CHAPTER III

RESULTS AND DISCUSSION

In this study, rice husks from five strains of rice cultivar, including Chai-nat 1 (CN), Look Daeng Pattani (LD), Leb Nok Pattani (LN), Go Ko 1 (GK) and Jasmine (JM), were extracted by organic solvents. Hexane, dichloromethane (CH_2Cl_2), ethylacetate (EtOAc), and methanol (MeOH) crude extract comprise about 1-2% (w/w) of the dry rice husks (Table 3.1). Among hexane, CH_2Cl_2, EtOAc, and MeOH extract, MeOH extract gave the highest % yield for CN, LD and Gk. Only the Jasmine rice husk shows maximum % yield in EtOAc extract.

Table 3.1 Amount (% yield) of extracts in each strains of rich husk

Strains	% yield of extract in each solvents (wt/wt)				
	Hexane	CH_2Cl_2	EtOAc	MeOH	total
CN	0.19	0.40	0.11	1.44	2.14
LD	0.22	0.28	0.12	1.04	1.66
GK	0.04	0.21	0.80	0.18	1.23
LN	0.20	0.26	0.11	0.60	1.17
JM	0.15	0.22	0.25	0.09	0.71

3.1 Screening of antioxidant, anti-tyrosinase and UV screening activity from crude rice husks.

Screening for antioxidant activity, anti-tyrosinase activity and UV screening activity in all crude extracts indicated that the CH_2Cl_2 and EtOAc crude extracts of the CN and LD strains possessed potent free radical scavenging effect, moderate anti-tyrosinase activity and UV absorption property while LN and JM possessed potent antioxidant activity in MeOH crude extract, moderate anti-tyrsinase activity in CH_2Cl_2 crude extract and some UV (290-400 nm) absorption property in EtOAc and MeOH crude extract. The rest, GK, showed moderately antioxidant and anti-tyrosinase activities in CH_2Cl_2 and EtOAc crude extracts and exhibited some UV absorption property in EtOAc and MeOH (Table 3.2).

In conclusion, CN and LD strains showed the most interesting biological activities in their CH_2Cl_2 and EtOAc crude extracts. CN gave the higher yield of extraction than LD, therefore the CN strain was selected for further study.

Table 3.2 Primary screening tests of antioxidant, anti-tyrosinase and UV screening activities in various crude extracts (different solvents) from five rice husks

Rice husk name	Solvent	DPPH radical scavenging activity	Anti-tyrosianase activity	UV absorption activity
		TLC autographic	TLC autographic	λ_{max} (nm)
Chai-nat I	Hexane	-	+	-
(CN)	CH_2Cl_2	++	++	310
(ชัยนาท)	EtOAc	+++	++	315
	MeOH	++	+	314
Look daeng Patani	Hexane	-	++	285
(LD)	CH_2Cl_2	++	++	288
(ลูกแดงปัตตานี)	EtOAc	+++	++	315
	MeOH	++	+	315
Leb-nok	Hexane	-	++	-
(LN)	CH_2Cl_2	+	++	-
(เล็บนก)	EtOAc	+	++	311
	MeOH	+++	+	285
Jasmine	Hexane	_	+	
(JM)	CH_2Cl_2	++	++	-
(หอมมะลิ)	EtOAc	++	+	311
	MeOH	+++	+	312
Gor Kor I	Hexane	-	+	
(GK)	CH_2Cl_2	++	++	-
(กข)	EtOAc	++	++	312
	MeOH	++	-	314
BHT		+++	NT	NT
Kojic acid		NT	+++	NT

Note: +++ = Strong activity, ++ = Moderate activity, + = Weak activity, - = No activity

NT = Do not test

BHT (80 µg/ml) using as standard for antioxidant activity

Kojic acid (40 µg/ml) using as standard for anti-tyrosinase activity

All crude extracts used 1000 µg/ml

3.2 Extraction and isolation

Powdered CN husk (3.7 kg) was extracted by maceration at room temperature with EtOAc and CH_2Cl_2. The extracts were filtered and evaporated under reduced pressure to obtain EtOAc crude extract (7.12 g, 0.1% w/w yield) and CH_2Cl_2 extract (9.58 g, 0.26% w/w yield). Both EtOAc and CH_2Cl_2 extract gave similar TLC pattern (solvent system: 7%MeOH in CH_2Cl_2) hence these crude extracts were combined to afford 16.70 g of extract defined as CN I crude extract. This is about 0.45% (w/w) from the powdered rice husk raw material.

3.3 Biological activities of CN I

The CN I crude extract was tested for its antioxidant activity, anti-tyrosinase activity and UV screening activity. The extract showed potent free radical scavenging effect towards DPPH, moderate tyrosinase inhibition activity and UV screening activity. The following paragraph details the three activities.

Five hundred μg/ml CN I crude extracts showed good for radical scavenging capacity. The radical scavenging experiment was carried out using the TLC autographic assay [75]. The method involved dropping sample onto TLC plate and then spraying an entire plate with DPPH radical solution. The active compounds' spots will appear as a white-yellow spot against purple background. The intensity of white-yellow color depends on the amount and nature of radical scavenger present in the sample. In the experiment, a serial dilutions of CN I crude extract (500 and 750 μg/ml) and a 40 μg/ml of BHT solution were tested and compared. The result indicated that activity of CN I at concentration 500 μg/ml was equivalent to activity of BHT at concentration of 40 μg/ml.

The tyrosinase inhibition experiment was carried out using the post TLC developing technique [77]. Sample was spotted onto TLC and then sprayed tyrosinase and L-tyrosine solution. Active sample spots will appear as white spot against brownish-purple background. The intensity of white color depends on the amount of sample and nature of tyrosinase inhibitor present in the sample. The result indicating that CN I at concentration of 1000 μg/ml showed equivalent anti-tyrosinase activity to that of kojic acid at concentration of 20 μg/ml.

CN I crude extract exhibited UV absorption band at 290 nm with shoulder around 320 nm. In addition, CN I crude extract also exhibited the absorption band around 540 nm (visible region) (Figure 3.1).

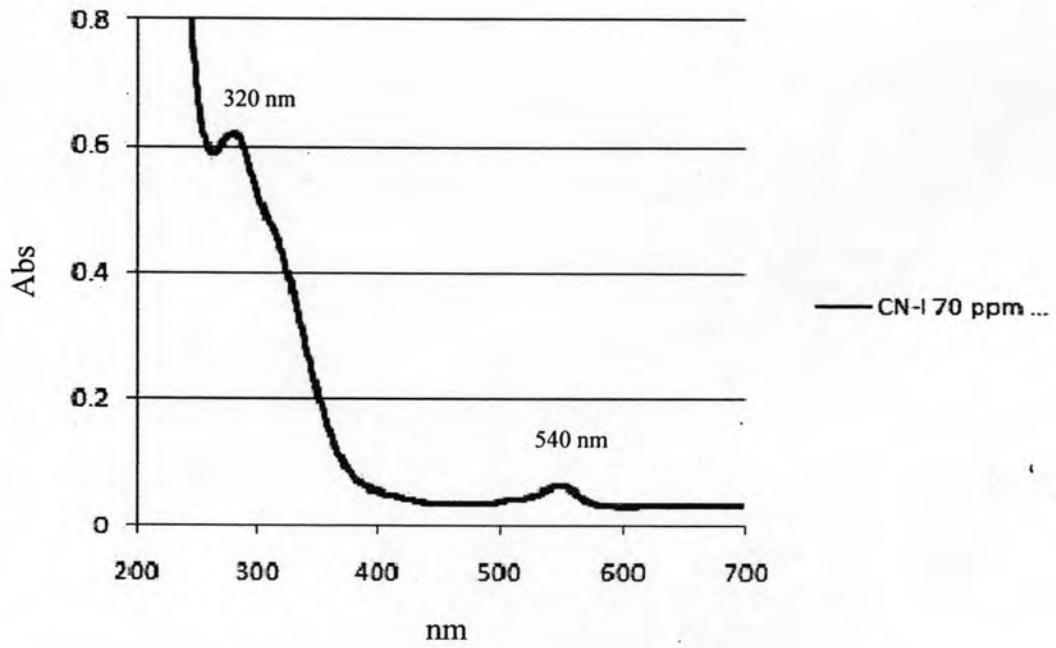


Figure 3.1 UV spectrum of CN I at concentration 70 μg/ml

Since CN I crude extract possessed potent antioxidant activity, a little tyrosinase inhibition activity and some UV-VIS (280-800 nm) absorption property, this extract was further isolated using chromatographic technique in order to identify chemical constituents of CN I.

The isolation of CN I was performed by silica gel column chromatography and obtained 8 fractions (CN I-1 to CN I-8). These eight fractions were tested for their antioxidant activity, anti-tyrosinase activity and UV screening activity. Results from biological activities testing of all separated fractions were used as a guide for further isolation of active compound.

3.4 Biological activities of CN I to CN 8

Antioxidant and anti-tyrosinase activity of CN I-1 to CN I-8 is shown in Table 3.1.

Table 3.3 Biological activities of CN I-1 to CN I-1

Samples	Concentration (µg/ml)	Antioxidant activity	Anti-tyrosinase activity	% yield (wt/wt)
		TLC autographic	Post TLC technique	
CN I-1	1000	-	++	5.71
CN I-2	1000	+++	-	4.28
CN I-3	1000	+	-	2.57
CN I-4	1000	+	-	9.28
CN I-5	1000	++	-	5.00
CN I-6	1000	+++	-	4.28
CN I-7	1000	+++	-	32.86
CN I-8	1000	+++	-	2.14
CN I crude	500	++	NT	
CN I crude	750	++	-	
CN I crude	1000	NT	+	
Kojic acid*	20	NT	++	
Kojic acid	40	NT	+++	
BHT**	40	++	NT	
BHT	80	+++	NT	

Note: +++ = Strong activity

++ = Moderate activity

\+ = Weak activity

\- = No activity

NT = Do not test

* Kojic acid using as standard for anti-tyrosinase activity

** BHT using as standard for antioxidant activity

Only CN I-2, CN I-6, CN I-7 fractions exhibited the absorption in both UVA and UVB. CN I-8 fraction also showed the absorption band in visible region with maximum absorption at 544 nm (Figure 3.2).

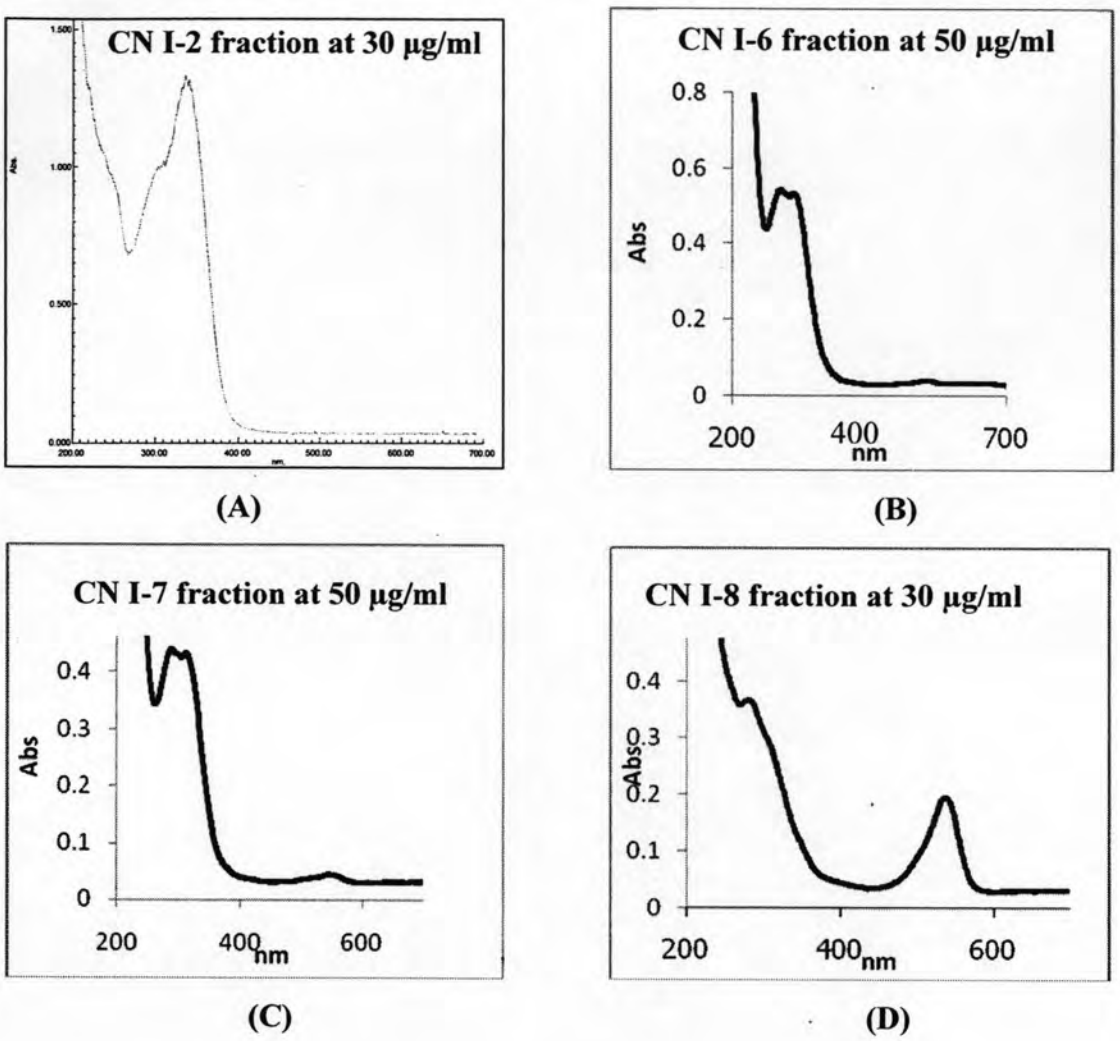


Figure 3.2 UV spectrum of some separated fractions. A) CN I-2 fraction at concentration 30 µg/ml, B) CN I-6 fraction at concentration 50 µg/ml, C) CN I-7 fraction at concentration 50 µg/ml and D) CN I-8 at concentration 30 µg/ml

In conclusion, CN I-1 showed the inhibitory effect toward tyrosinase while CN I-2, CN I-6 and CN I-7 exhibit strongly free radical scavenging activity and UV absorption property. The CN I-8 also exhibited the absorption at 544 nm. Among these five interesting fractions, only CN I-1, CN I-2 and CN I-8 were further isolated in this project because of TLC profiles of CN I-1 and CN I-2 were quite clean and well isolated spots while CN I-8 gave the absorption in the visible region from the purple color compound in fraction.

Isolation of CN I-1, CN I-2 and CN I-8

Isolation of CN I-1 by silica gel column chromatography yielded 3 fractions, CN I-1/1, CN I-1/2 and CN I-1/3. Since, the most non-polar fractions (CN I-1/1) gave white precipitate when it was dissolved in MeOH thus CN I-1/2 was further purified by precipitation using cold MeOH (Scheme 3.1). The white precipitate was identified as dipentyl phthalate (11 mg, 0.16% w/w) and the MeOH soluble compound (yellow oil) was identified as 5-decene (8 mg, 0.11% w/w).

Isolation of CN I-2 by silica column chromatography yielded 4 fractions, CN I-2/1, CN I-2/2, CN I-2/3 and CN I-2-4. The moderate polar fraction (CN I-2/3) was further purified by semi-preparative HPLC (Scheme 3.2) to obtain 4-hydroxy-3-methoxycinnamaldehyde (1.5 mg, 0.22% w/w) at retention time 20 min.

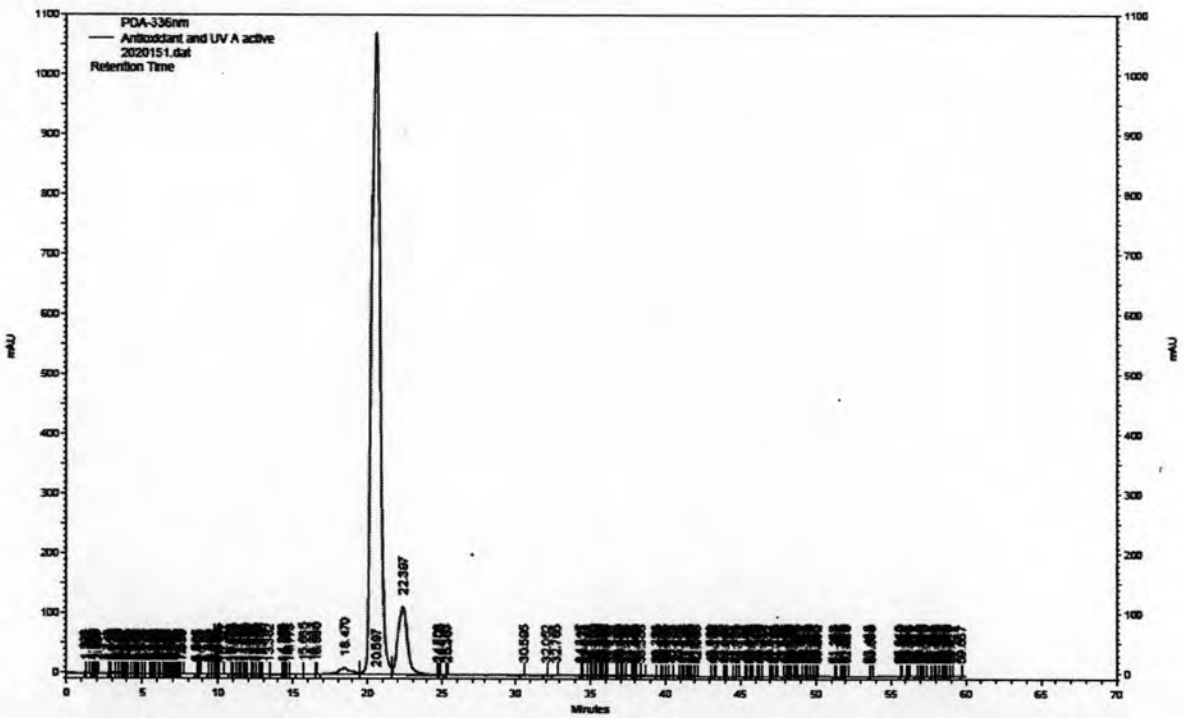


Figure 3.3 Semi-preparative HPLC chromatogram obtained from separation of CN I-2/3 fraction under condition described in the experimental section.

Isolation of CN I-8 by C18 column chromatography yielded 3 fractions, CN I-8/1, CN I-8/2 and CN I-8/3 (Scheme 3.3). The moderate polar fraction (CN I-8/2) was further purified by semi-preparative HPLC. Two fractions (CN I-8/2-1 and CN I-8/2-2) were obtained at retention time of 24 and 27 min (figure 3.4). Dissolving CN I-

8/2-2 in CH_2Cl_2 provided two parts CH_2Cl_2 soluble part and in CH_2Cl_2 insoluble part. The CH_2Cl_2 soluble in part was identified and expected as anthracene.

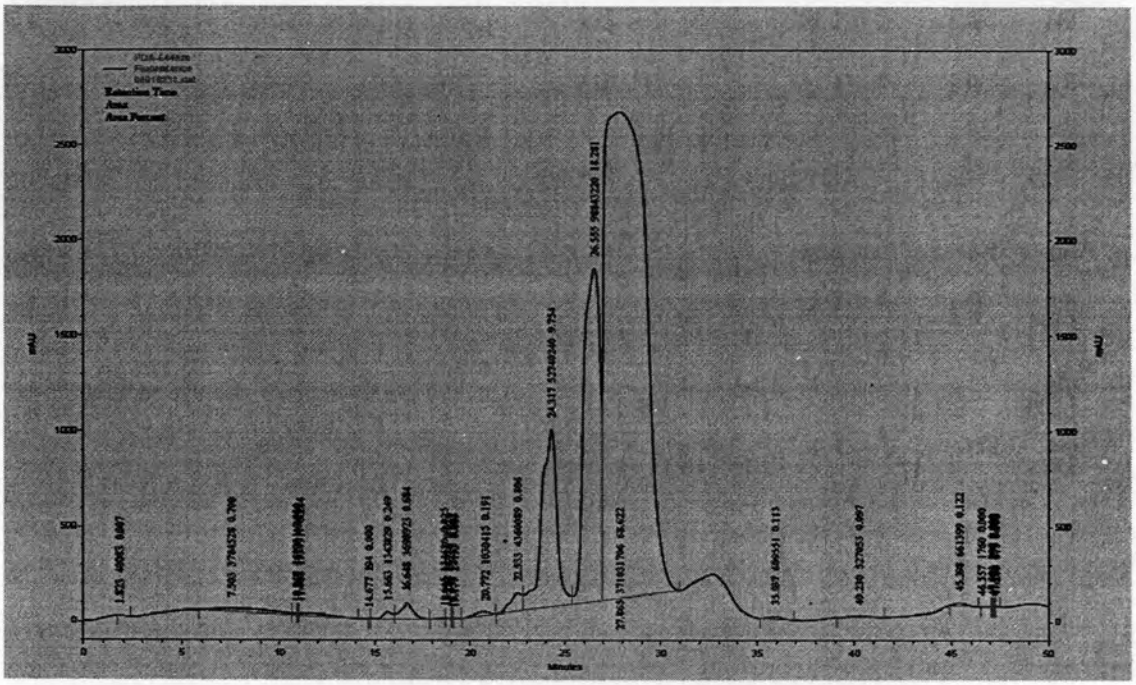
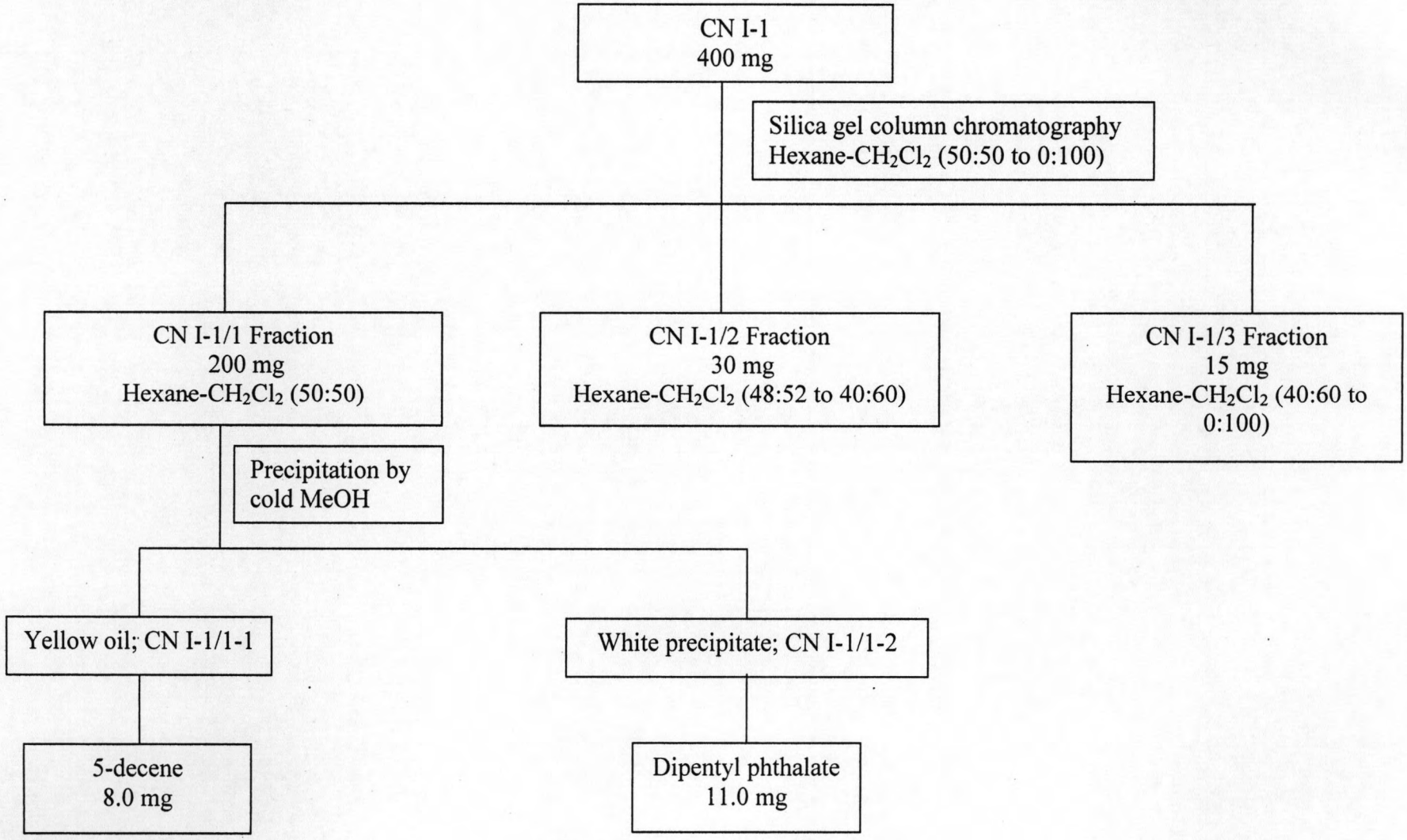
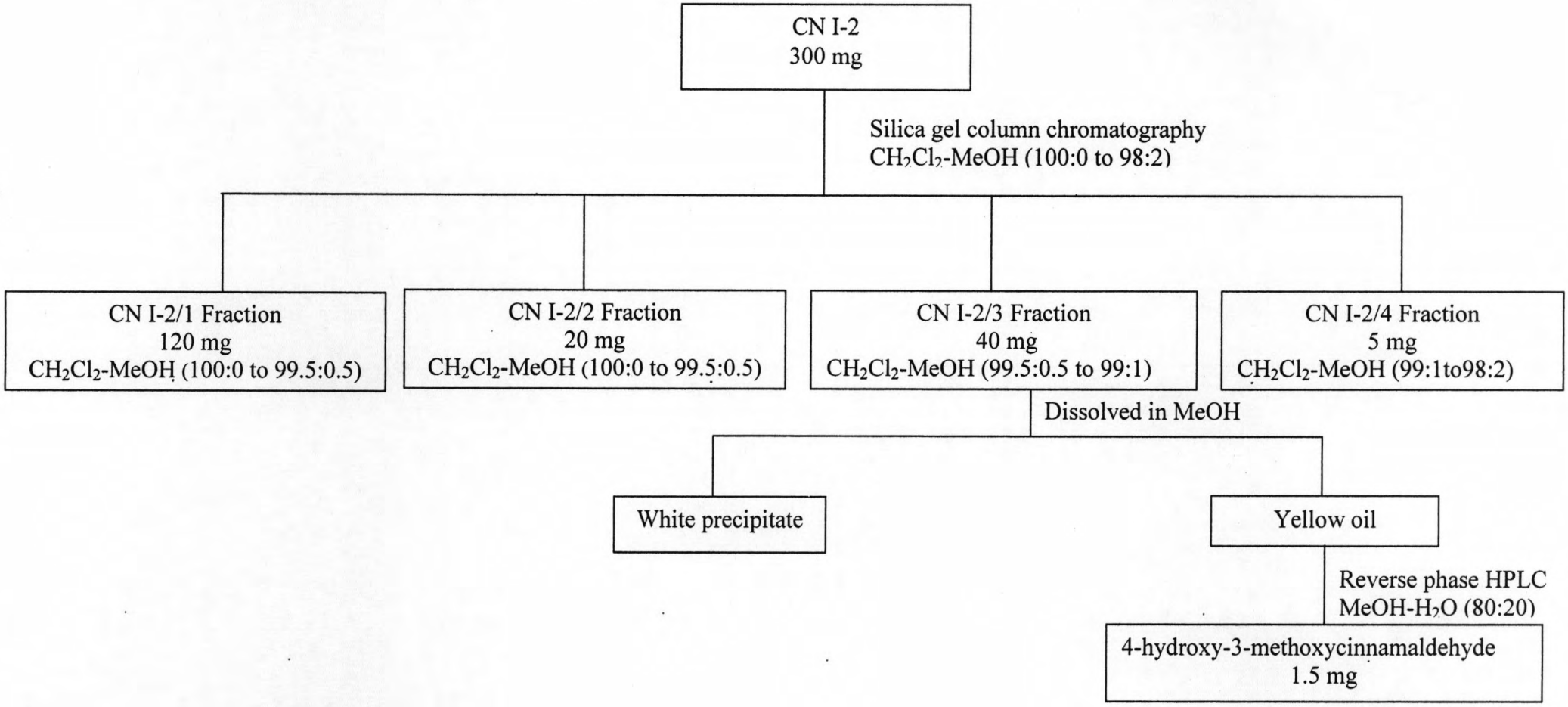


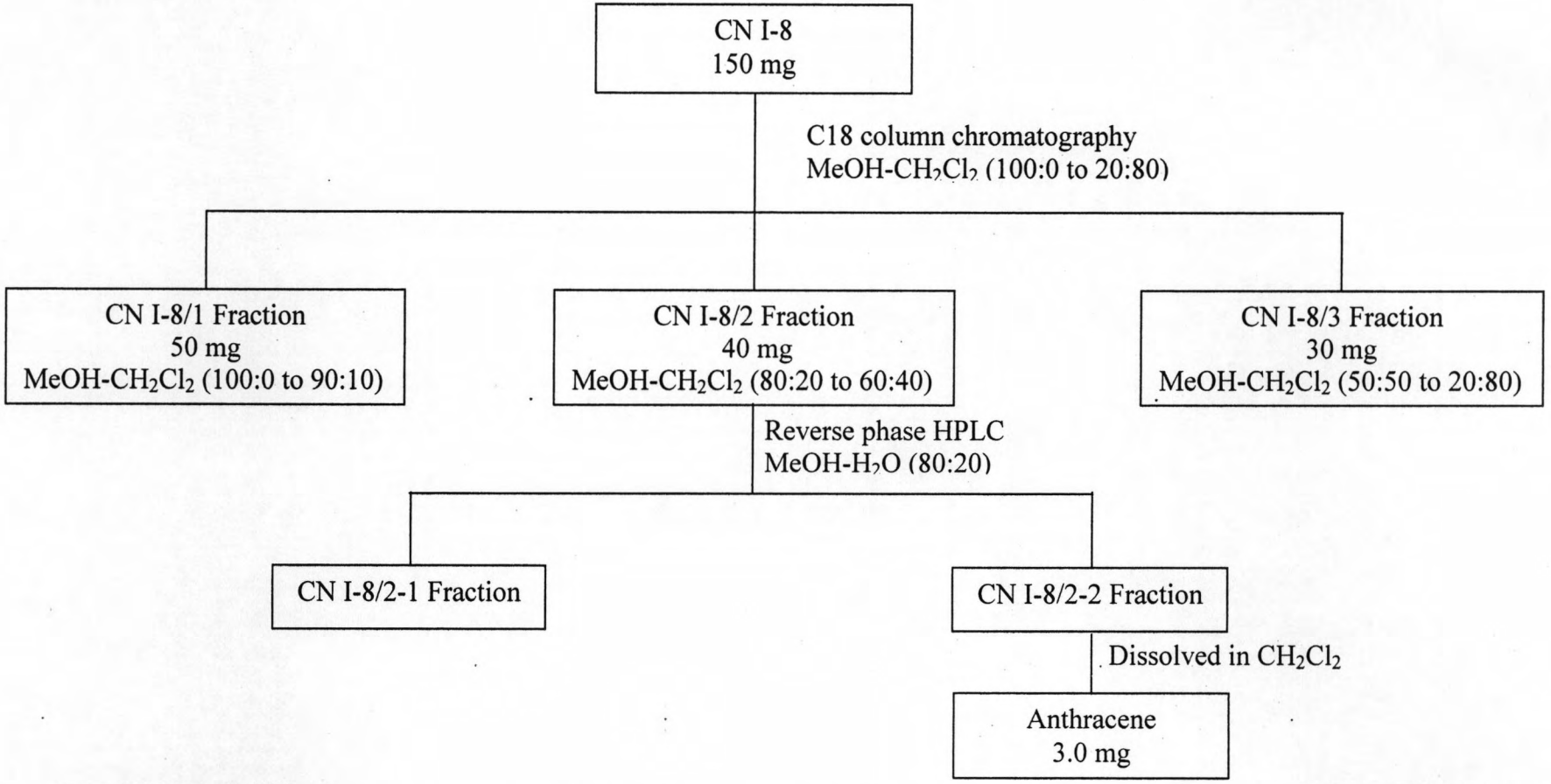
Figure 3.4 Semi-preparative HPLC chromatogram obtained from separation of CN I-8/2 fraction under condition described in the experimental section



Scheme 3.1 Isolation of CN I-1



Scheme 3.2 Isolation of CN I-2



Scheme 3.5 Isolation of CN I-8

3.5 Characterization of isolated compound

3.5.1 Dipentyl phthalate

CN I-1/1-2 (11 mg) was obtained as a pale yellow sticky semi-solid from CN I-1 of CN I crude extract. R_f (SiO_2, 4%MeOH in CH_2Cl_2) value was 0.7. IR spectrum showed absorption band of C=O stretching at 1725 cm^{-1} and C=C stretching vibration of aromatic ring at 1460 cm^{-1}.

The ^{13}C NMR spectrum of CN I-1/1-2 (Figure C-1, Appendix C, Page 77) contained nine resonances resulting from one methyl, four methylene, two methine and one quaternary carbon (a carbonyl of ester) (Table 3.4). The proton NMR of CN I-1/2-2 (Figure C-2, Appendix C, Page 78) show double aromatic protons at δ_H 7.7 and 7.5 (H-3 and H-4), one methyl proton at δ_H 0.89 ppm (H-5') and four methylene proton at δ_H 4.2 ppm (H-1'), 1.6 ppm (H-2'), 1.3 ppm (H-3') and 1.17 ppm (H-4') (Table 3.4). The direct connectivity (one bond) of protons and carbon atoms were deduced from a HSQC spectrum (Figure C-3, Appendix C, Page 79).

Table 3.4 1D and 2D NMR spectroscopic data for dipentyl phthalate in $CDCl_3$

position	δ_H, mult. (*J* in Hz)	δ_C	COSY	HMBC
1		167		
2		132.2		
3	7.7 (1H, d, 5.06)	128.7	H-4	C-1,C-3, C-4
4	7.5 (1H, d, 5.05)	130.8	H-3	C-3
1'	4.2 (2H, t, 6.18)	68	H-2'	C-1, C-2', C-3',C-4'
2'	1.6 (2H, M)	38.6	H-4'	
3'	1.3 (2H, M,)	29.2		C-5'
4'	1.17 (2H, M)	23	H-2', H-5'	
5'	0.89 (3H, t, 14.76)	14	H-2', H-4'	C-4'

The ^{1}H-^{1}H cosy spectrum (Figure C-4, Appendix C, Page 80) revealed the presence of the following connectivity as shown in Figure 3.5

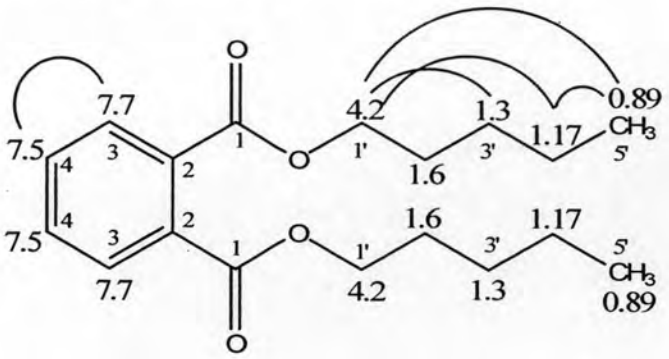


Figure 3.5 The COSY correlation of dipentylphthalate

The HMBC correlation (Figure 3.6 and Figure C-5, Appendix C, Page 81) were observed between the proton signals at δ_H 7.7 (H-3) to carbon signal at δ_c 132.2 ppm (C-2), 128.7 ppm (C-3) and 130.8 ppm (C-4), between the proton signals of H-4 to carbon signal at δ_c 128.7 ppm (C-3), H-3 (δ_H 7.7) to carbonyl carbon of ester (C-1), which led to the aromatic ester part of CN I-1/2-2 structure. The HMBC correlation of the proton signals of H-1' (δ_H 4.2 ppm) to C-2', C-3' and C-4', H-3' to C-5' and H-5' to C-4' led to the aliphatic part of the CN I-1/2-2 structure. HMBC correlation of H-1' (δ_H 4.2 ppm) to C-1 (δc 167 ppm) and H-3 (δ_H 7.7 ppm) to C-1 indicated that the two partial structures of CN I-1/2-2 were connected via an ester. This agree with the C=O stretching in the IR spectrum (Figure C-6, Appendix C, Page 82). Consequently, the structure of CN I-1/1-2 was determined as dipentyl phthalate.

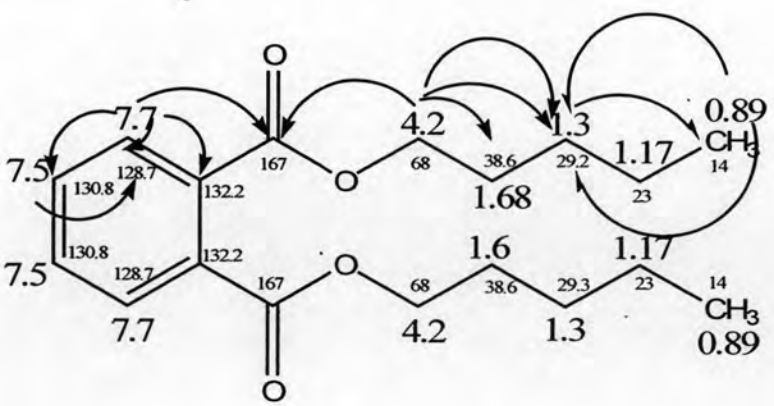


Figure 3.6 Key HMBC correlation of dipentyl phthalate

The positive ESI mass spectrum (Figure 3.7) showed a $[M+H_2O+H]^+$ ion at *m/z* 325, thus confirming the dipentyl phthalate structure.

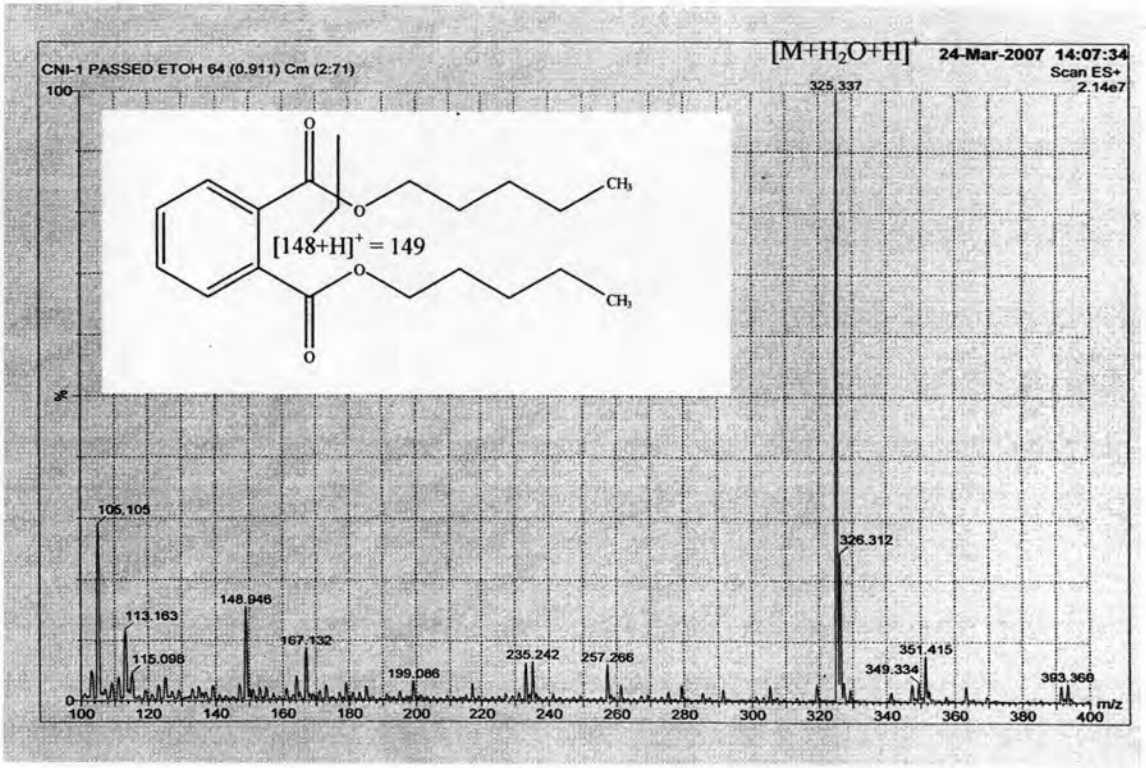


Figure 3.7 The positive ESI-MS spectrum of dipentyl phthalate

3.5.2 5-Decene

CN I-1/1-1 was obtained as yellow oil from CN I-2 of CN I crude extract. R_f (SiO_2, 3%MeOH/CH_2Cl_2) value was 0.6.

The ^{13}C NMR spectrum (Table 3.4 and Figure C-7, Appendix C, Page 83) in $CDCl_3$ of CN I-1/1-1 contained 5 resonances resulting from one methyl, three methylene and one methine carbon.

The 400 MHz ^1H NMR spectrum (Table 3.5 and Figure C-8, Appendix C, Page 84) showed five signals of one methyl proton at δ_H 0.8 ppm (3H, t, 7.02 Hz), three methylene proton at δ_H 1.1-1.3 (2H) and δ_H 2.0 ppm (2H, q, 5.928 Hz) and one methine proton at δ_H 5.3 ppm (1H).

Table 3.5 1D and 2D NMR spectroscopic data of 5-alkene in $CDCl_3$

position	δ_H, mult. (*J* in Hz)	δ_C	COSY	HMBC
1	0.8 (3H, t, 7.02)	14	H-2 to H-3	C-2 to C-3
2-3	1.1-1.3	28-32	H-4	C-1, C-4
4	2.0 (2H, q, 5.93)	27.1	H-2 to H-4, H-5	C-6, C-2 to C-3
5	5.3 (1H)	130	H-4	C-5

The ^1H-^1H COSY spectrum (Figure C-9, Appendix C, Page 85) revealed the presence of the following connectivity as shown in Figure 3.8

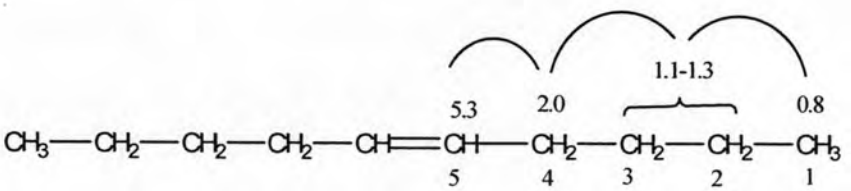


Figure 3.8 The COSY correlation of 5-decene

The HMBC correlations (Figure 3.9 and Figure C-10, Appendix C, Page 86) were observed between the proton signals at δ_H 0.8 ppm (H-1) to carbon signals at δ_c 28-32 ppm (C-2 to C-3), between proton signals at δ_H 1.1-1.3 ppm (H-2 to H-3) to carbon signals at δ_c 27.1 ppm (C-4), between proton signals at δ_H 2.0 ppm(H-4) to carbon signals at δ_c 28-32 ppm (C-2 to C-3) and δ_c 130 ppm (C-5) and between proton signals at δ_H 5.3 ppm (H-5) to carbon signals at δ_c 27.1 ppm (C-4). The combination with chemical shift of protons and carbons, structure of CN I-1/1-1 was determined as 5-decene.

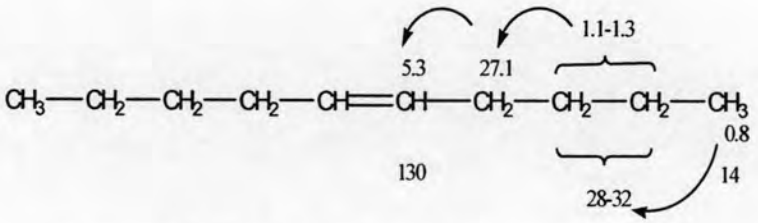


Figure 3.9 Key HMBC correlation of 5-decene

The positive ESI mass spectrum showed a molecular peak at a $[M+2H_2O+H]^+$ ion of *m/z* 177 (Figure 3.10).

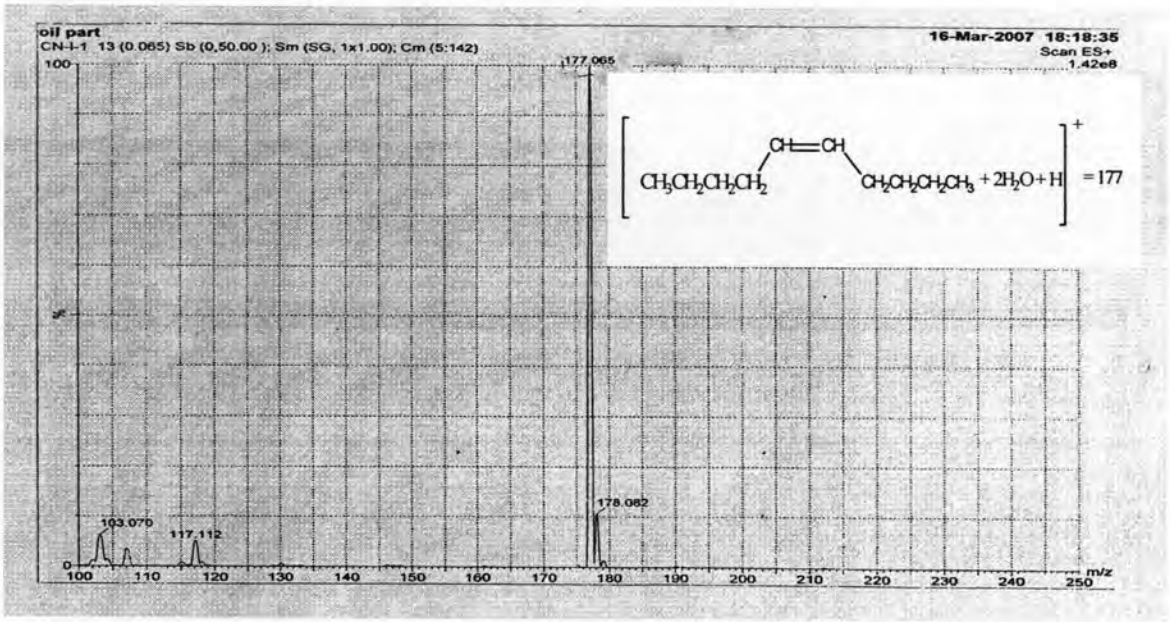


Figure 3.10 The positive ESI-MS spectrum of 5-decene

The compound was subjected to anti-tyrosinase activity test. The result clearly indicated that the compound could not inhibit tyrosinase even at the concentration of up to 2000 µg/ml.

3.5.3 4-Hydroxy-3-methoxycinnamaldehyde

CN I-2/3-2 (1.5 mg) was obtained as pale yellow solid from fraction CN I-2 of CN I crude extract. The R_f (SiO_2 3%MeOH in CH_2Cl_2) values was 0.58. The IR spectrum showed absorption bands for hydroxy (3335 cm^{-1}), carbonyl group of aldehyde (1634 cm^{-1}), and aromatic (1573 and 1500 cm^{-1}) (Figure C-11, Appendix C, Page 87).

The ^{13}C NMR spectrum (Figure C-12, Appendix C, Page 88) in $CDCl_3$ of CN I-2/3-2 contained 10 resonances resulting from one methyl, five methine and three quaternary carbons (δ_C 194 ppm, C-3') (Table 3.4).

The 400 MHz ^1H NMR spectrum (Figure C-13, Appendix C, Page 89) showed three signals of an aromatic protons at δ_H 6.96 ppm (1H, d, J = 8.1 MHz), δ_H 7.08 ppm (1H, s) and δ_H 7.13 ppm (1H, d, J = 8.1 MHz), one methoxy signal at δ_H 3.95 ppm (3H, s) and one phenolic proton at δ_H 5.95 ppm (1H, s). Ethylene proton appeared as doublet at δ_H 7.42 ppm (1H, J = 15.82 MHz) and a doublet of doublet at

δ_H 6.58 ppm (1H, J = 8.01, 15.88 MHz) whereas carbonyl proton appeared at δ_H 9.68 ppm (1H, J = 7.63 MHz) (Table 3.6).

The direct connectivities (one bond) of proton and carbon atoms were deduced from a HSQC spectrum (Figure C-14, Appendix C, Page 90). One proton signal (δ_H 5.95, s, br) (Table 3.6) was associated with hydroxyl group due to missing HSQC cross-peak and the IR spectrum of this hydroxyl group is broaden, which is resulted from hydrogen bonding with moisture in the sample.

Table 3.6 1D and 2D NMR spectroscopic data of 4-hydroxy-3-methoxycinnamaldehyde in $CDCl_3$

position	δ_H, mult. (J in Hz)	δ_C	COSY	HMBC
1		127		
2	7.08 (1H,s)	109	H-1'	C-3, C-4, C-6, C-1'
3		147		
3a	3.95 (OCH_3, s)	56		C-3
4		149		
4a	5.95 (OH, s)			C-4, C-5
5	6.96 (1H, d, 8.08)	115	H-6, H-1'	C-1, C-4
6	7.13 (1H, d, 8.12)	124	H-5, H-1'	C-2, C-4, C-1'
1'	7.42 (1H, d, 15.82)	154	H-2'	C-3'
2'	6.58 (1H, dd, 8.01 and 15.88)	126	H-3'	
3'	9.68 (1H, d, 7.63)	194	H-2'	C-2', C-3'

The ^{1}H-^{1}H COSY spectrum (Figure C-15, Appendix C, Page 91) revealed the presences of the following connectivities of ; CH (2) to CH(1'), CH (5) to CH (6) and CH (1'), CH (6) to CH (5) and CH (1'), CH (1') to CH (2'), CH (2') to CH (3') and CH (3') to CH (2'), as shown in Figure 3.11

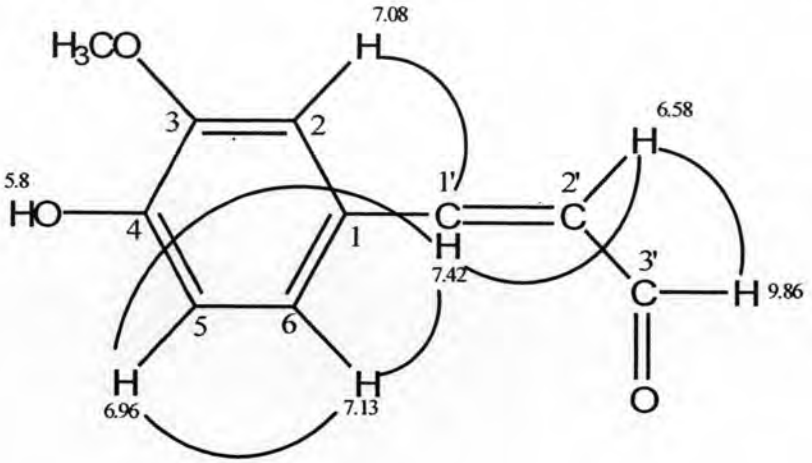


Figure 3.11 The COSY correlation of 4-hydroxy-3-methoxycinnamaldehyde

The HMBC (Figure C-16, Appendix C, Page 92) correlations (Figure 3.12) were observed between a ethylene proton signal δ_H at 7.42 ppm (H-1') to a carbonyl carbon signal δ_C at 194 ppm (C-3'), aldehyde proton δ_H at 9.86 ppm (H-3') to ethylene carbon signal δ_C at 126 ppm (H-2') and aldehyde proton δ_H at 9.86 ppm to aldehyde carbon δ_C at 194 ppm (C-3'), which led to the partial struture in the conjugated aldehyde part of the CN I-2/3-2. In addition, HMBC correlations between proton signal δ_H 7.08 ppm (H-2) to carbon signal δ_C 147 ppm (C-3), 149 ppm (C-4), 124 ppm (C-6) and 154 ppm (C-1'), proton signal δ_H 6.96 ppm (H-5) to carbon signal δ_C 127 ppm (C-1) and 149 ppm (C-4), proton signal δ_H 7.13 ppm (H-6) to carbon signal δ_C 109 ppm (C-2), 149 ppm (C-4) and 154 ppm (C-1'), methoxy proton δ_H 3.95 ppm to carbon signal δ_C 147 ppm (C-3) and hydroxy proton δ_H 5.95 ppm to carbon signal δ_C 149 ppm (C-4) and 115 ppm (C-5). In combination with chemical shift of these protons and carbons demonstrated unequivocally methoxy and hydroxy were located at C-3 and C-4 on benzene moiety of CN I-2/3-2. The HMBC correlation from H-2 and H-6 to C-1' demonstrated unequivocally that the conjugated aldehyde part was located at C-1 of benzene moiety. Consequently, the structure of CN I-2/3-2 was determined as 4-hydroxy-3-methoxycinnamaldehyde. The common name of 4-hydroxy-3-methoxycinnamaldehyde is coniferaldehyde.

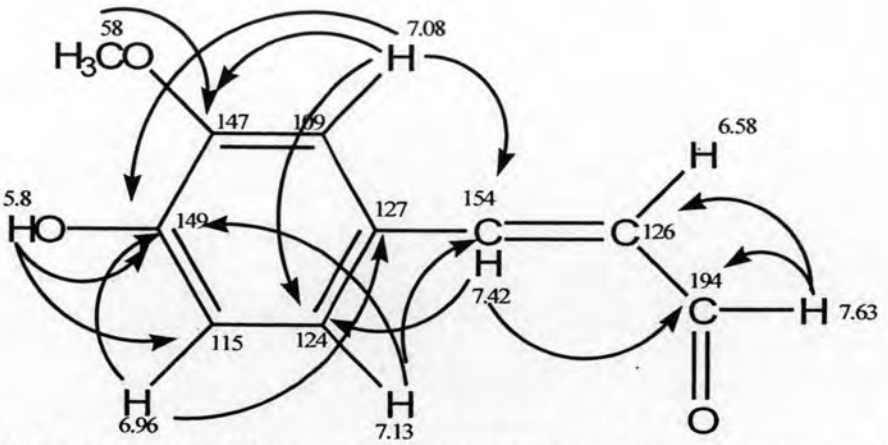


Figure 3.12 The key HMBC correlations of 4-hydroxy-3-methoxycinnamaldehyde

The positive ESI mass spectrum showed a molecular peak at a $[M-1]^-$ ion of m/z 177 (Figure 3.13).

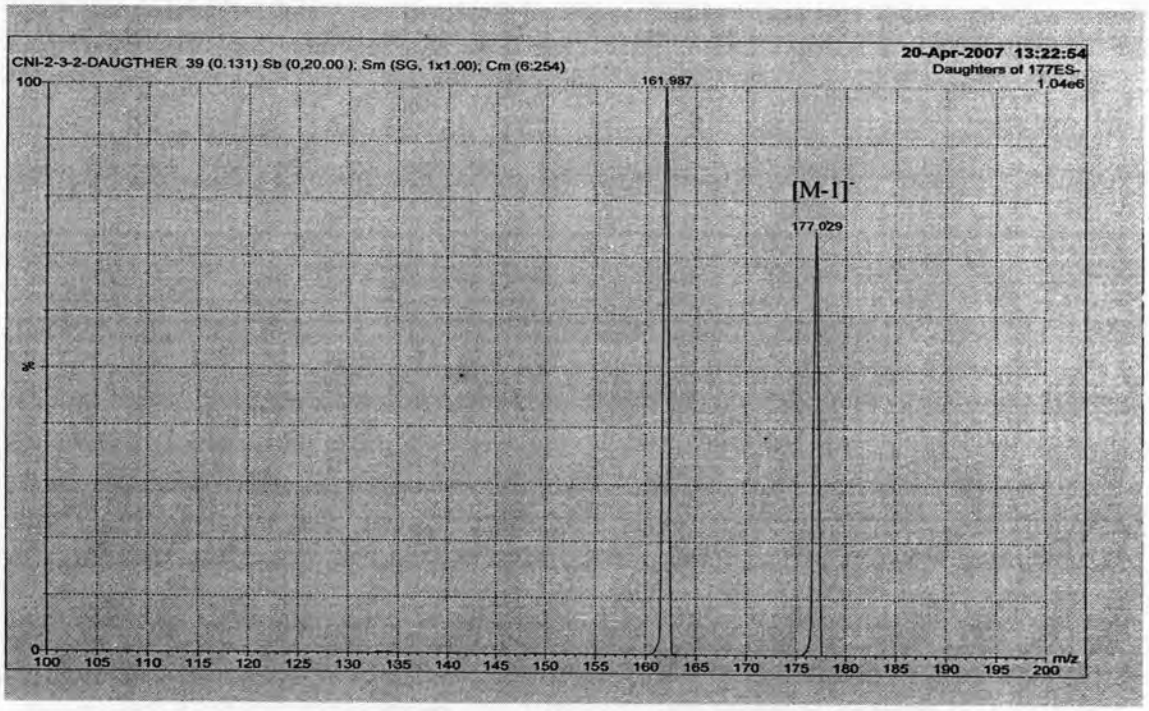


Figure 3.13 The negative ESI-MS spectrum of 4-hydroxy-3-methoxycinnamaldehyde

3.5.4 anthracene derivative

CN I-8/2-1 (3.0 mg) was obtained as purple solid from CN I-8 of CN I crude extract. CN I-8/2-1 was analyzed by using NMR spectroscopy. From the ^{1}H NMR data found that CN I-8/2-1 is an anthracene derivative. Unfortunately this compound easily decomposed hence its structure could not be identified. Figure 3.14A and 3.14B showed the NMR of the freshly obtained CN I-8/2-1 and the 20 day old CN I-8/2-1.

Obvious appearance of resonance at 5.4 and 4.2 ppm could be seen in the older sample, thus indicating formation of decomposition compound.

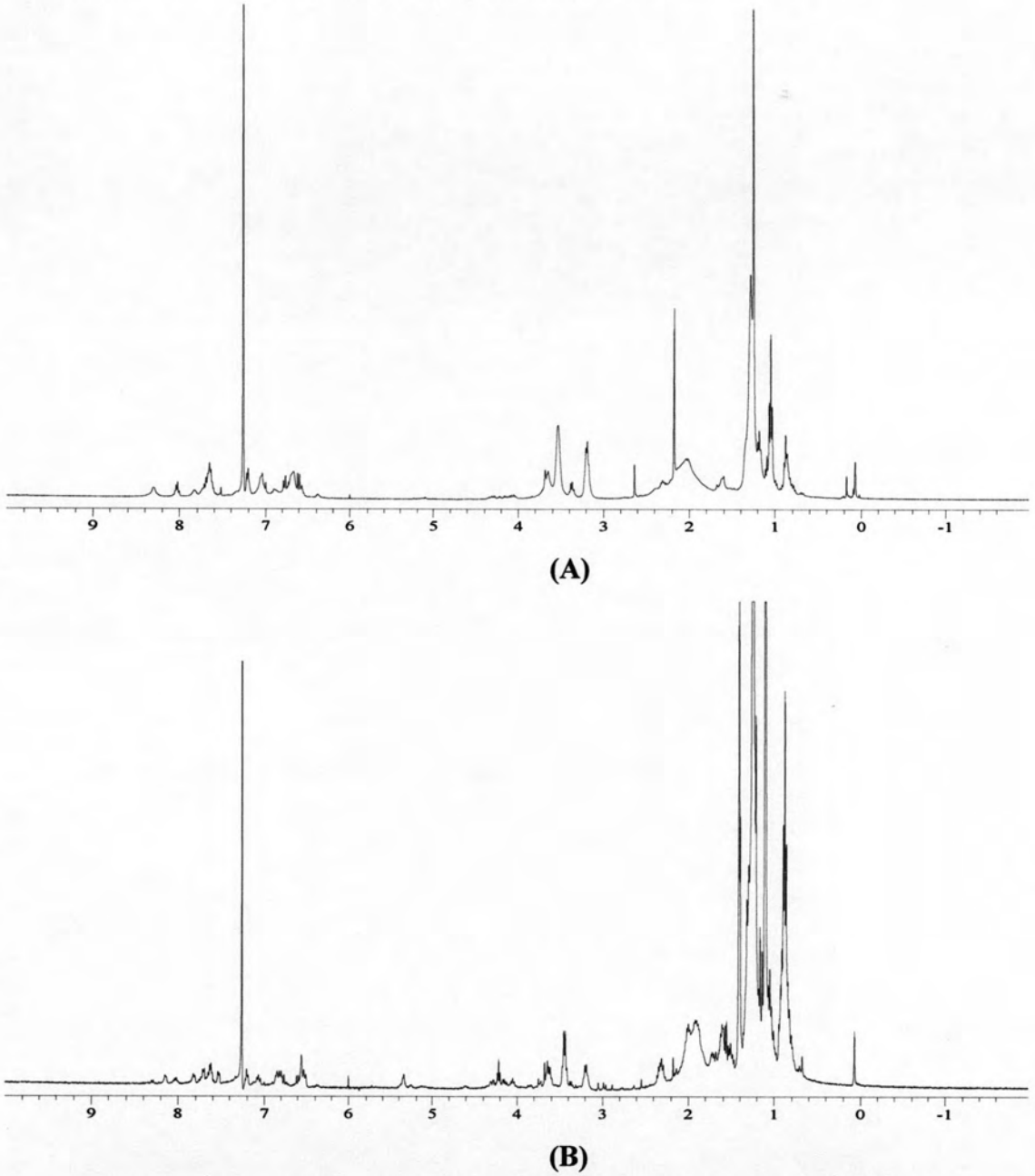


Figure 3.14 NMR spectrums of (A) freshly CN I-8/2-1 and (B) A 20 days old CN I-8/2-1

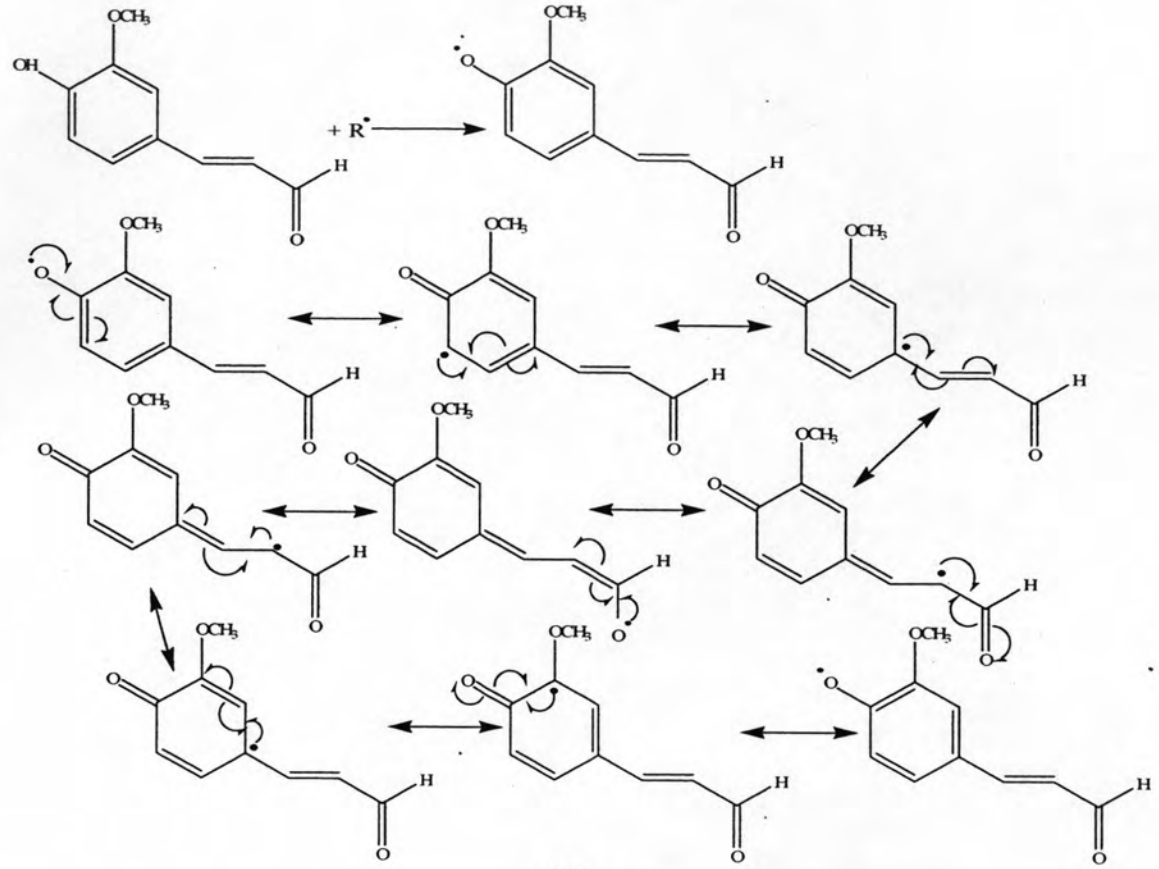
3.6 The biological activities of isolated compounds

3.6.1 Antioxidant activity (DPPH radical scavenging activity)

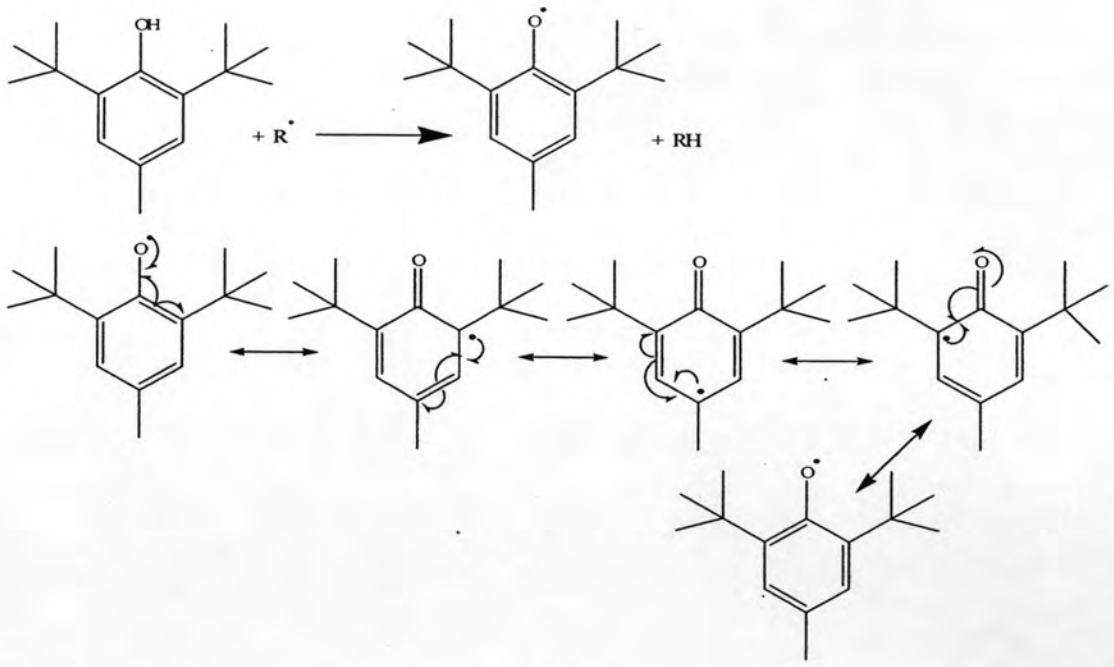
Antioxidant activity of 4-hydroxy-3-methoxycinnamaldehyde was tested by TLC autograph method. BHT was used as a control. The test was performed at various concentrations of 4-hydroxy-3-methoxycinnamadehyde and BHT. It was found that 4-hydroxy-3-methoxycinnamadehyde and BHT showed comparable activity. A more specific spectroscopic technique was then carried out and the result indicate the IC_{50} values of 64.49 and 42.3 μg/ml for 4-hydroxy-3-methoxycinnamadehyde and BHT, respectively (Figure D-1, Appendix D, Page 94).

The radical scavenging activity of 4-hydroxy-3-methoxycinnamaldehyde should be attributed to the phenolic functionality. Both BHT and 4-hydroxy-3-methoxycinnamaldehyde structure contain a phenolic functionality site on aromatic ring. This phenolic moiety is a well known free radical trap. Its mechanism of action is shown in figure 3.15.

It should be noted here that 4-hydroxy-3-methoxycinnamaldehyde also possess aldehyde functionality. This group has reducing property, thus, 4-hydroxy-3-methoxycinnamaldehyde may act as an antioxidant through both radical scavenging mechanism and reducing agent. Antioxidant tested as described above involves only radical scavenging activity of the compound. However, aldehyde is an active functional group, thus, extract containing component with this functional group should be kept and handle with care in order to avoid oxidation.



(A)



(B)

Figure 3.15 Resonance effects of (A) 4-hydroxy-3-methoxycinnamaldehyde and (B) Butylated hydroxytoluene (BHT)

4-Hydroxy-3-methoxycinnamaldehyde or coniferaldehyde has been found in the heartwood of *Gliricidia sepium*, a shade plant in the tea plantations of Sri Lanka [79] and berries of *Pimenta dioca* [80]. In addition, it has been found in ripe fruit and senescent leave of *Melia azedarach* L. [81]. Plant tissue culture of both *Artemisia annua* and *Tanacetum parthenium* were found to produce coniferaldehyde derivatives which have not been reported previously from parent plants [82]. Many biological activities such as antifungal [81], antibacterial [81], and antioxidant activity [80] have been attributed to conniferaldehyde derivatives.

In addition, many other cinnamic aldehyde derivatives have been reported. For example, *p*-hydroxycinnamaldehyde was reported to be an antibacterial substance [83], 2-hydroxycinnamaldehyde (HCA) and 2-methoxycinnaldehyde was reported as potent antitumor agents [84]. HCA has also been shown to inhibit the growth of human cancer cell lines including breast, leukemia, ovarin, lung and colon tumor cell [85]. In the present, the cytotoxicity of 4-Hydroxy-3-methoxycinnamaldehyde to human skin cell has not been reported.

3.6.2 UV screening activity

The UV absorption spectrum of 4-hydroxy-3-methoxycinnamaldehyde shows the maximum absorption peak at 339 nm with a molar absorptivity of 32000 $cm^{-1}M^{-1}$ (Figure 3.16). This absorption is in the UVA region.

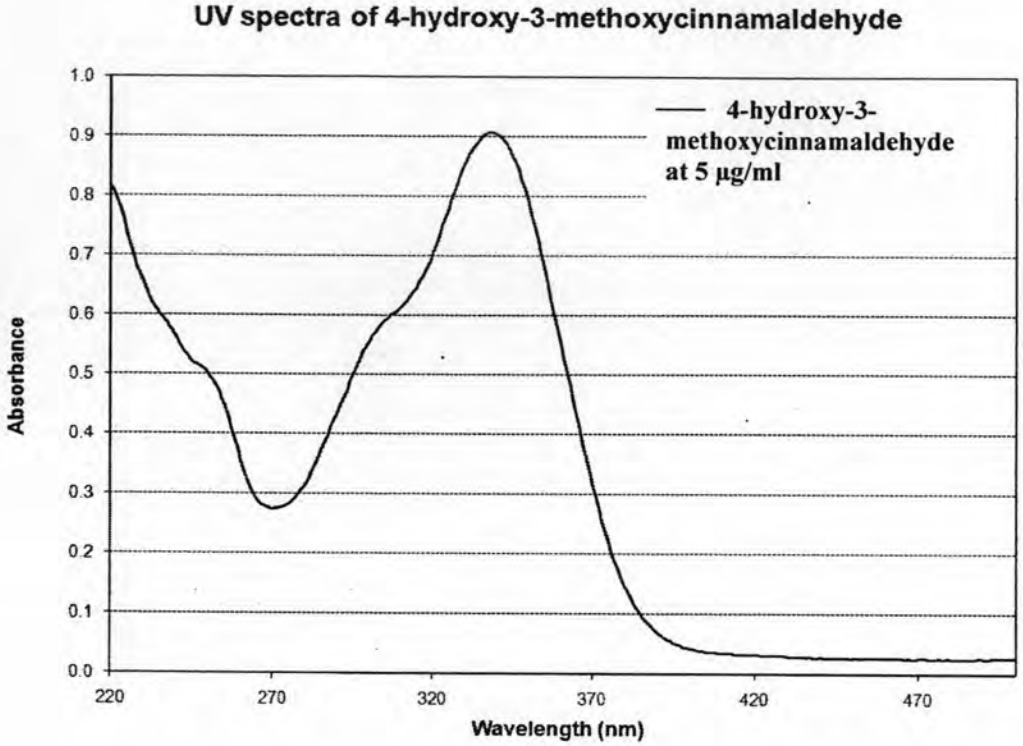


Figure 3.16 UV spectrum of 4-hydroxy-3-methoxycinnamaldehyde at 5 µg/ml

The structure of 4-hydroxy-3-methoxycinnamaldehyde contains large conjugated systems which bring about a bathochromic shift. In the presence of the conjugated double bonds, the electronic energy levels of a chromophore move closer together. As a result, the energy required to produce a transition from an occupied electronic energy level to an unoccupied level decreases, and the wavelength of the light absorbed becomes longer. Moreover, the substitution of unshared electron, hydroxyl and methoxy, on the molecule of 4-hydroxy-3-methoxycinnamaldehyde can increase the length of the π system through resonance.

3.6.3 Anti-tyrosinase activity

Dipentyl phthalate isolated from of CN I-1 showed detectable anti-tyrosinase activity at minimal concentrations 750 µg/ml. The post anti-tyrosinase TLC technique was used in this experiment. It should be noted here that, the determination of anti-tyrosinase activity by using spectrophotometer which involved measuring the absorption of at 492 nm can not be used here because the sample also shows absorption at this wavelength. Anti-tyrosinase activity of dipentyl phthalate at

concentration of 750 μg/ml was equivalent to that of reference kojic acid at concentration of 30 μg/ml. The minimum quantity of dipentyl phthalate required to inhibit tyrosinase activity on TLC plate was 3.75 μg. By comparison, the reference kojic acid was active at 0.15 μg. These results firmly indicated that dipentyl phthalate was about 25 folds less active in inhibition of tyrosinase than kojic acid.

Many phthalates such as butyl benzyl phthalate, di-2-ethylhexyl phthalate and diisodecyl phthalate (DIDP), are used as plasticizer to make vinyl soft and flexible [86]. Some phthalates such as dibutyl phthalate (DBP), dimethyl phthalate (DMP) and diethyl phthalate (DEP) have been a key ingredient in fragrances and nail polish. They are used primarily at concentrations of less than 10% as plasticizers in products such as nail polishes (to reduce cracking by making them less brittle) and hair sprays (to help avoid stiffness by allowing them to form flexible film in the hair) and as solvents and perfume fixatives in various other products [87].