>t</i> , 1H = 7,1)	127.8	-	127.8
6'	-	-	-	-	2,24 ( <i>s</i> , 3H)	20.3
7	7.17 ( <i>t</i> , 1H, J=7.8)	128.5	7,16 ( <i>d</i> , 1H= 7,1)	124.3	7,02 ( <i>dd</i> , 1H, 8,45)	129.5
8	7.15 ( <i>dd</i> , 1H, J=3.3; J=7.8)	117.3	-	125.7	7,09 ( <i>t</i> , 1H, 8,45 )	117.0
8'	-	-	2,37 ( <i>s</i> , 3 H )	16.0	-	-
9	-	151.9	-	150.1	-	149.8
10	-	125.9	-	126.6	-	134.5
11	-	140.4	-	140.6	-	140.7
12	7,32 ( <i>dd</i> , 1H, J=2,0 Hz; J= 6,5 Hz)	127.7	6,98 ( <i>dd</i> , 1 H , J = 7.8)	129.2	7.15 ( <i>dd</i> , 1H, 2,0; 7,0)	129.3
13	7,37 ( <i>dd</i> , 1H, J=6,5 Hz; J=7,0 Hz)	129.3	7,36 ( <i>dd</i> , 1H, J= 7.1)	129.9	7,34 ( <i>dd</i> , 1H, 2,0; 7,0)	129.5
14	7,10 ( <i>t</i> , 1H, J=7,0 Hz)	127.8	7,15 ( <i>t</i> , 1H, J= 7,14)	127.7	7,29 ( <i>t</i> , 1H, 7,0)	128.8
15	7,37 ( <i>dd</i> , 1H, J=6,5 Hz; J=7,0 Hz)	129.3	7,36 ( <i>dd</i> , 1H, J= 7.1)	129.9	7,34 ( <i>dd</i> , 1H, 2,0; 7,0)	129.5
16	7,32 ( <i>dd</i> , 1H, J=2,0 Hz; J=6,5 Hz)	127.7	6,98 ( <i>dd</i> , 1 H , J = 7.8)	129.2	7,15 ( <i>dd</i> , 1H, 2,0; 7,0)	129.3

Cinnamic acid and its natural analogues are known for the treatment of cancer for over centuries. Herein, we observed evaluation especially for the dihydrocoumarin derivatives and their anticancer potentials. In our laboratory, dihydrocoumarin derivatives have been tested in vitro cytotoxic potential against P388 Leukemia cells and HeLa cervical cancer cells. Biological activities of cinnamic acid and dihydrocoumarin derivatives were described in Table 3 below.

**Table 3.** Evaluation of Biological Activity

No	Compounds	Biological Activity	
		BSLT (LC <sub>50</sub> in ppm)	Cancer Cells
1	Methyl <i>trans</i> -cinnamate	199.53	P388 Leukemia cells IC <sub>50</sub> = 20.35 µg/mL
2	Cinnamic Acid	204.17	P388 Leukemia cells IC <sub>50</sub> = 48.85 µg/mL
3	4-Phenylchroman-2-one	112.72	HeLa cells > 50 % inhibition
4	8- Methyl-4-phenylchroman 2-one	110.80	P388 Leukemia cells IC <sub>50</sub> = 68.42 µg/mL
5	6- Methyl -4-phenylchroman 2-one	93.33	P388 Leukemia cells IC <sub>50</sub> = 37.85 µg/mL

## 5. CONCLUSION

Hydrolysis reaction of methyl *trans*-cinnamate to cinnamic acid was produced with a yield of 83.6%. Reaction of cinnamic acid with phenol, *p*-cresol and *o*-cresol using *p*-toluensulfonat was produced 4-phenylchroman-2-one, 8-methyl-4-phenylchroman-2-one and 6-methyl-4-phenyl chroman-2-one in 56.2 %, 41.2 % and 55.2 % yield respectively. This method is very simple for the dihydrocoumarin natural product synthesis. And Evaluation their biological activity results that they have potency as candidate anti-cancer.

## 6. ACKNOWLEDGMENT

We thank the financial support for this work from the Competitive Project of Indonesian Institutes of Sciences (LIPI).

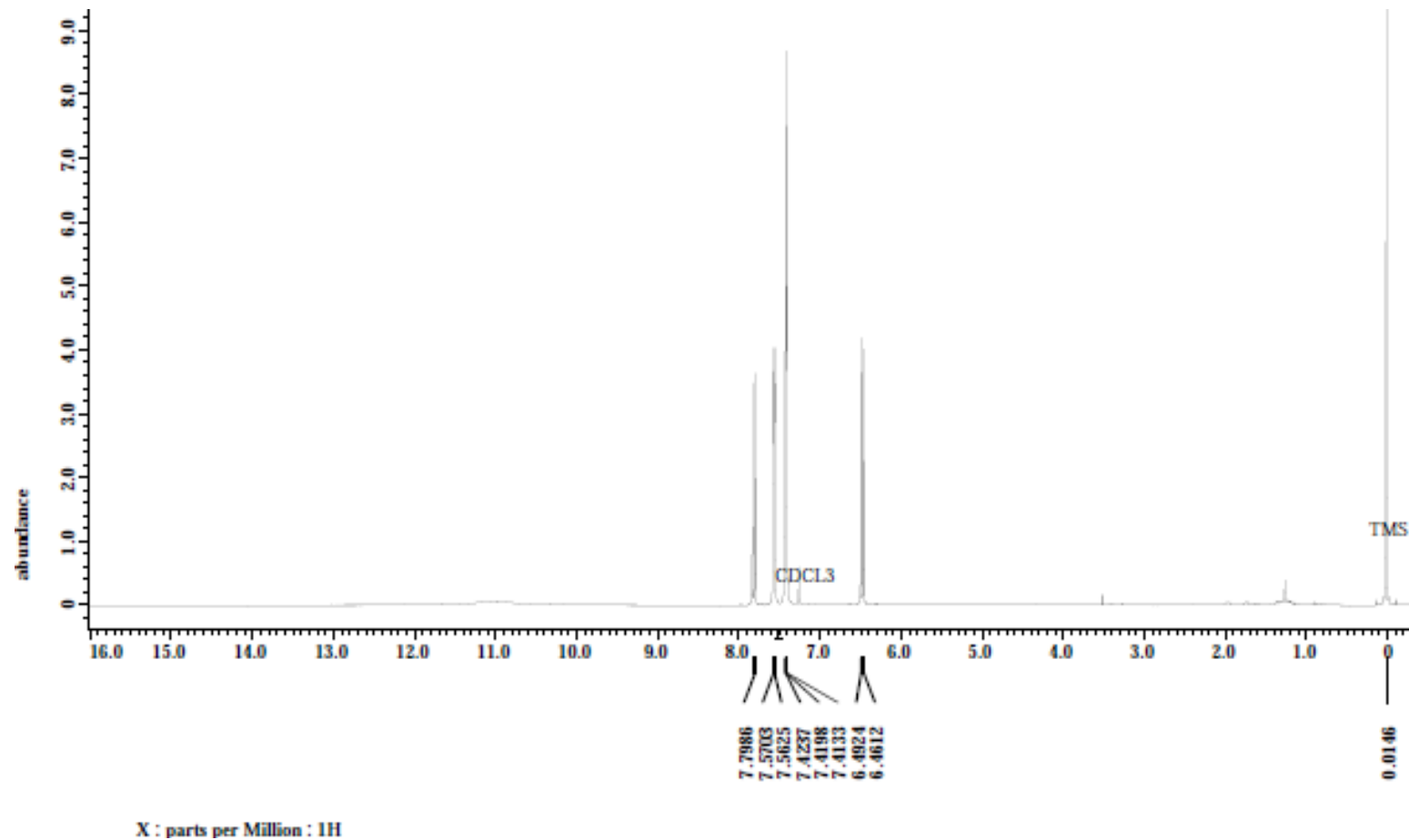
## 7. REFERENCES

- [1] Sinaga, Erna. *Alpinia galanga* (L.) Willd., Lengkuas. Pusat Penelitian dan Pengembangan Tumbuhan Obat UNAS (P3TO UNAS). [http://www.warintek.ristek.go.id/pangan\\_kesehatan/tanaman\\_obat\\_/depkes/Bunglai\\_%20laki.pdf](http://www.warintek.ristek.go.id/pangan_kesehatan/tanaman_obat_/depkes/Bunglai_%20laki.pdf), tanggal 10 Mei 2012.
- [2] Ernawati, Teni, Deana Wahyuningrum, Minarti, Yulia Anita, Lia Meilawati, dan Puspa D. Lotulung, "Development of Raw Materials for Drugs by Using Methyl *trans* Cinnamate Isolated from Galangal (*Alpinia malaccensis*) as Novel Approaches to Drug Discovery". In Proceeding of The Eijkman Institute Comes of Age: Vitamins, Genomics, dan Welfar. Jakarta: Eijkman Institute for Molecular Biology, PIV-5, pp 146, 2011.
- [3] Muchtaridi, Ikhsan Rambia, Ida Musfiroh. Kadar Metil Sinamat dari Batang, Daun dan Rimpang Tumbuhan Laja Gowah (*Alpinia Malaccensis* (Burm f.)) dengan GC/MS. Fakultas Farmasi Universitas Padjajaran, 2008.
- [4] Li, Q. F., S. L. Shi, Q. R. Liu, J. Tang, J. Song, dan Y. Liang. "Anticancer Effects of Ginsenoside Rg1, Cinnamic Acid, and Tanshinone in Osteosarcoma MG-63 Cells: Down Regulation and Cytoplasmic Trafficking of Neucleophosmin". *Int. J. Biochem. Cell Biol.* Vol. 40, pp.1918 – 1929, 2008.
- [5] Baltas, M. P. De, dan F. Bedos Belval. "Cinnamim Acid Derivatives as Anticancer Agents- A Review. *Current Medicinal Chemistry*", Vol.18, pp.1672 – 1703, 2011.
- [6] Bairwa, Ranjeet, Mariam S. Degani, "Novel Molecular Hybrid of Cinnamic Acids and Guanylhidrazones as Potential Antitubercular Agents", *Bioorganic and Medicinal Chemistry Letters*, Vol.20, pp.1623 – 1625, 2010.
- [7] Li, Kelin, Lindsay N. Foressee, dan Jon A. Tunge. "Trifluoroacetic Acid Mediated Hydroarylation: Synthesis of Dihydrocoumarins dan Dihydroquinolones", *J. Org. Chem.* Vol.70 (7), pp. 2881 – 2883, 2005.
- [8] Lin, Hsiu Chen, Shin Hui Tsai, Chien Shu Chen, "Structure Activity Relationship of Coumarin Derivatives on Xanthine oxidase Inhibiting and Free Radical-Scavenging Activities", *Biochemical Pharmacology*, Vol.75, pp.1416 – 1425, 2008.
- [9] Baba, Masaki, Yongri Jin, Atsuo Mizuno, Studies on Cancer Chemoprevention by Traditional Folk Medicines XXIV, "Inhibitory Effect of A Coumarin Derivative, 7-isopentenylcoumarin, Against Tumor-Prevention", *Biol. Pharm. Bull.* Vol. 25 (2), pp. 244 – 246, 2002.
- [10] Ekowati, Heny, Indwiani Astuti, dan Mustofa, "Anticancer Activity of Calanone on HeLa Cell Line", *Indo. J. Chem.* Vol.10(2), pp. 247 – 251, 2010.
- [11] Chuang, Jing-Yuang, Yung Feng Huang, Hsu Feng Lu, "Coumarin Induces Cell Cycle Arrest and Apoptosis in Human Cervical Cancer HeLa Cells Through A Mitochondria and Caspase-3 Dependent Mechanism and NF-kB Down-Regulation", *In vivo*, Vol. 2t, pp.1003 – 1010, 2007.

- [12] Sudalai, A. dan A. R. Jagdale. “*p*-toluenesulfonic acid Mediated Hydroarilation of Cinnamic Acids with Anisoles and Phenols Under Metal and Solvent-Free Conditions”, *Tetrahedron Letters*, Vol. 48, pp. 4895 – 4898, 2007.
- [13] Dey, P. M. dan J. B. Harborne, “*Methods in Plant Biochemistry: Assays for Bioactivity*”, Volume 6. London: Academic Press, pp. 1 – 30, 1991.
- [14] McLaughlin, Jerry L., Lingling L. Rogers, dan Jon E. Anderson, “The Use of Biological Assays to Evaluate Botanicals”, *Drug Information Journal*, Vol.32, pp. 513 – 524, 1998.
- [15] Wu, Bin, Jin-Shui Zhu, Yi Zhang, Wei-Ming Shen, dan Qiang Zhang, “Predictive Value of MTT Assay as An in vitro Chemosensitivity Testing for Gastric Cancer: One Institution’s Experience”, *World Journal Gastroenterology*, Vol.14 (19), pp. 3064 – 3068, 2008.
- [16] Sumaryono, Wahono dan Agung Eru Wibowo, “Aktivitas sitotoksik Ekstrak Etanol Daun *Aglaia elliptica* Blume terhadap Galur Sel Kanker Serviks (HeLa)”, *J. Ilmu Kefarmasian Indonesia*, Vol. 8(1), pp. 19 – 23, 2010.

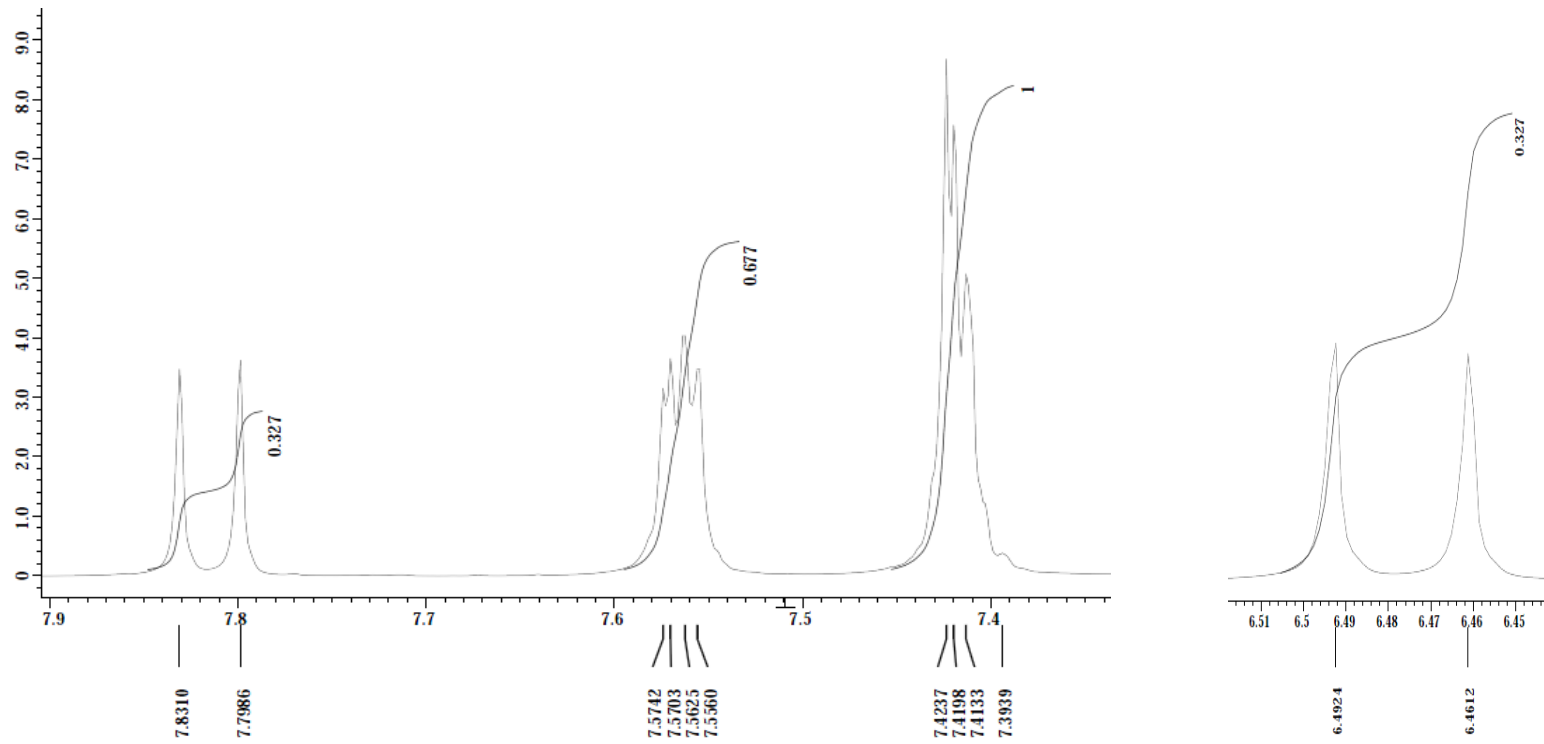
Supplementary Data

<sup>1</sup>H-NMR Data of Cinnamic acid

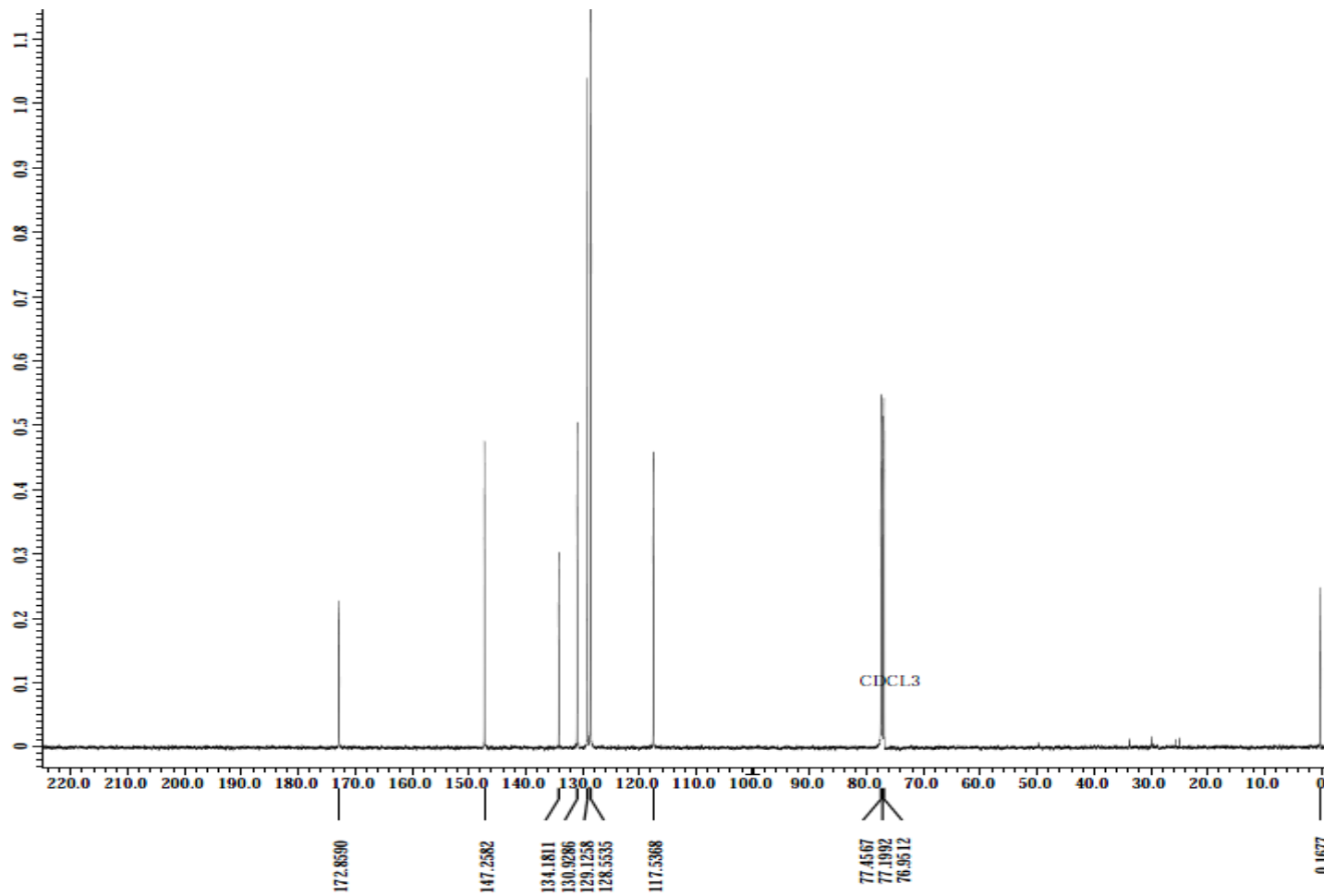




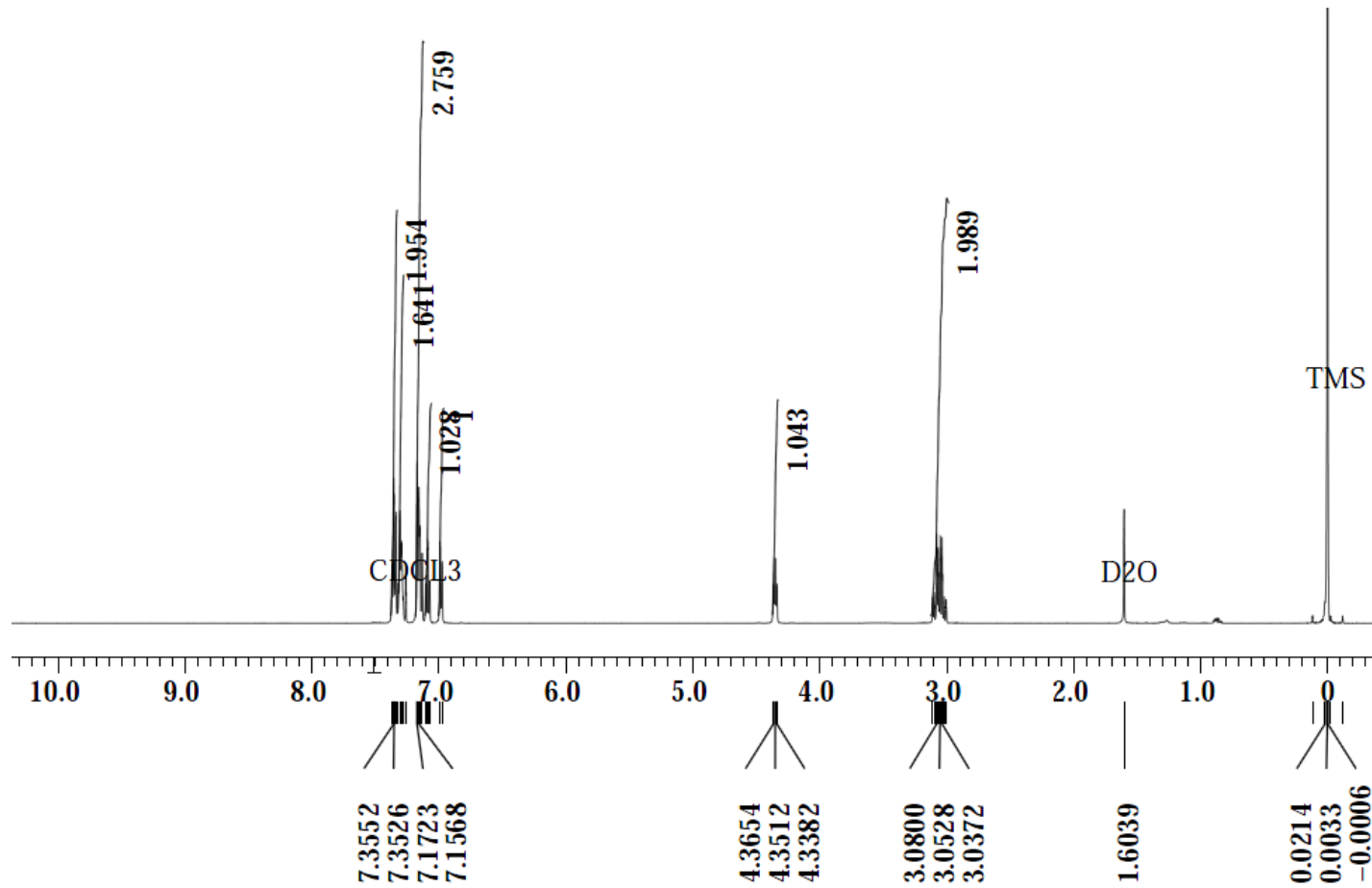
<sup>1</sup>H-NMR Data of Cinnamic acid



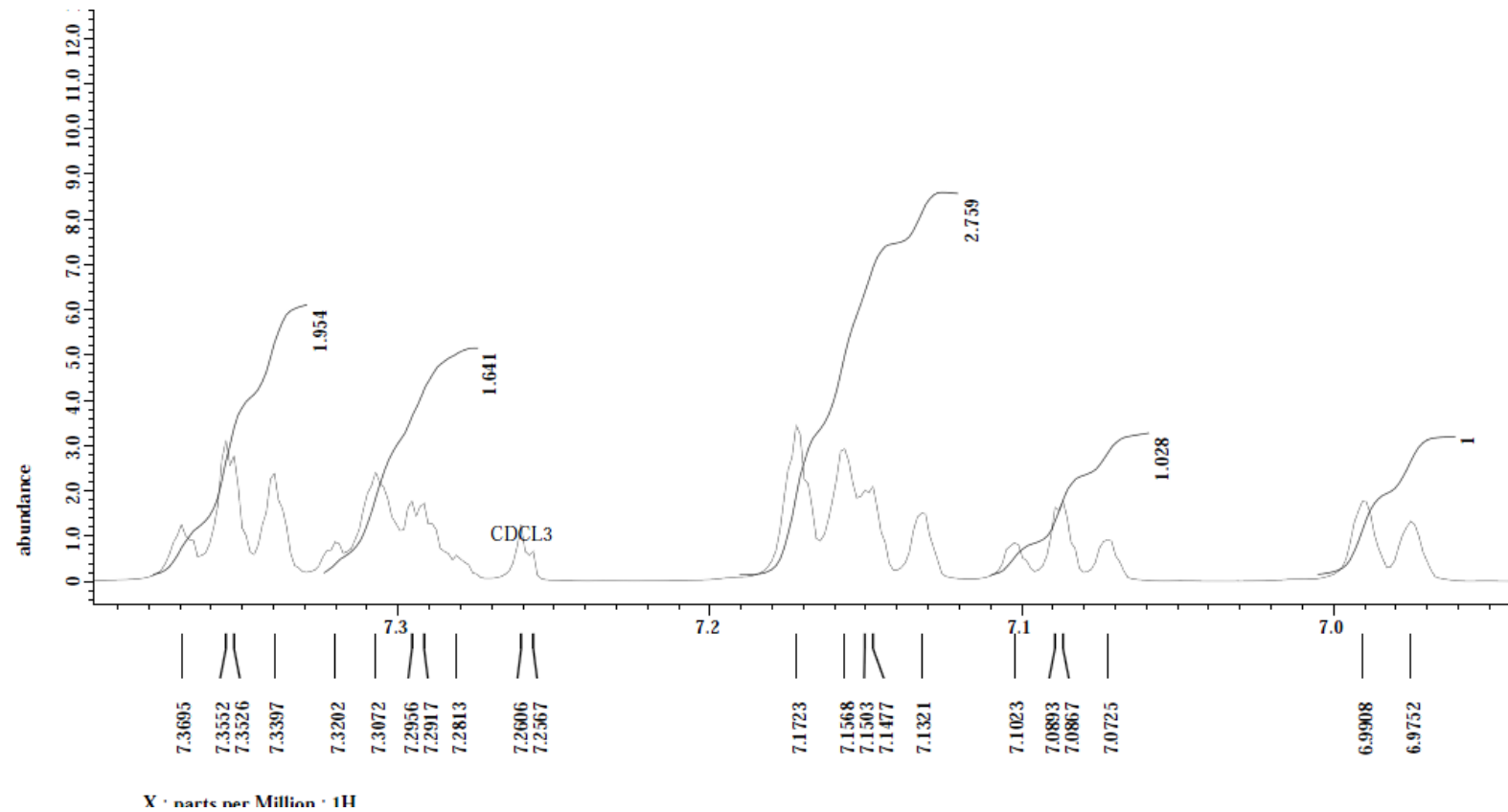
$^{13}\text{C}$ -NMR Data of Cinnamic acid



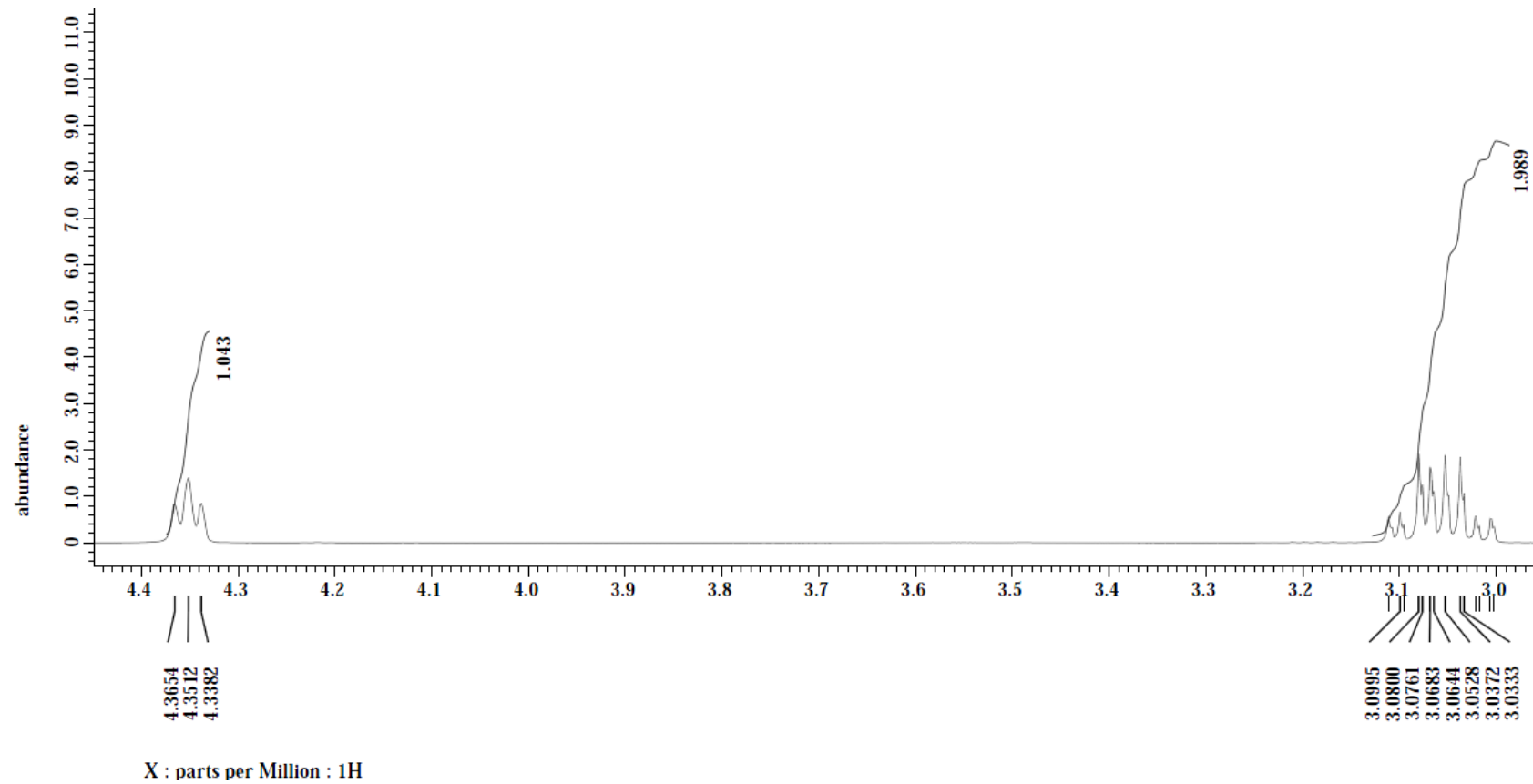
<sup>1</sup>H-NMR Data of 4-Phenylcroman-2-one



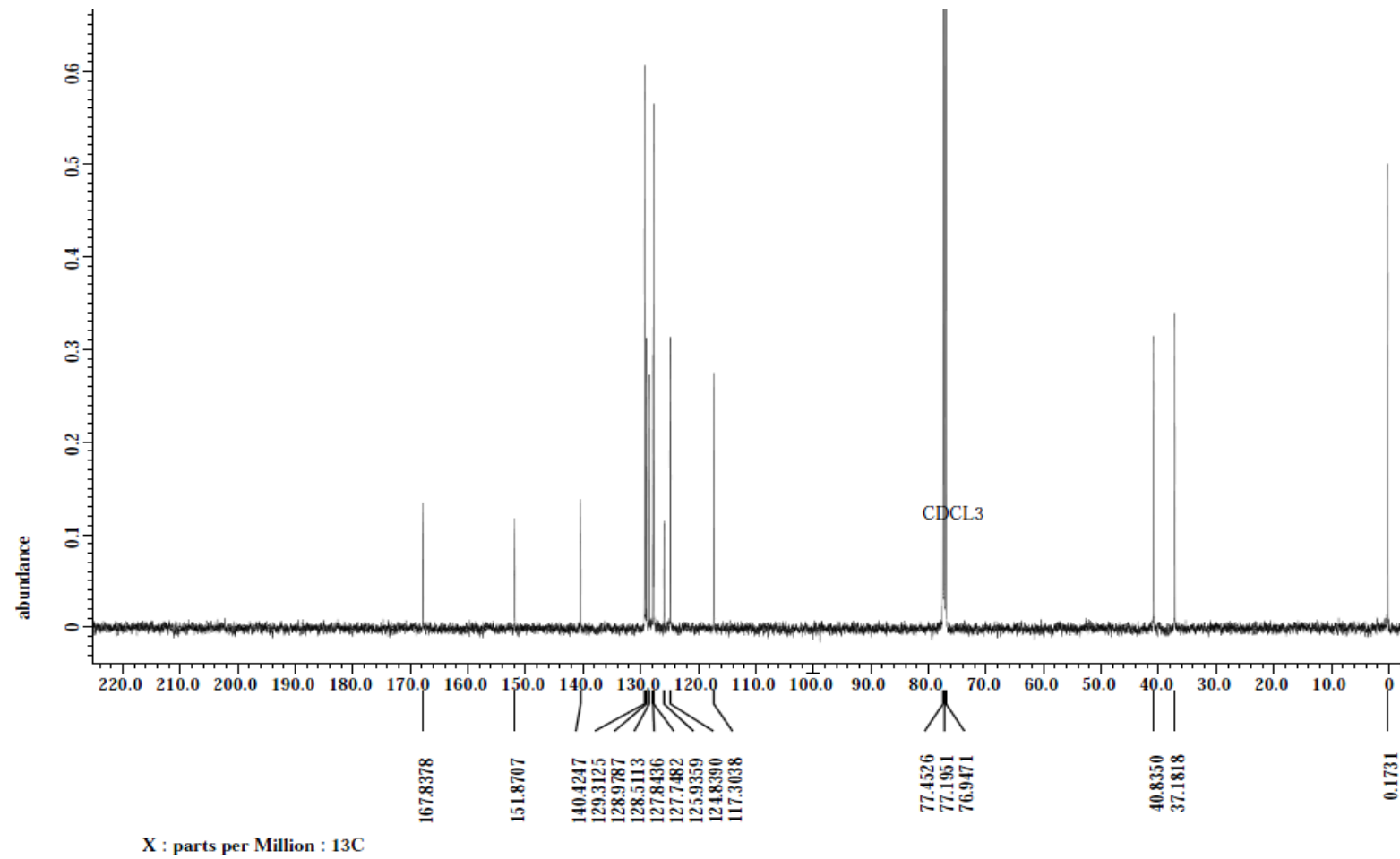
<sup>1</sup>H-NMR Data of 4-Phenylcroman-2-one



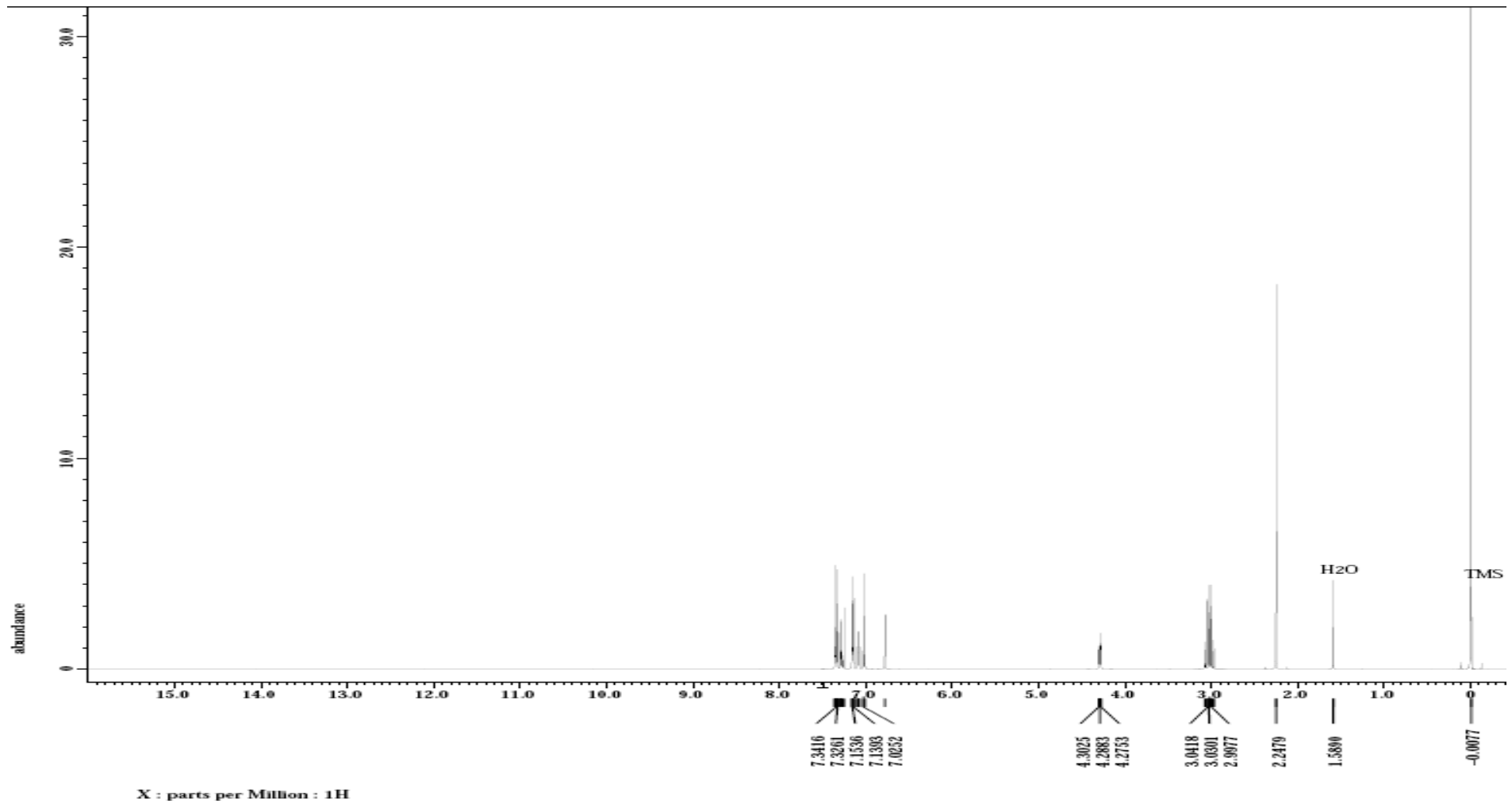
<sup>1</sup>H-NMR Data of 4-Phenylcroman-2-one



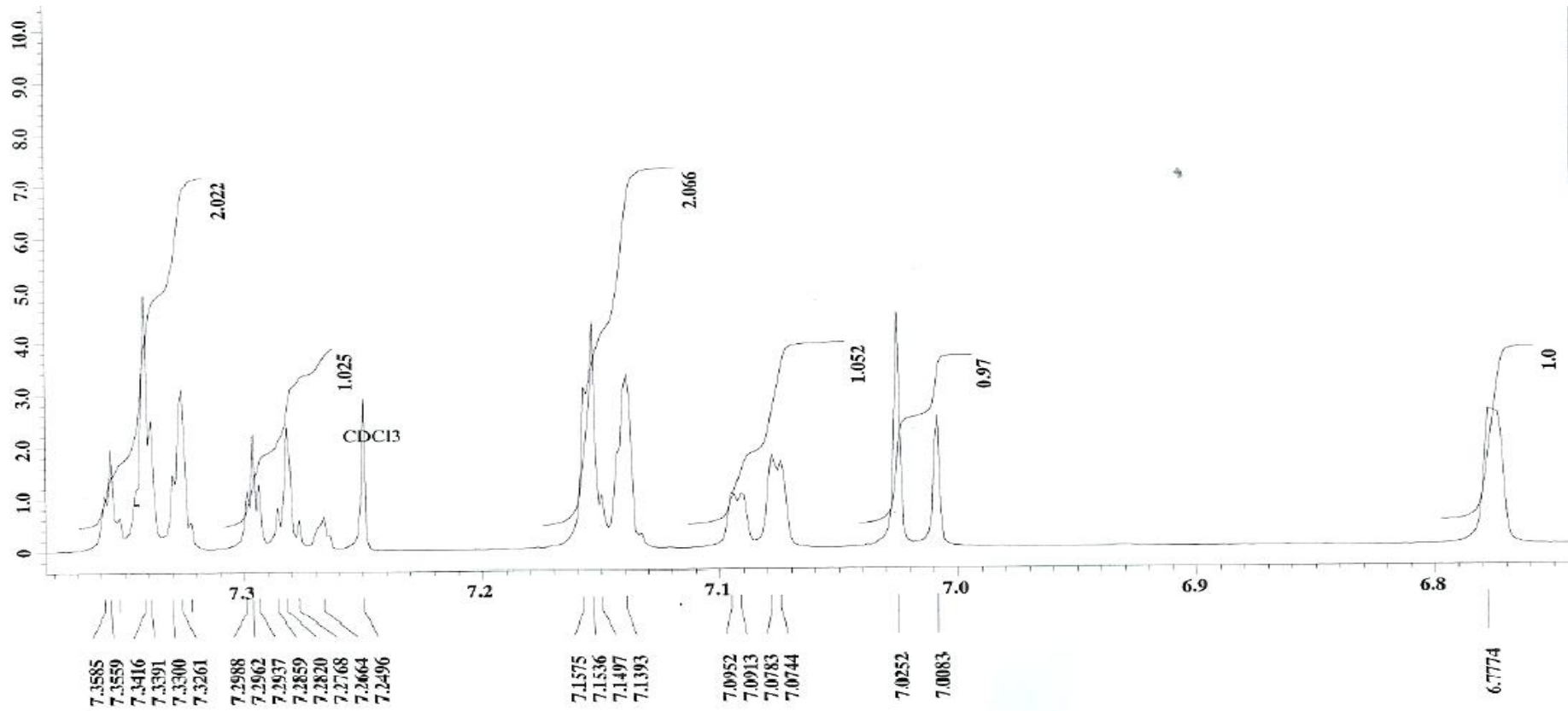
$^{13}\text{C}$ -NMR Data of 4-Phenylcroman-2-one



<sup>1</sup>H-NMR Data of 6-methyl-4-Phenylchroman-2-one

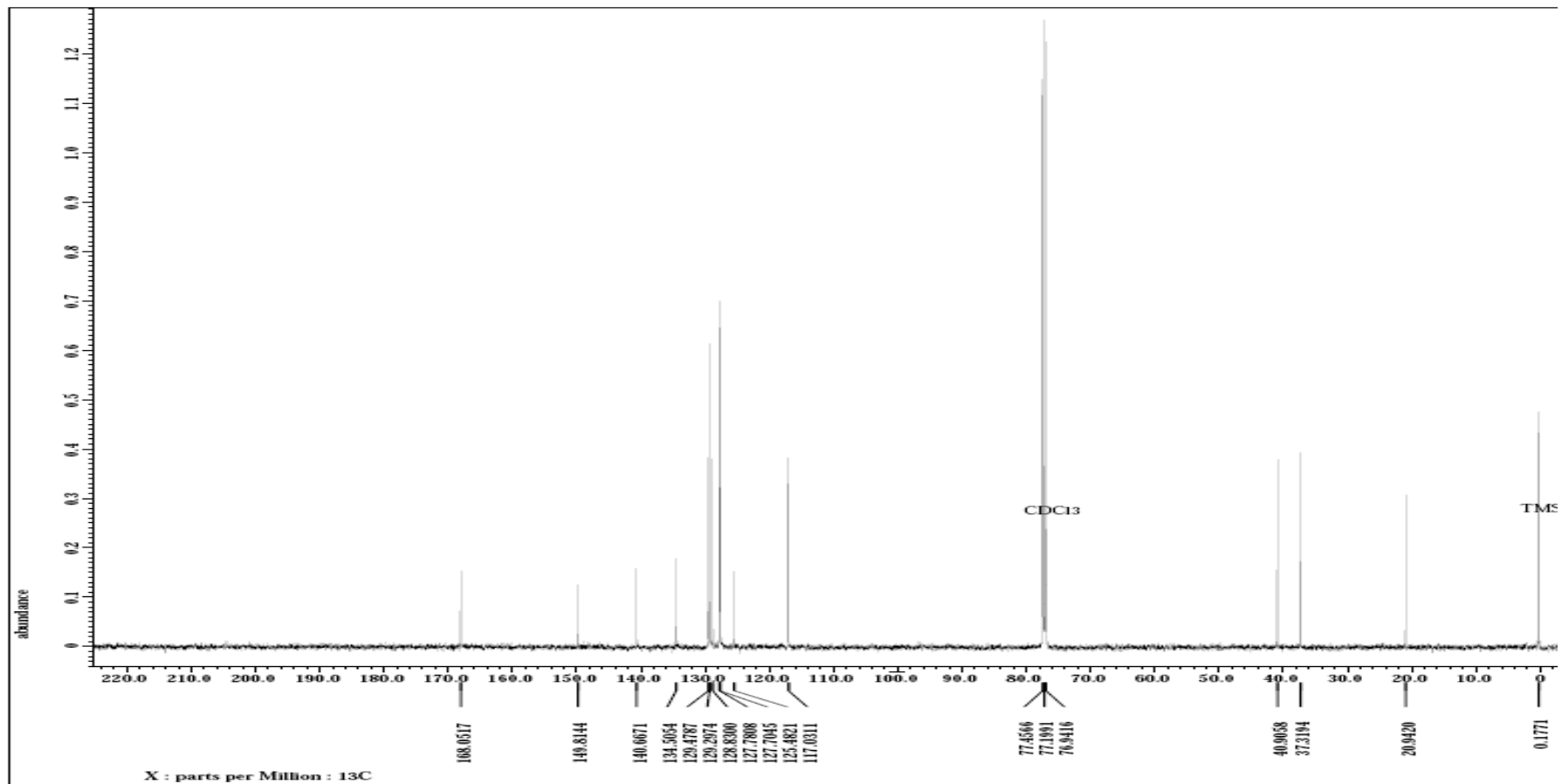


$^1\text{H-NMR}$  Data of 6-methyl-4-Phenylcroman-2-one

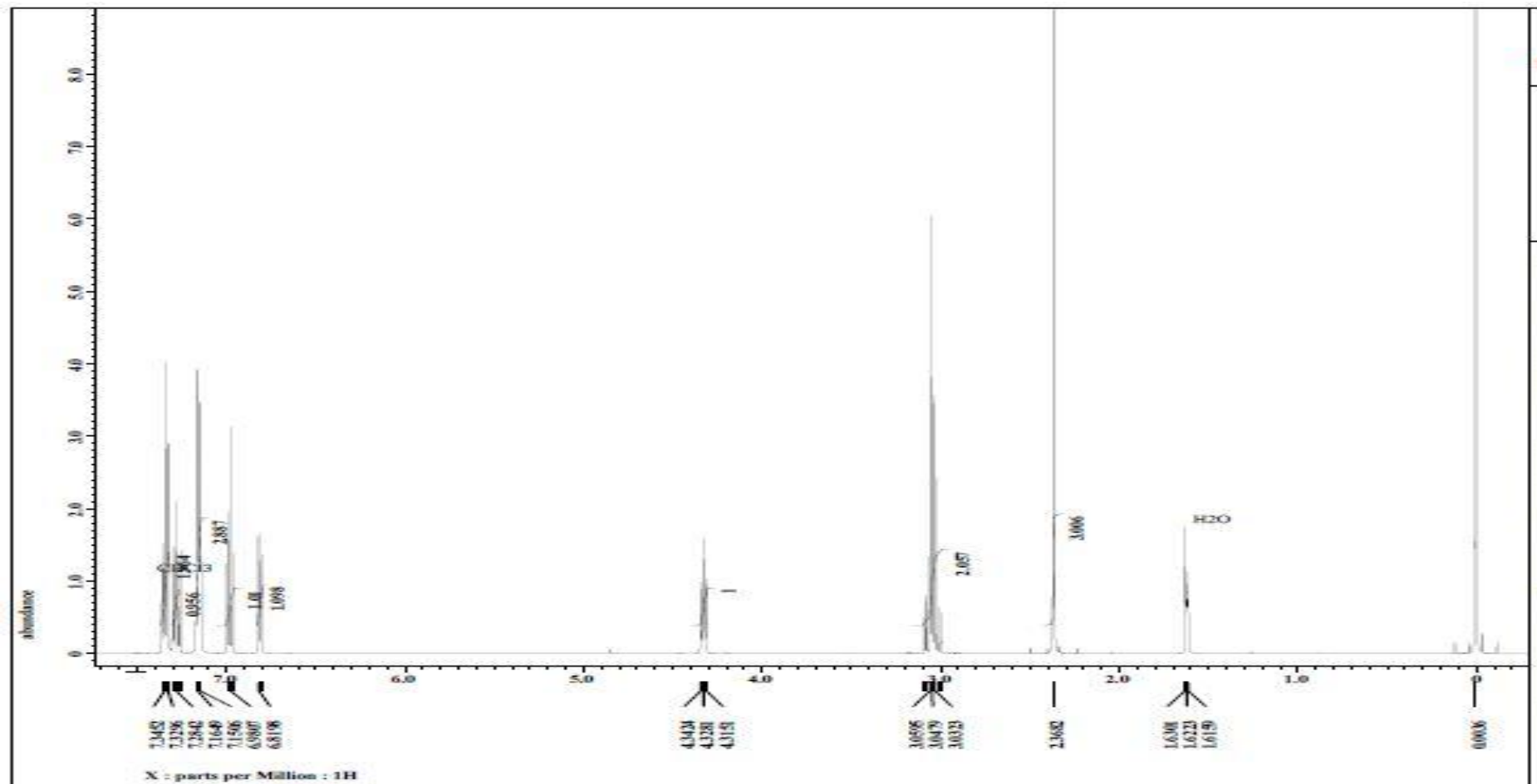




$^{13}\text{C}$ -NMR Data of 6-methyl-4-Phenylcroman-2-one



<sup>1</sup>H-NMR Data of 8-methyl-4-Phenylcroman-2-one



$^{13}\text{C}$ -NMR Data of 8-methyl-4-Phenylcroman-2-one

