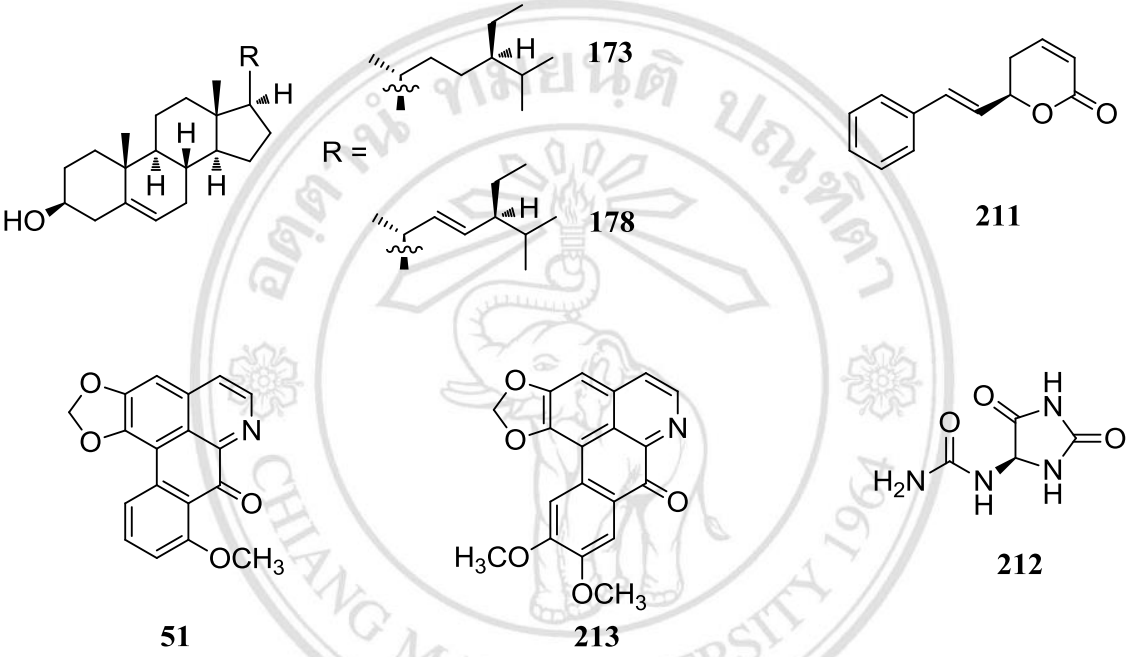
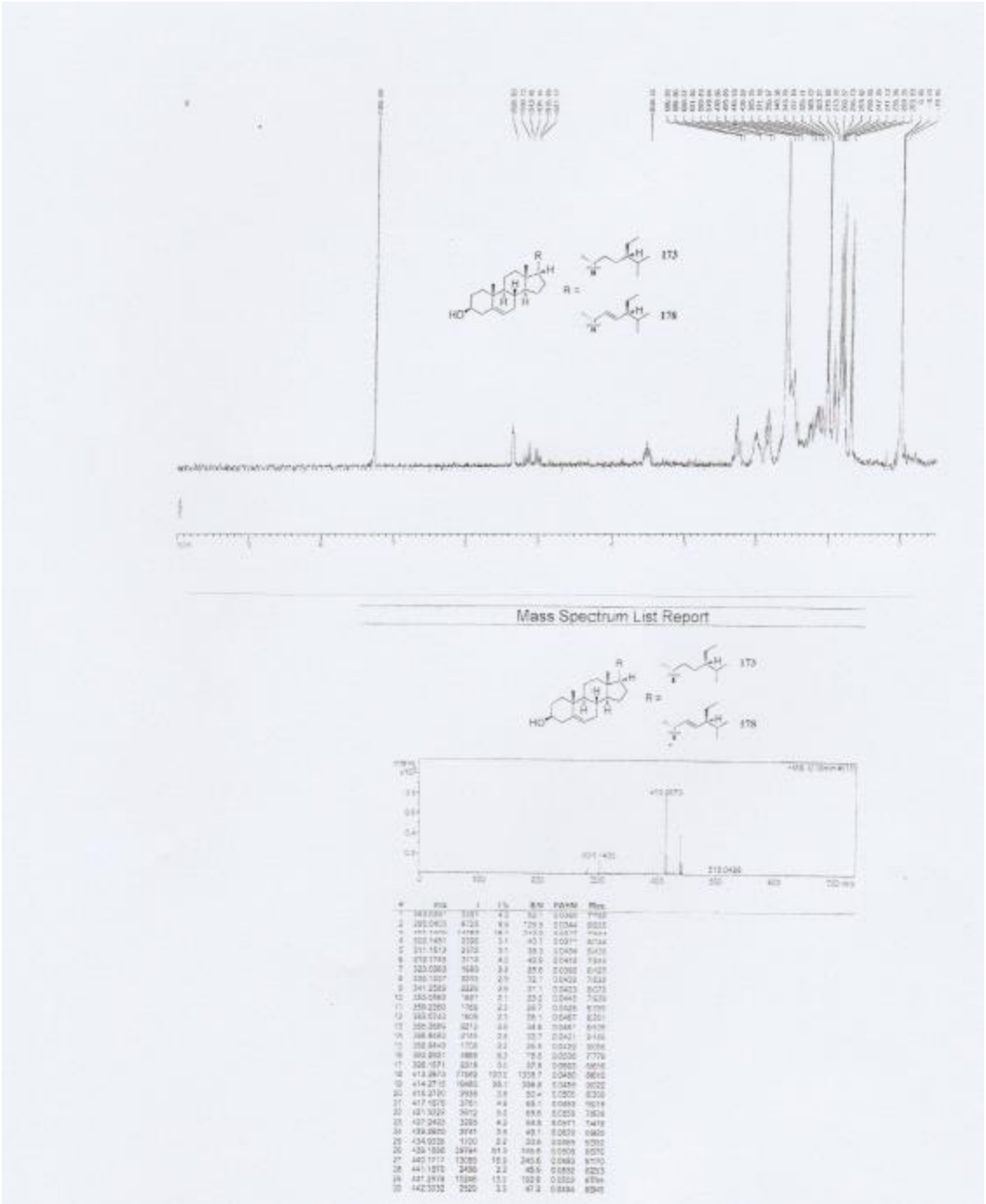
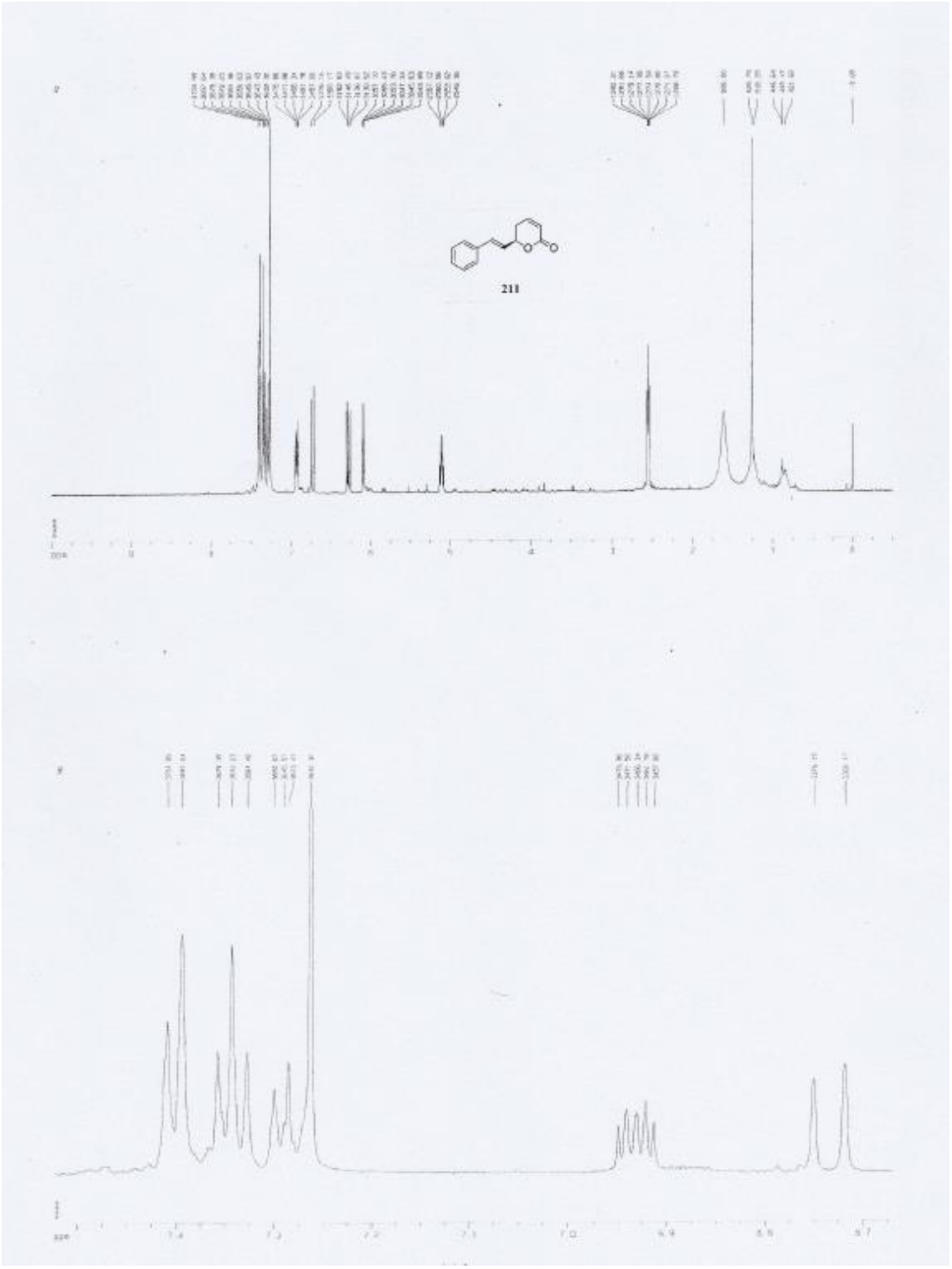
APPENDIX

APPENDIX A

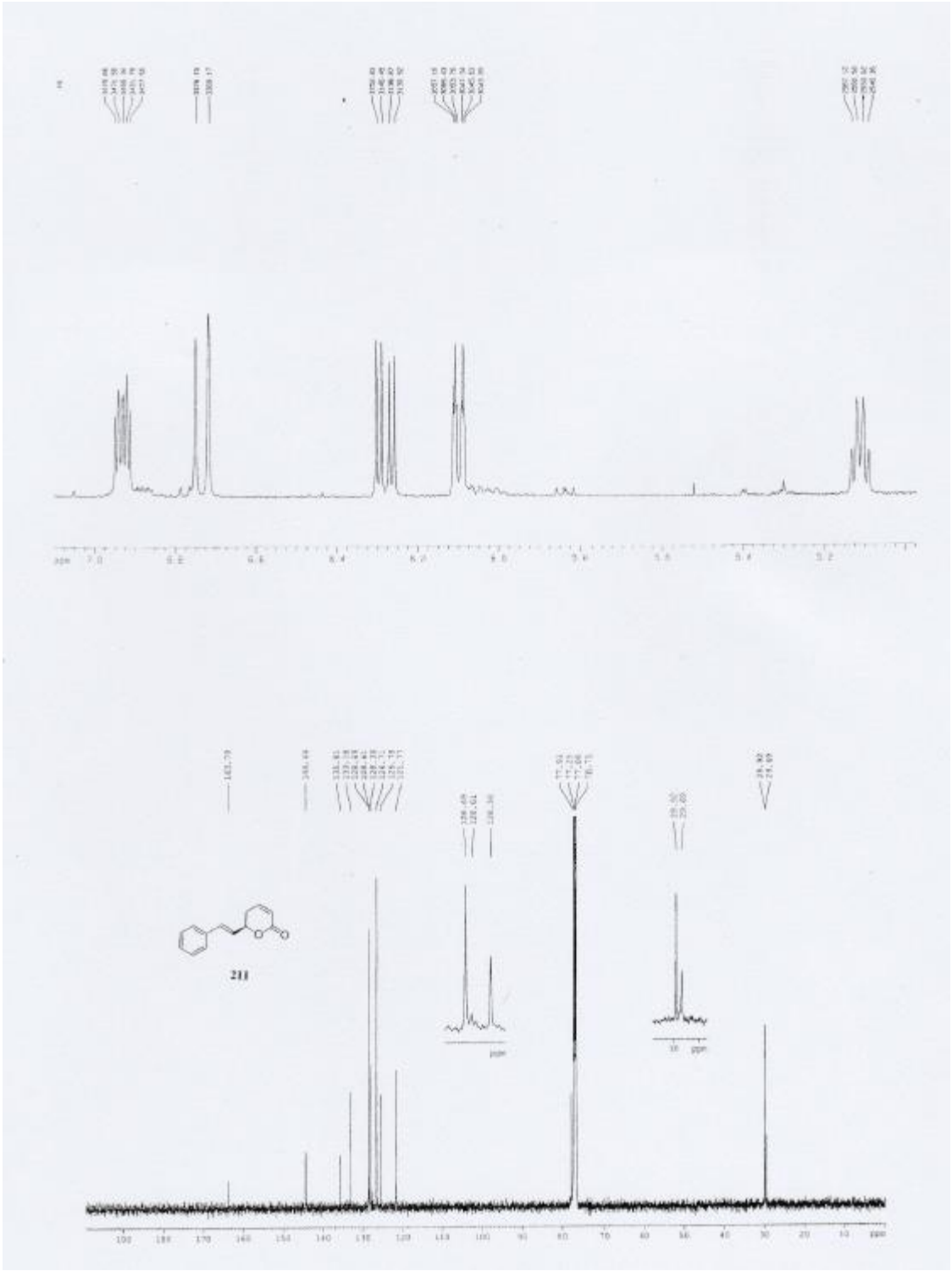
Spectrum Data of the Isolated Compounds from *P. evecta* var. *attopeuensis*

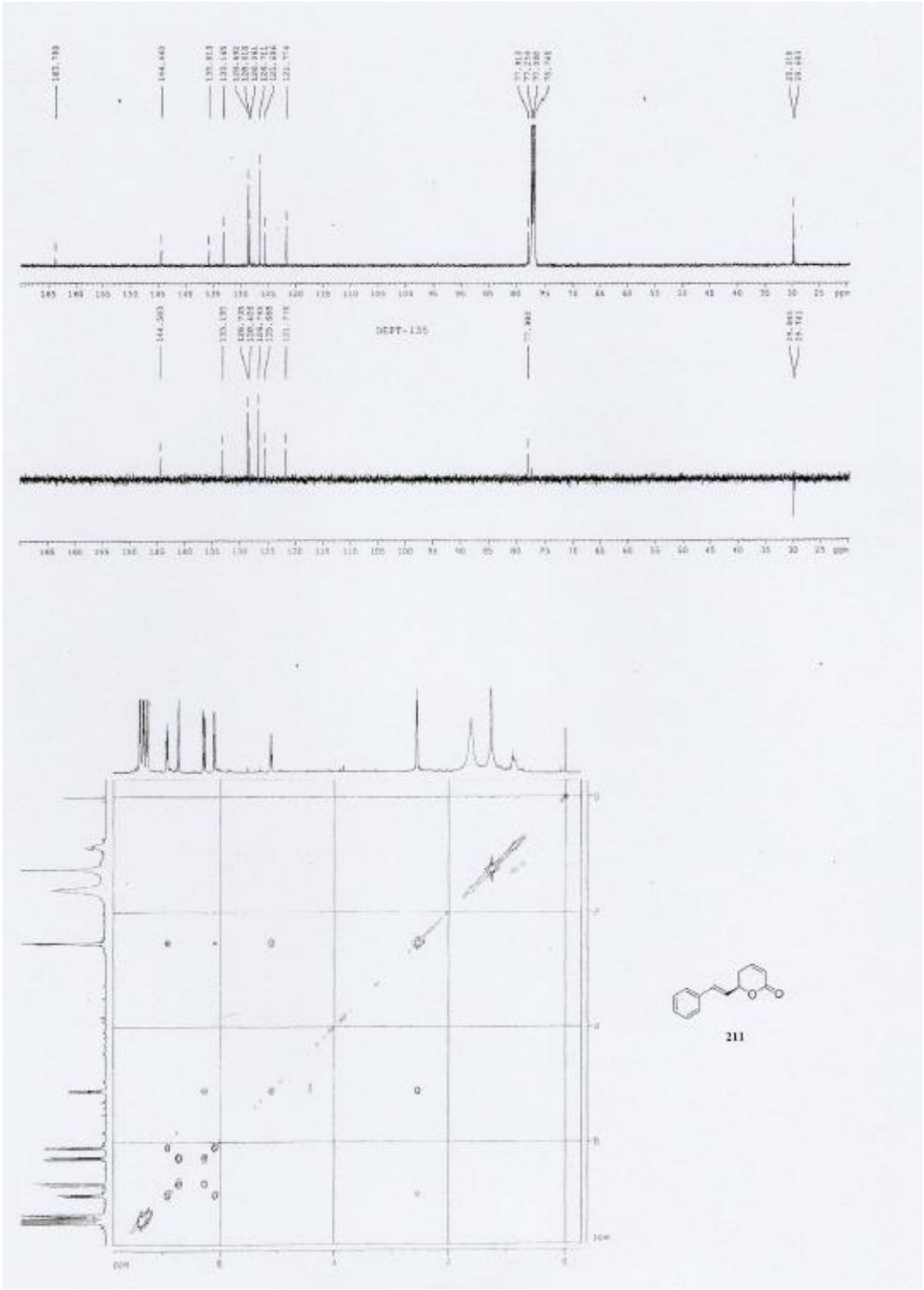



R
HO
R =
173
176
Mass Spectrum List Report

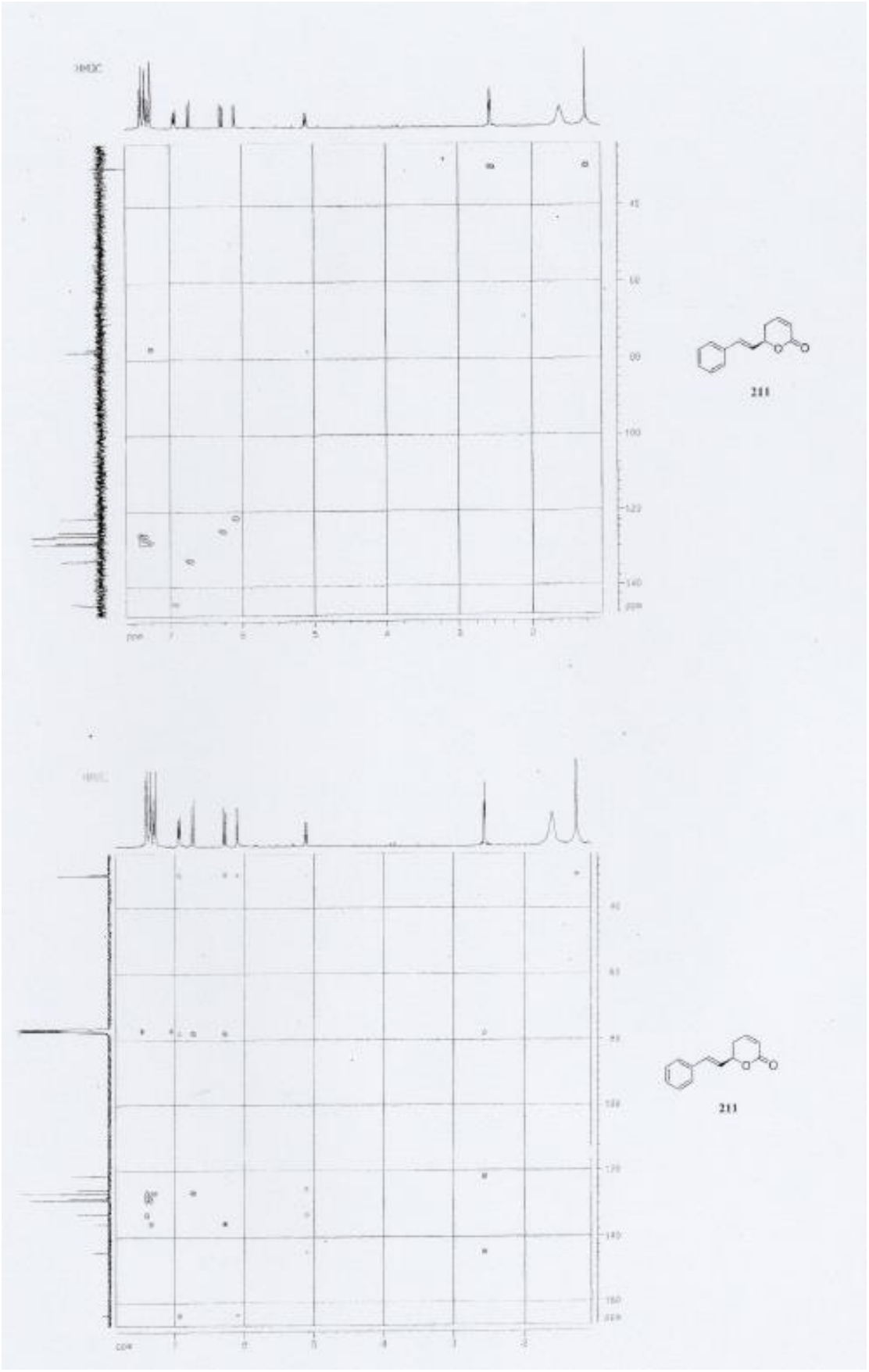


211



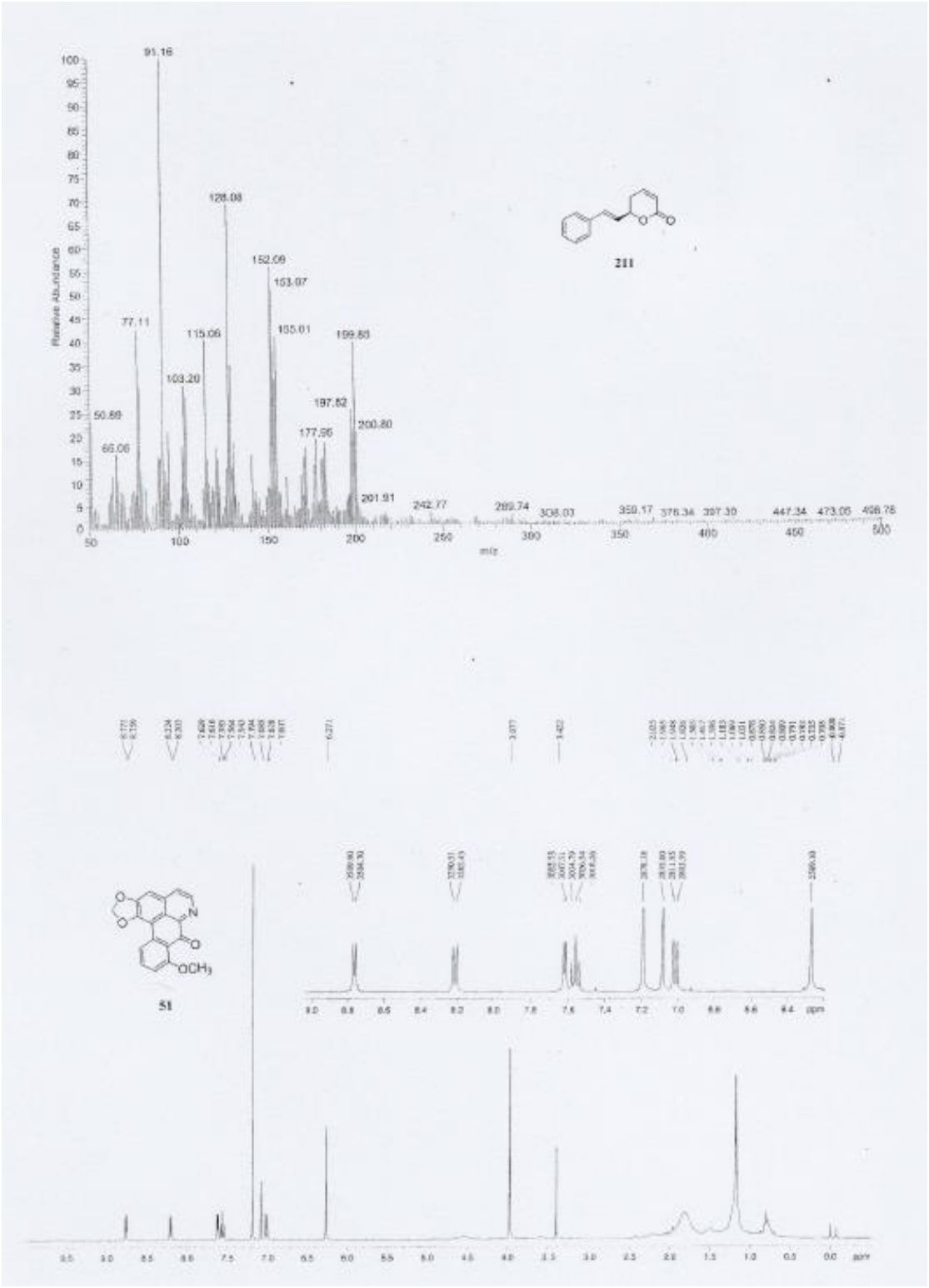


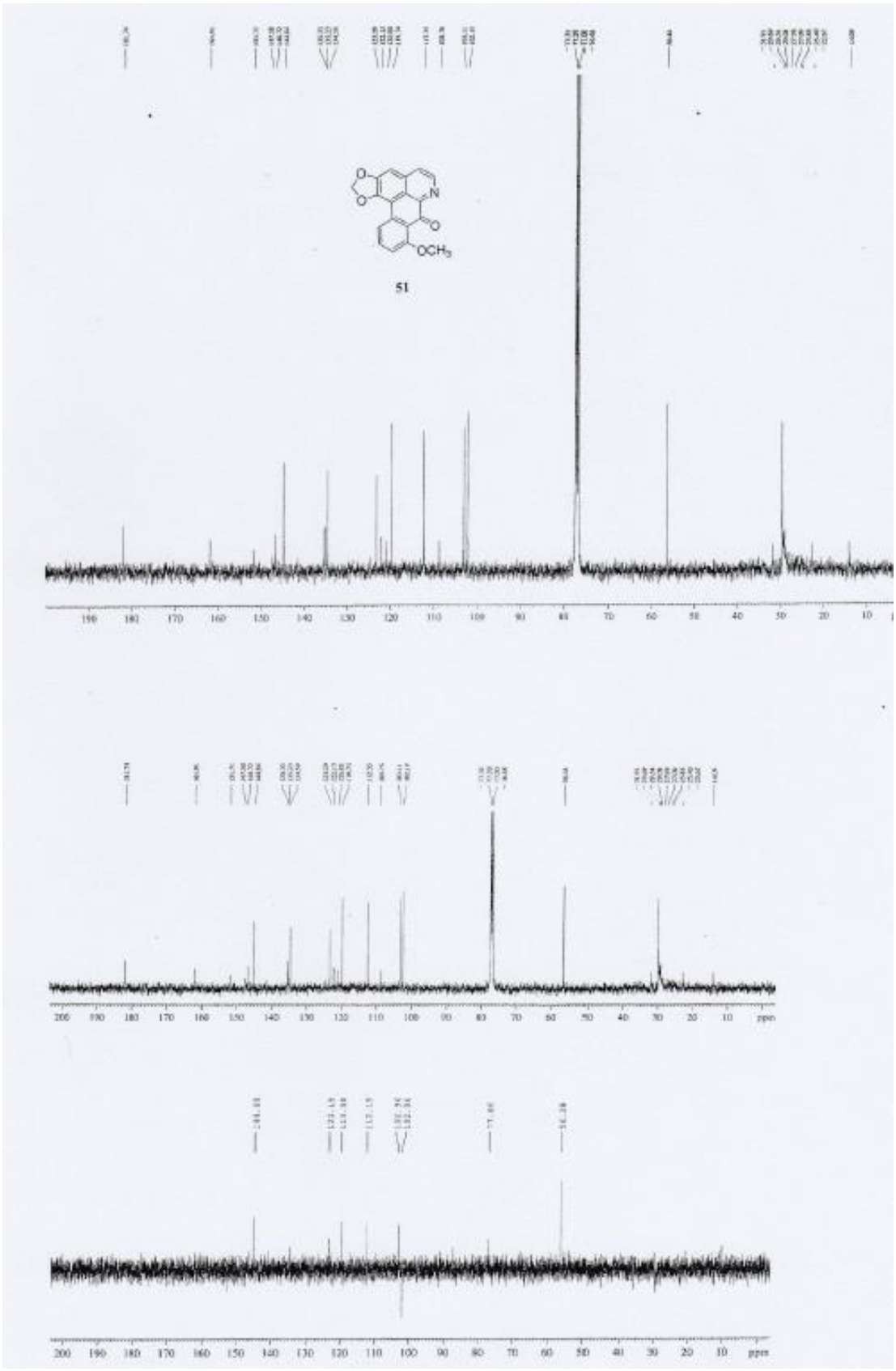
211



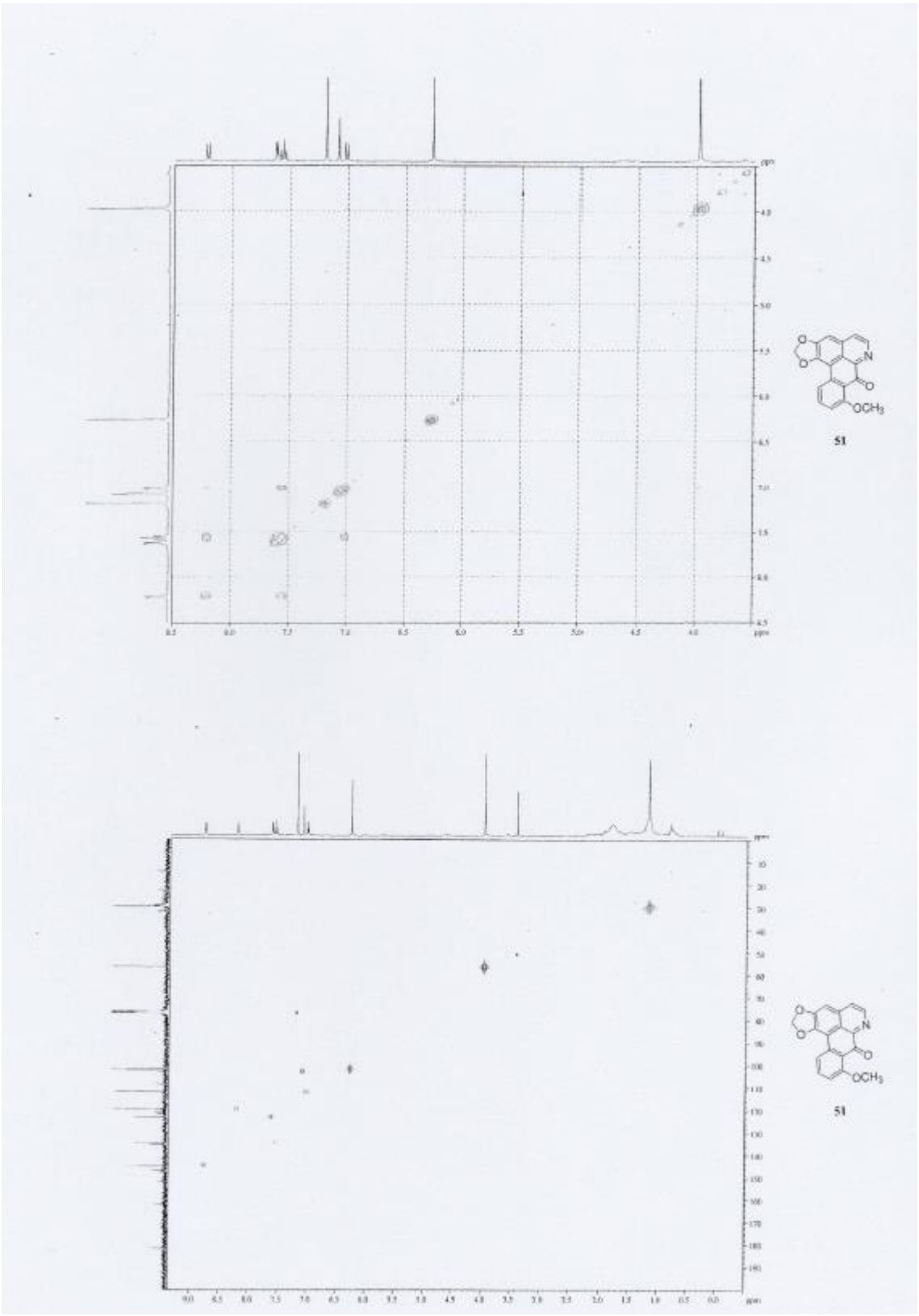
211

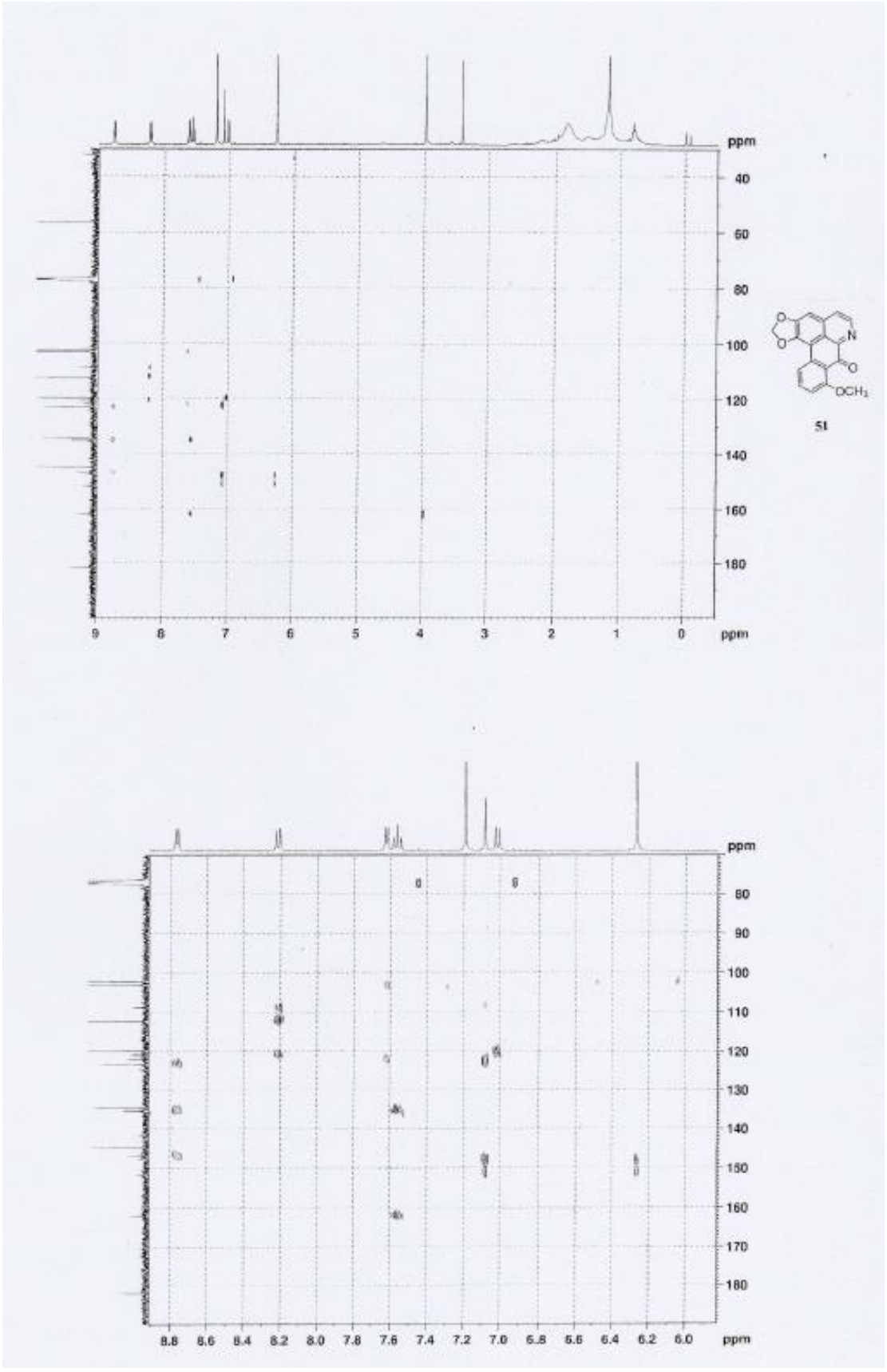
211

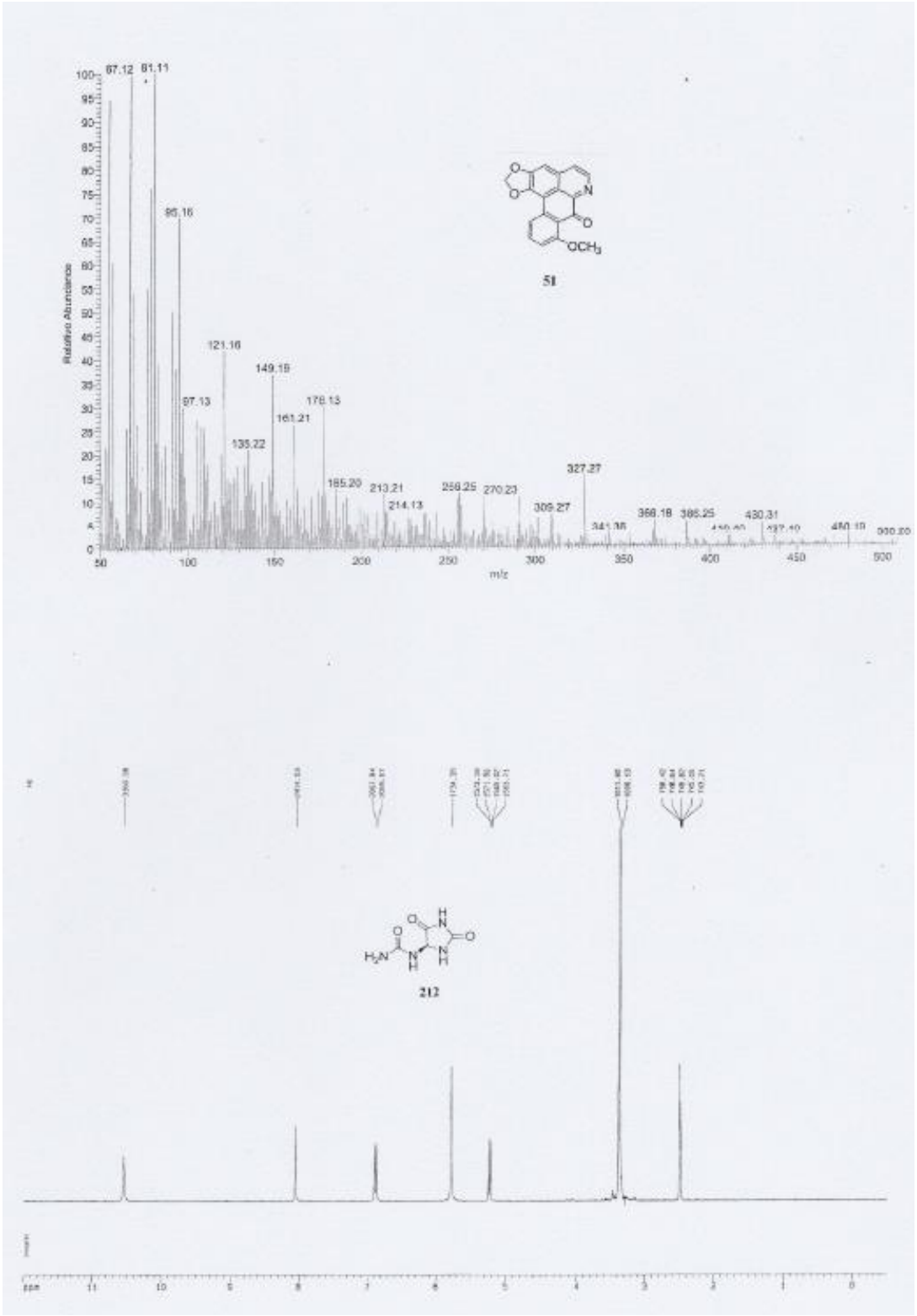

91.16
128.08
152.09
153.07
77.11
115.06
155.01
199.85
103.20
197.82
200.80
50.89
177.96
66.06
201.91
242.77
289.74
308.03
359.17
376.34
397.30
447.34
473.05
498.78
Relative Abundance
m/z
211
51
OCH3

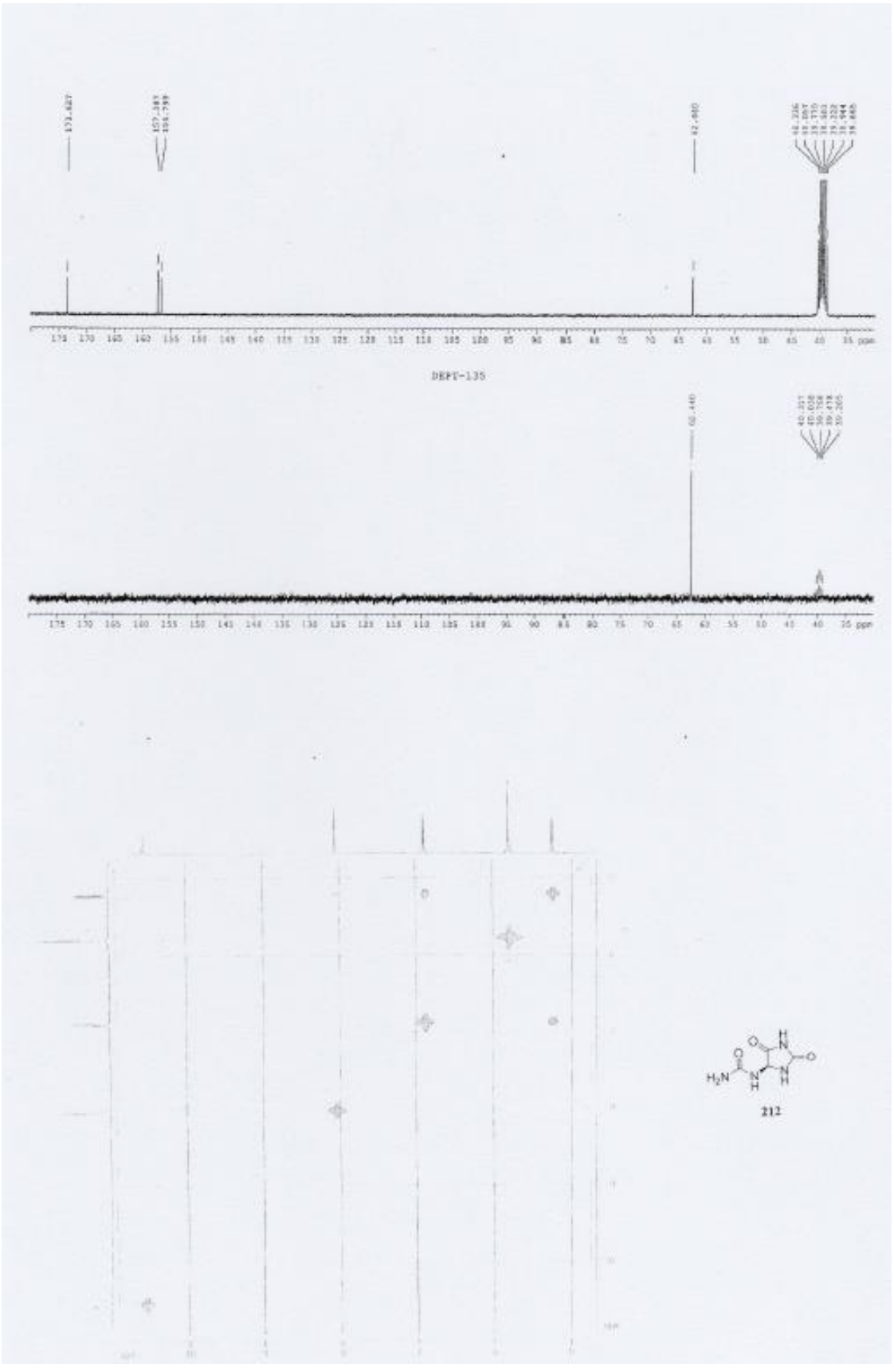


51

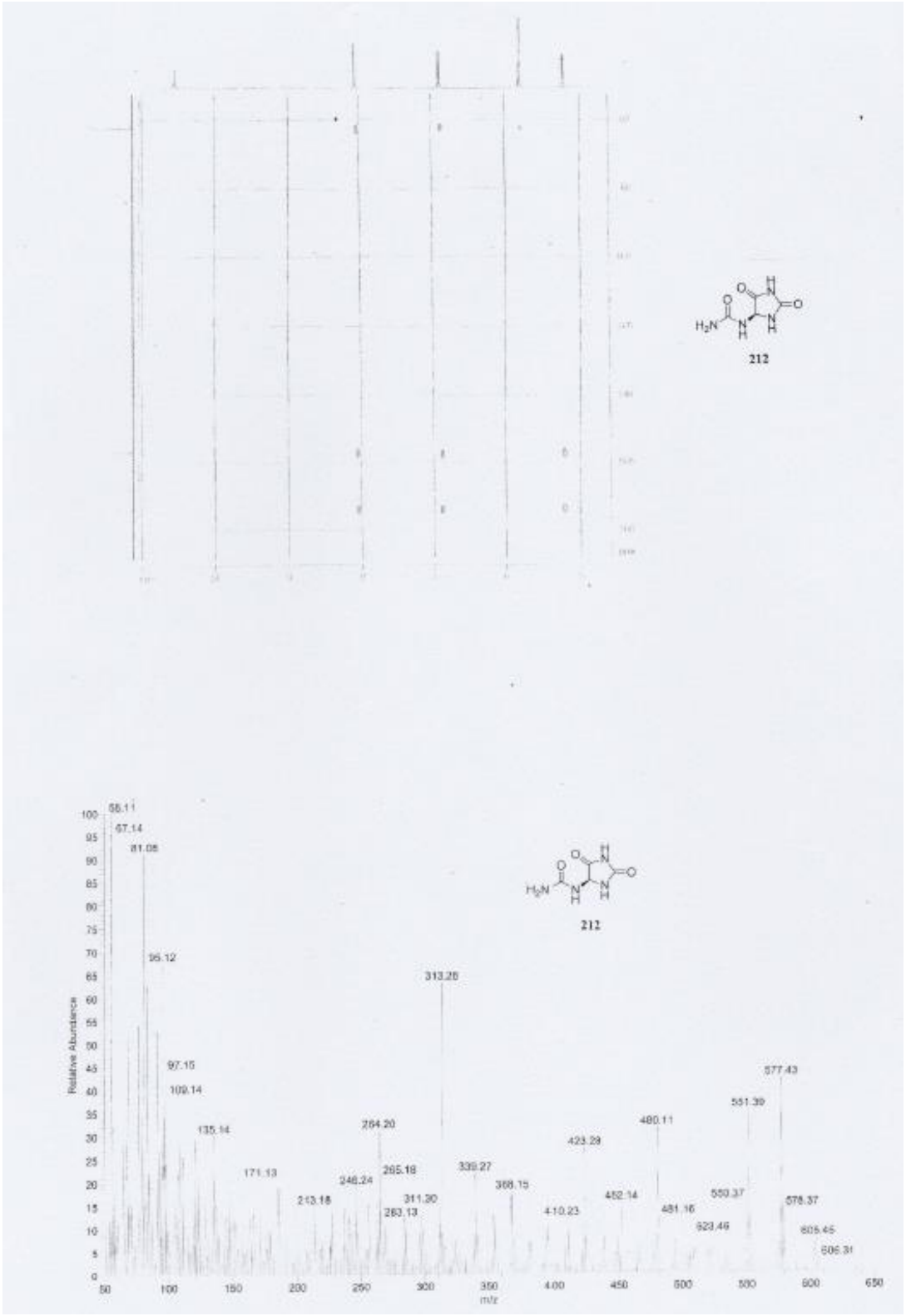

O
O
N
O
OCH3
51
O
O
N
O
OCH3
51

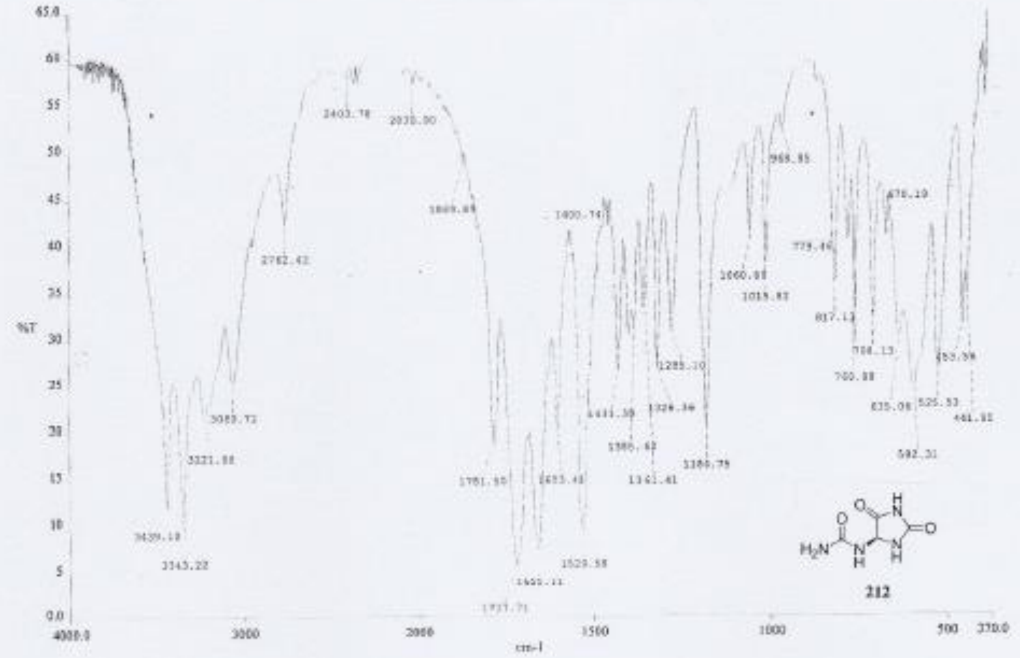

ppm
40
60
80
100
120
140
160
180
9
8
7
6
5
4
3
2
1
0
ppm
O
O
N
O
DCH3
51
ppm
80
90
100
110
120
130
140
150
160
170
180
8.8
8.6
8.4
8.2
8.0
7.8
7.6
7.4
7.2
7.0
6.8
6.6
6.4
6.2
6.0
ppm

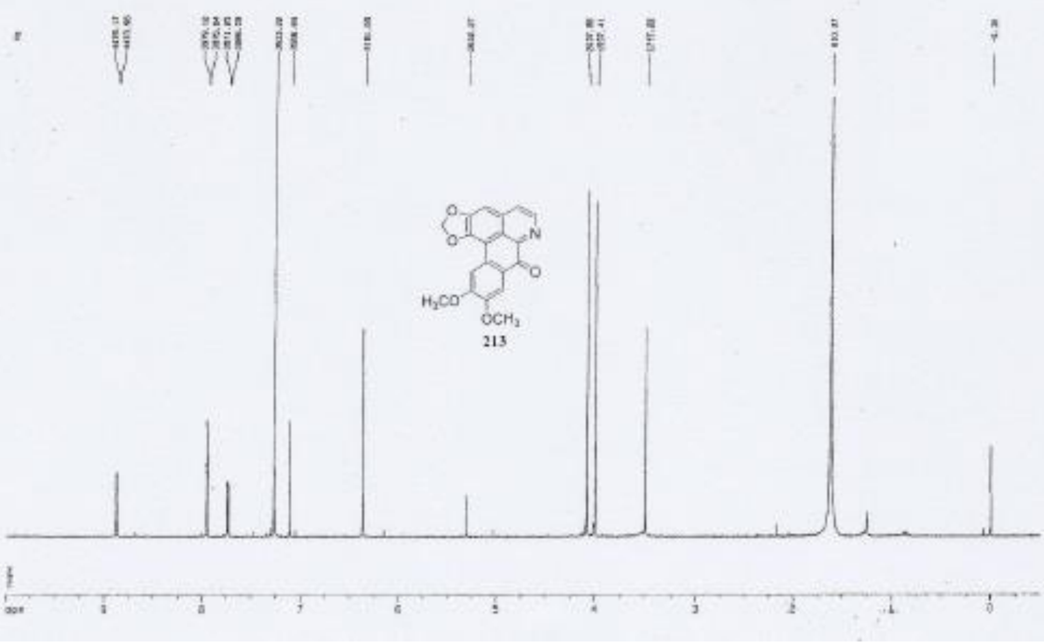

67.12
81.11
95.16
121.16
149.19
97.13
178.13
161.21
135.22
185.20
213.21
214.13
256.25
270.23
309.27
327.27
366.18
386.25
430.31
Relative Abundance
m/z
51
212

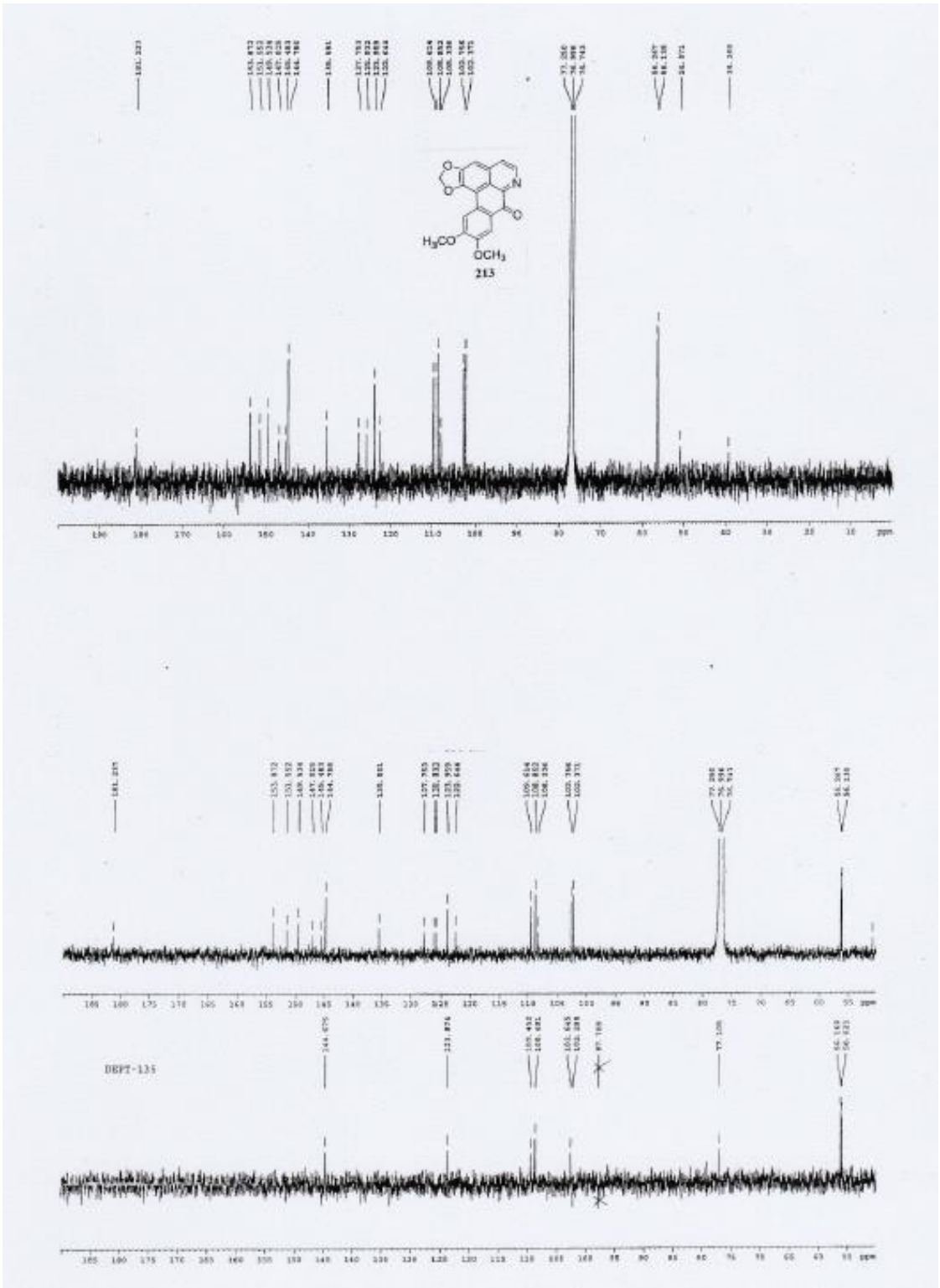


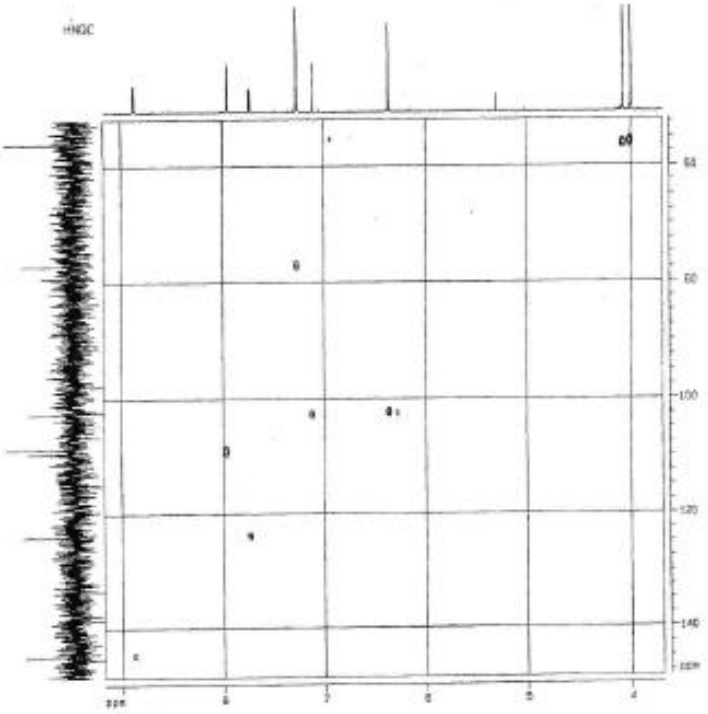
212

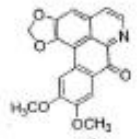

212
212
Relative Abundance
m/z


%T
cm-1
4000.0
3000
2000
1500
1000
500 370.0
212

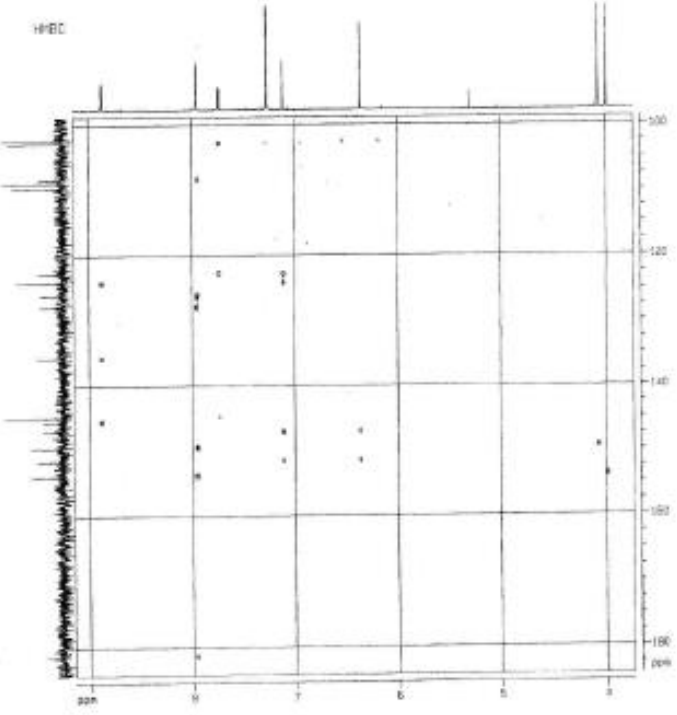

213

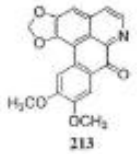

H3CO
OCH3
213
DEPT-135



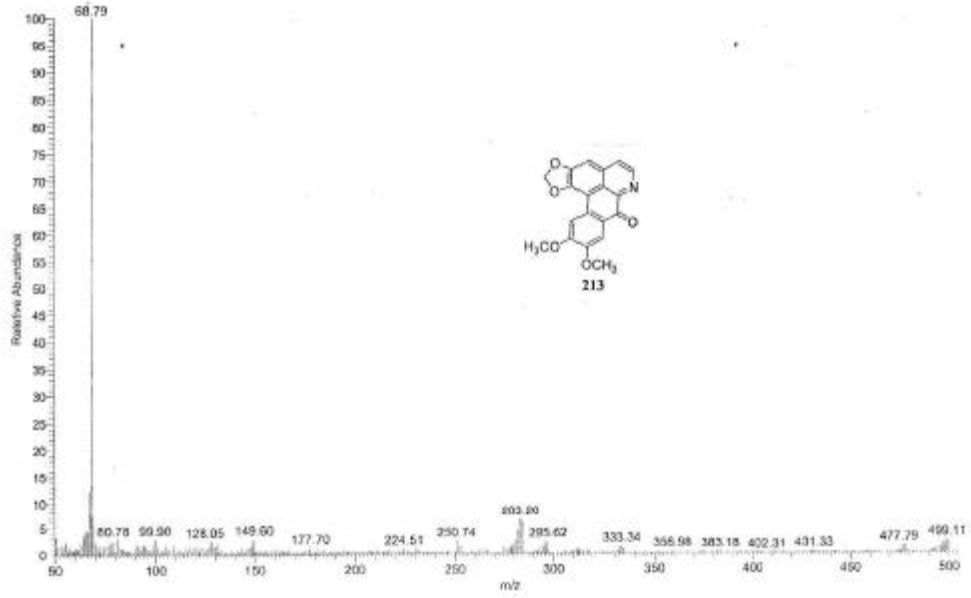


213



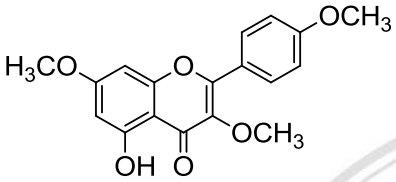


213

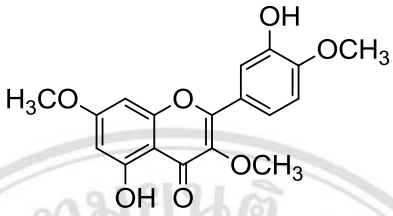


APPENDIX B

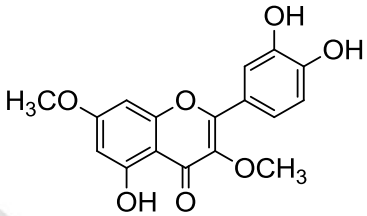
Spectrum Data of the Isolated Compounds from *P. bullata*



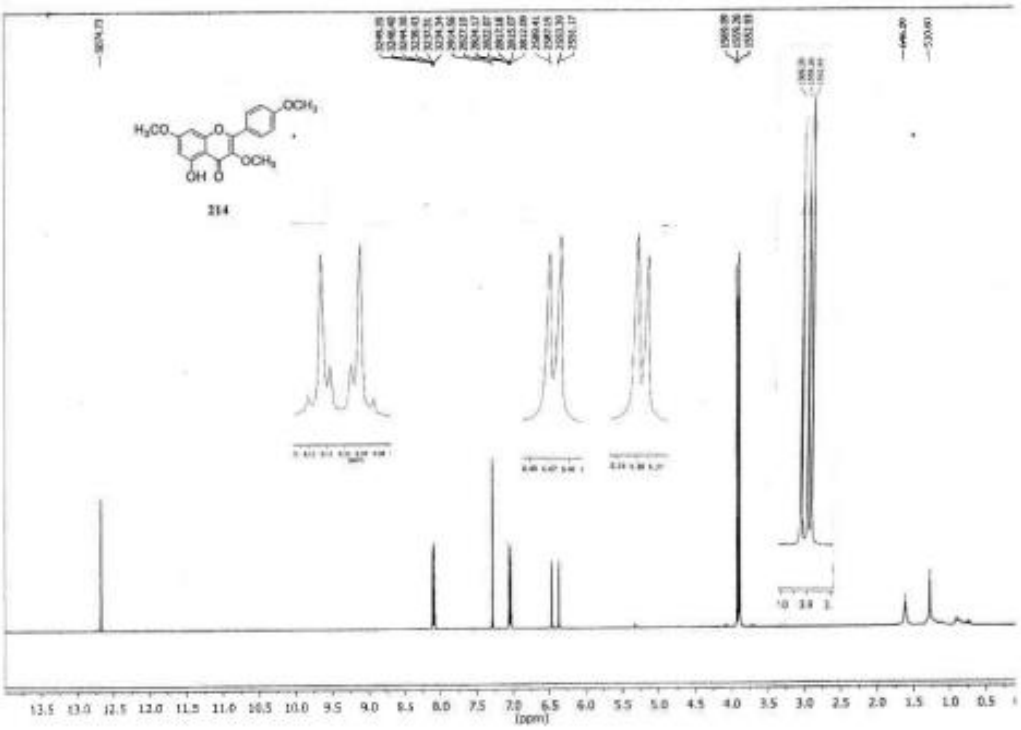
214

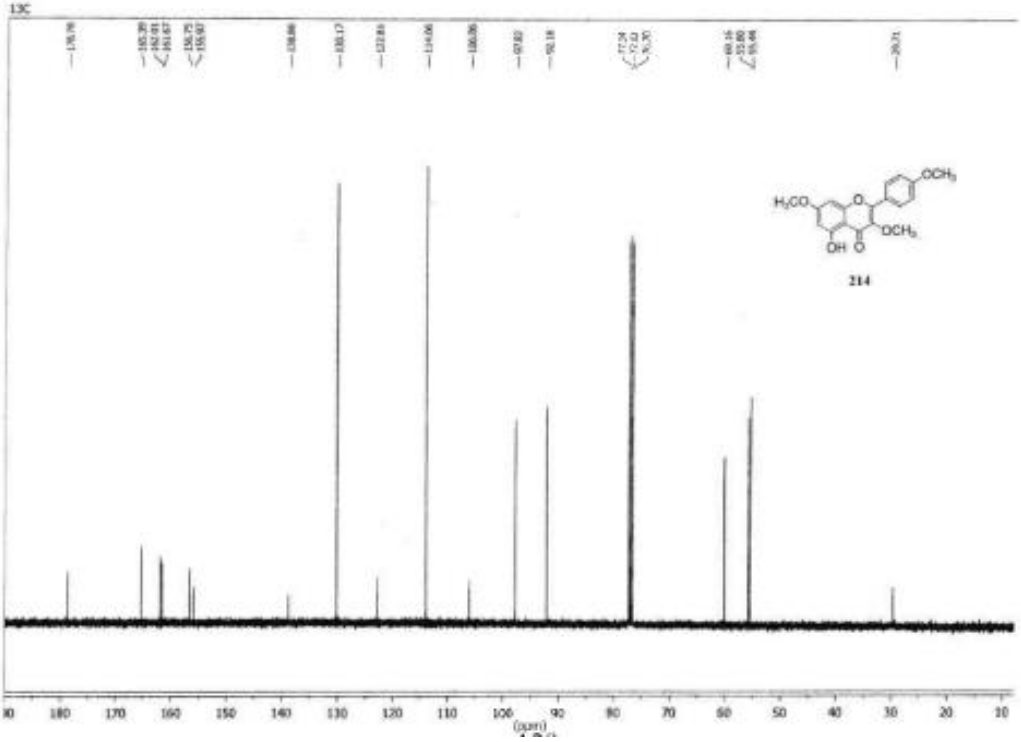


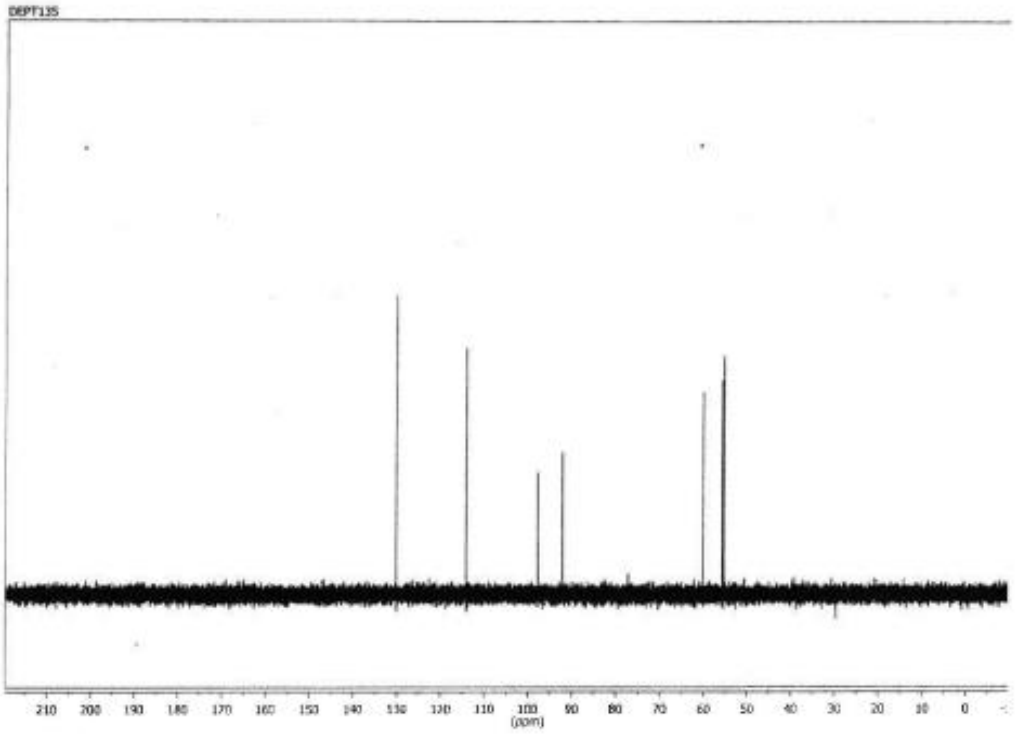
215

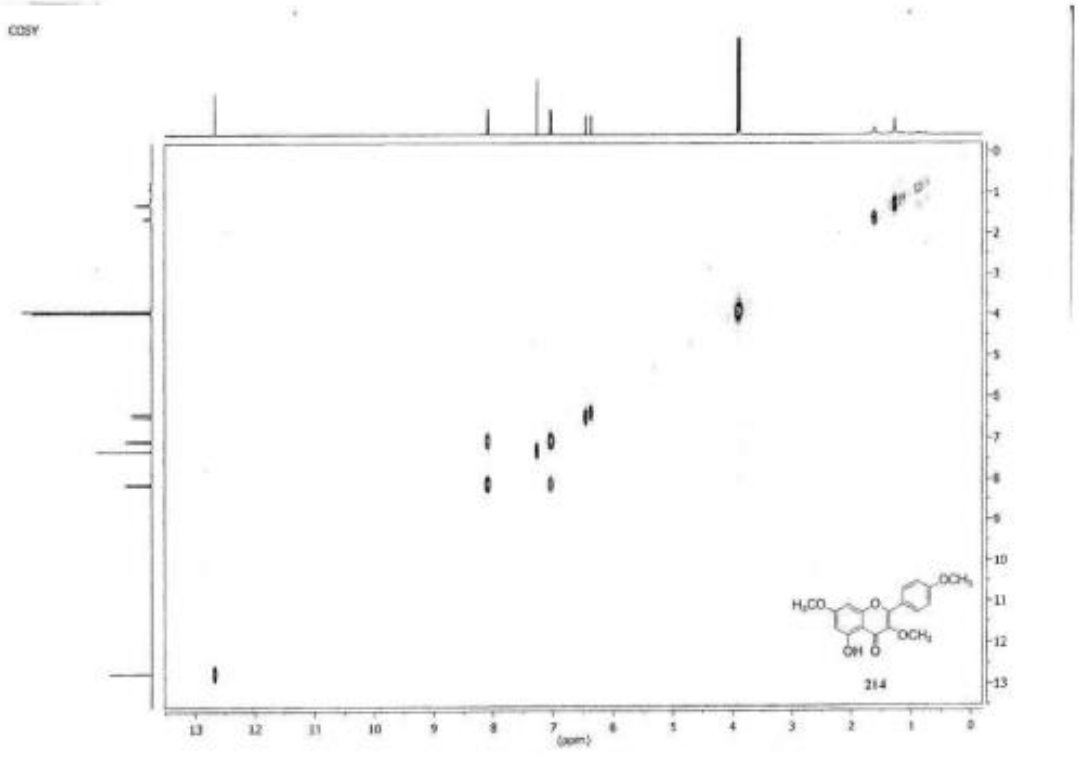


216

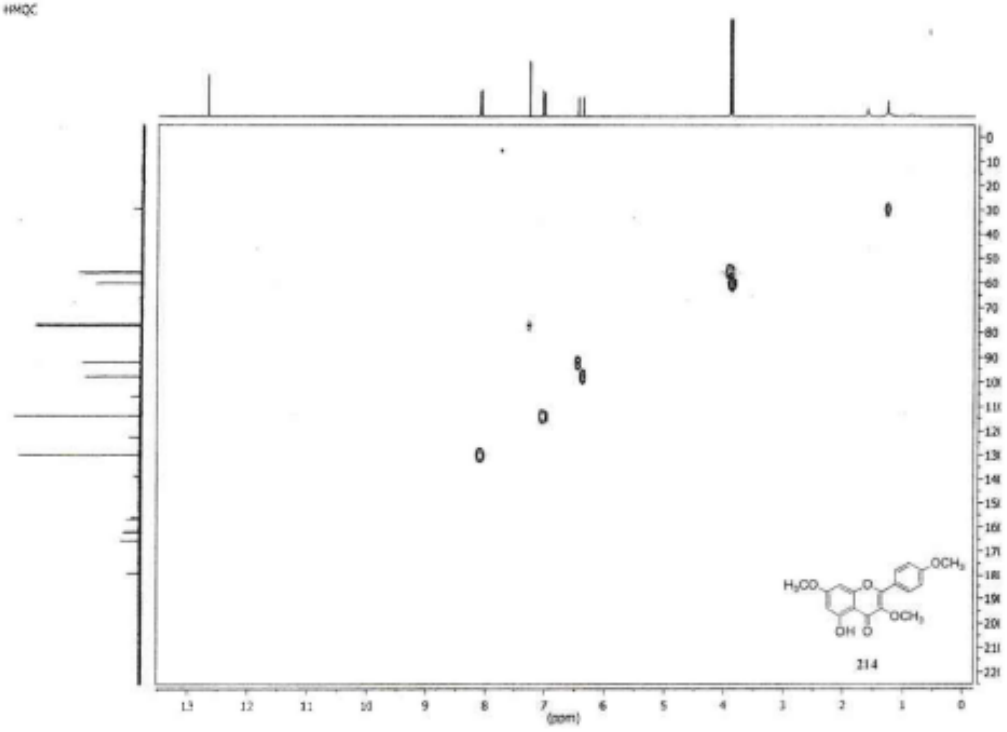

214
(ppm)


13C
214
(ppm)

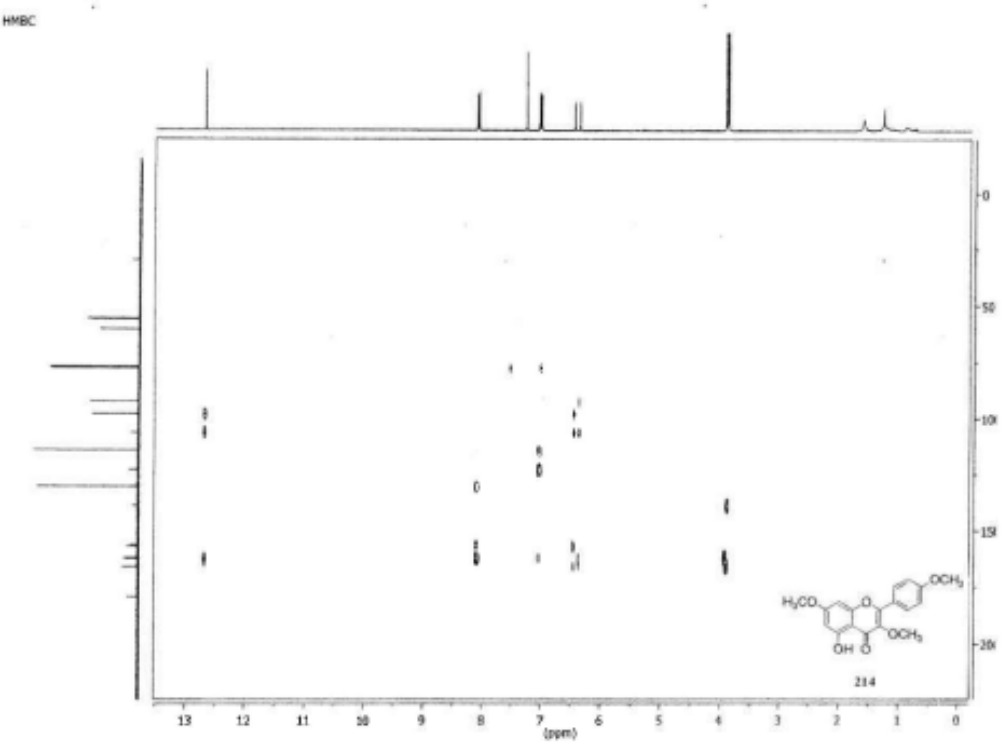

DEPT135
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
(ppm)

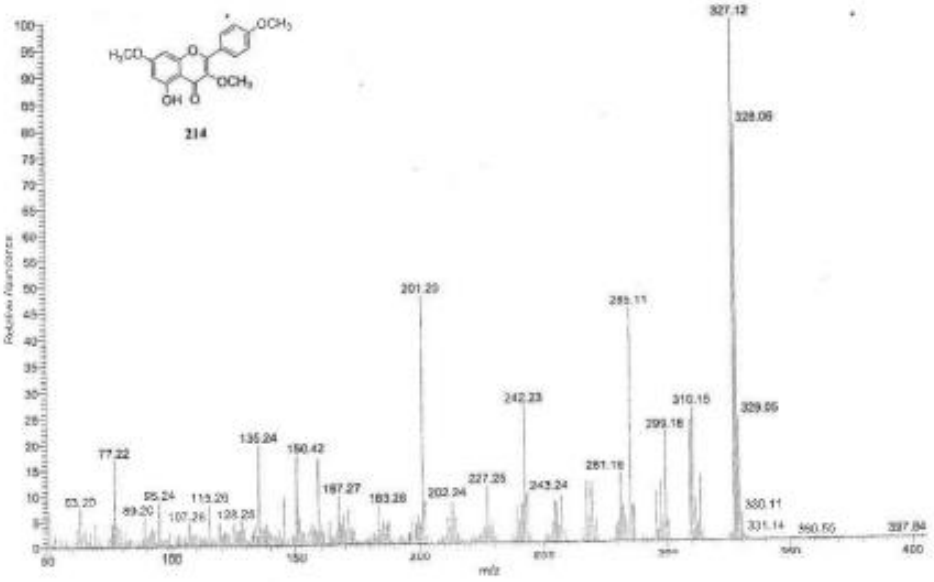

COSY
13 12 11 10 9 8 7 6 5 4 3 2 1 0
(ppm)
H3CO
OCH3
OCH3
OH O
214

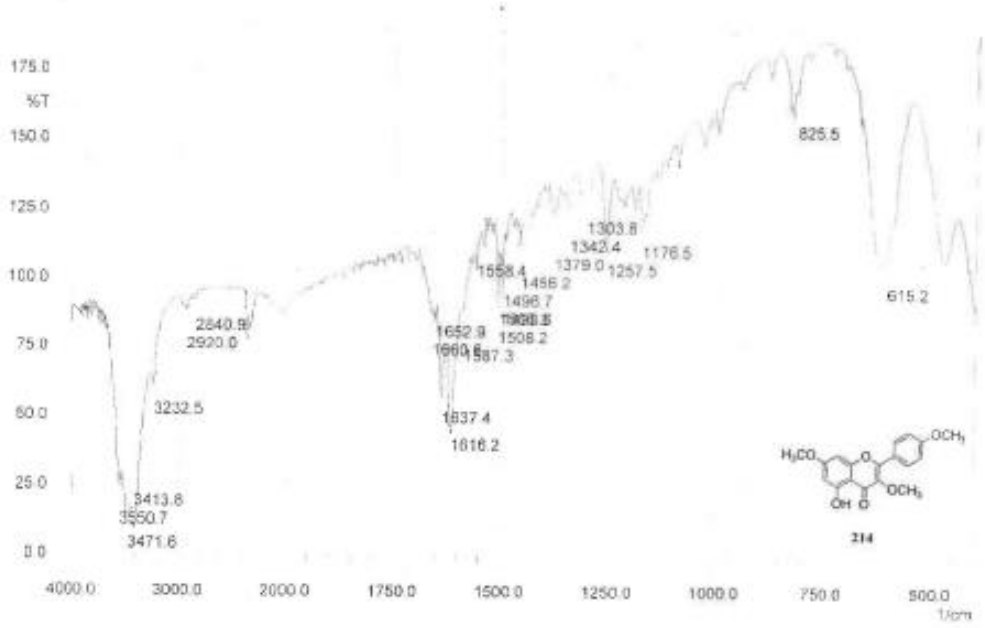
HMQC

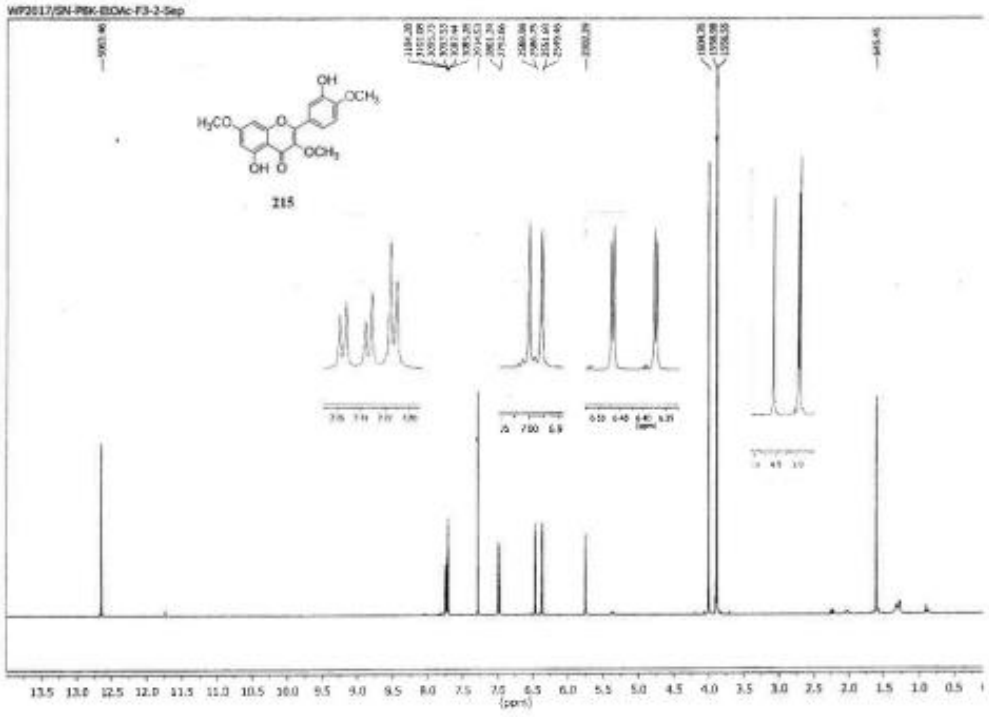


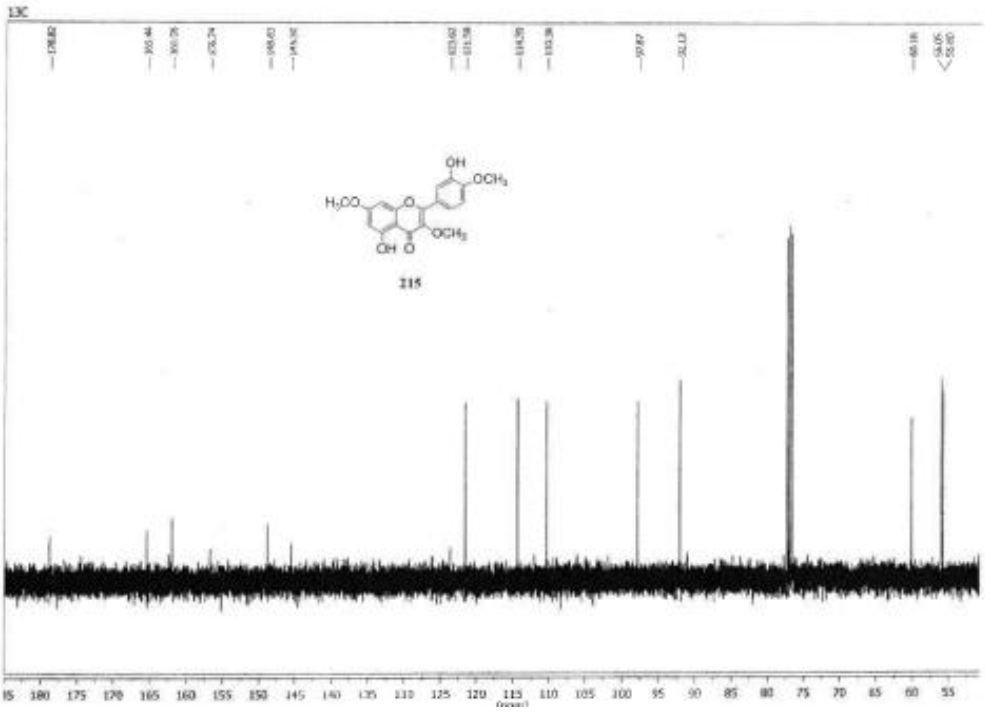
HMBC

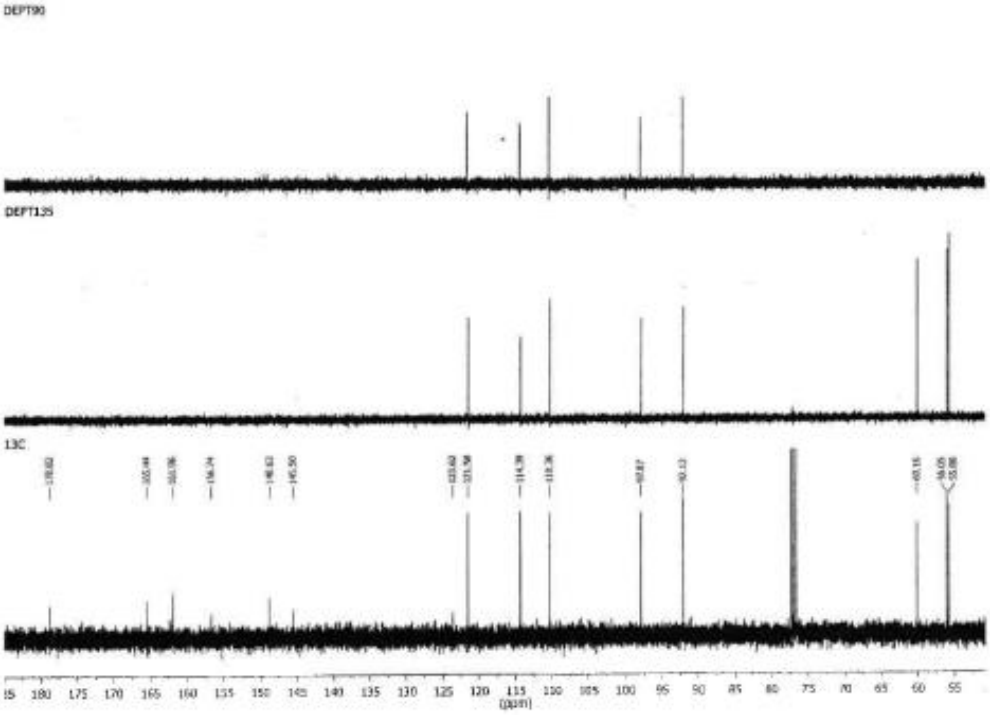


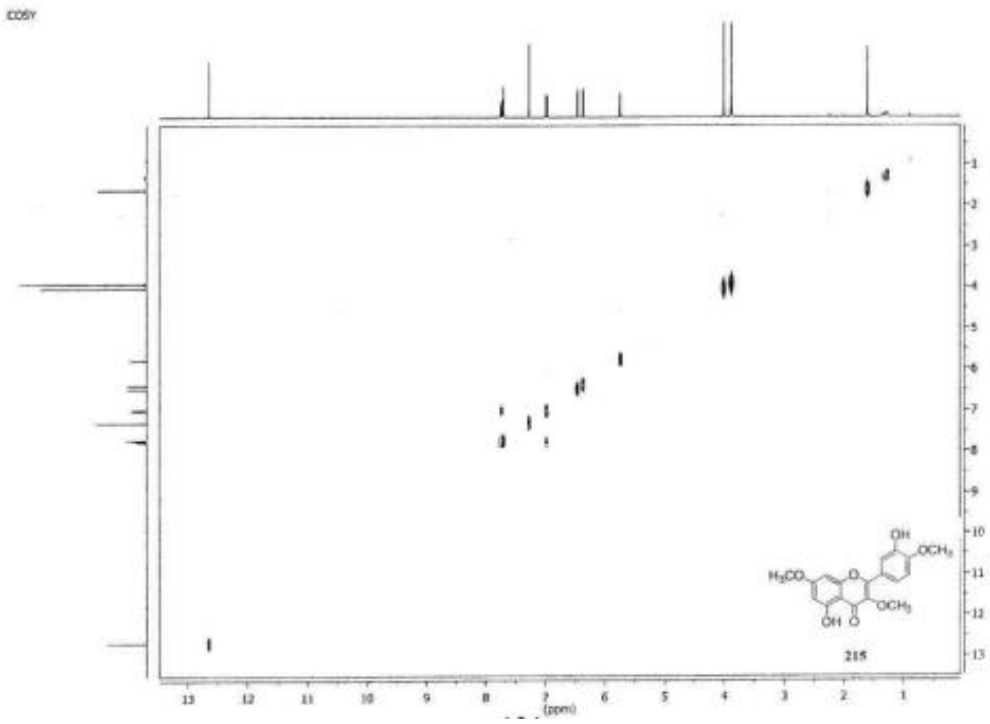

214
327.12
328.09
329.05
201.20
285.11
242.23
310.15
293.18
135.24
190.42
77.22
187.27
183.28
202.24
227.25
243.24
261.16
330.11
331.14
Relative Abundance
m/z

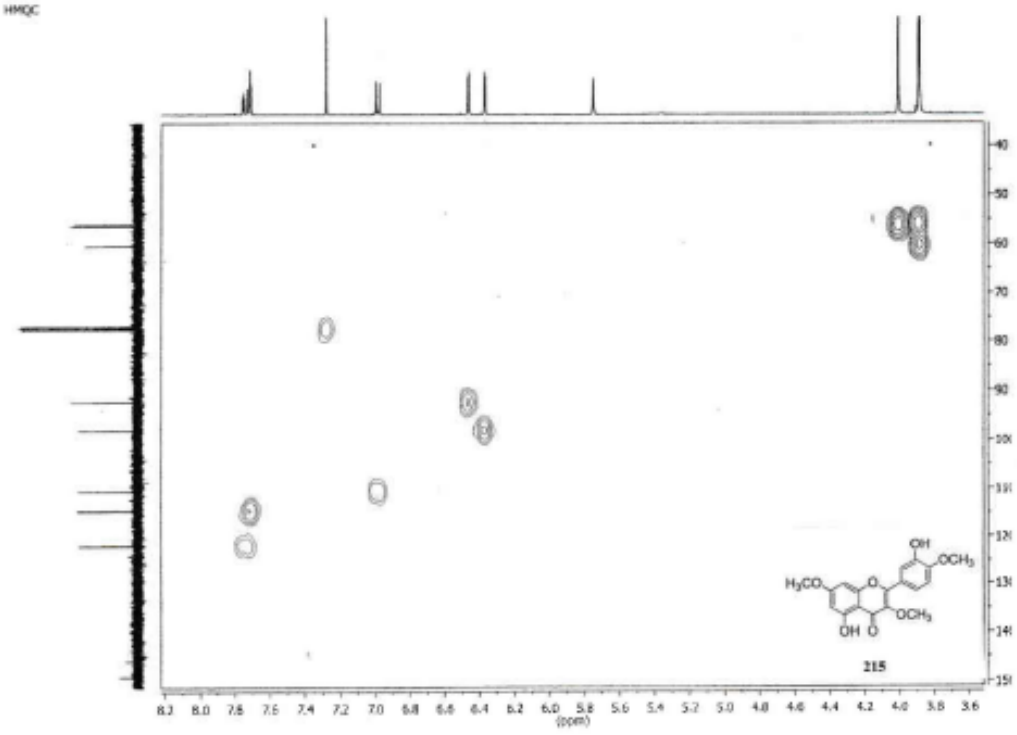

%T
825.5
1303.8
1342.4
1379.0
1176.5
1257.5
1558.4
1486.2
1496.7
1508.2
615.2
2840.9
2920.0
1652.9
1587.3
3232.5
1837.4
1616.2
3413.8
3450.7
3471.6
214
4000.0
3000.0
2000.0
1750.0
1500.0
1250.0
1000.0
750.0
500.0
1/cm

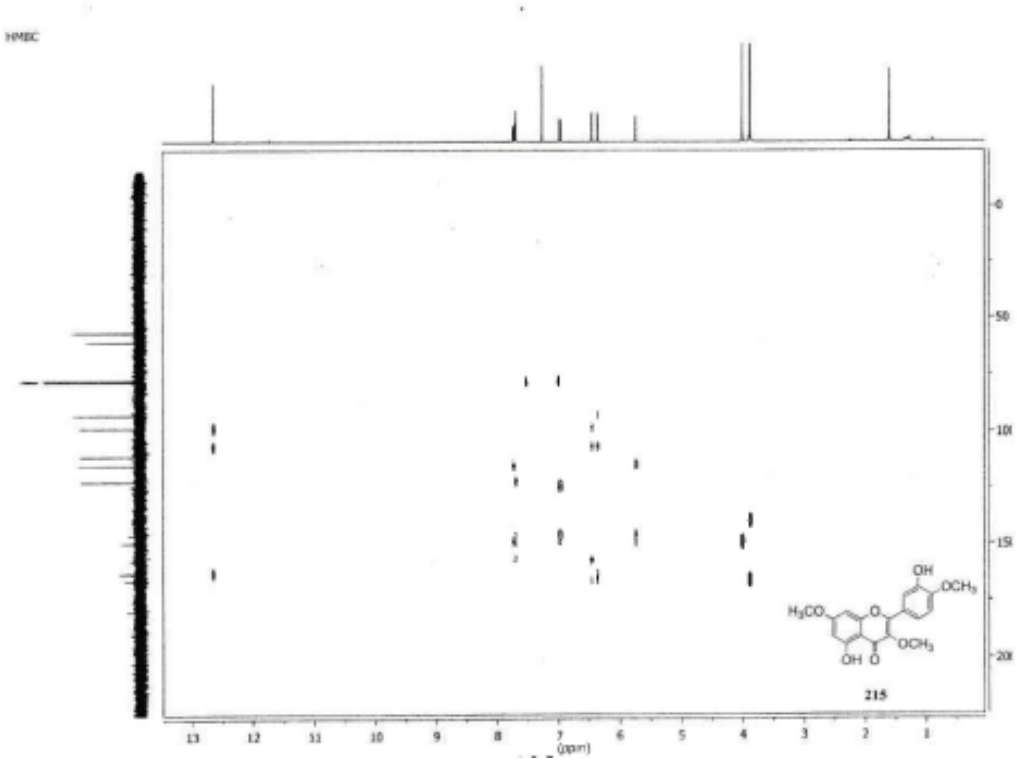

WP2017/SN-PBK-EtOAc-F3-2-Sep
OH
OCH3
H3CO
O
OCH3
OH O
215

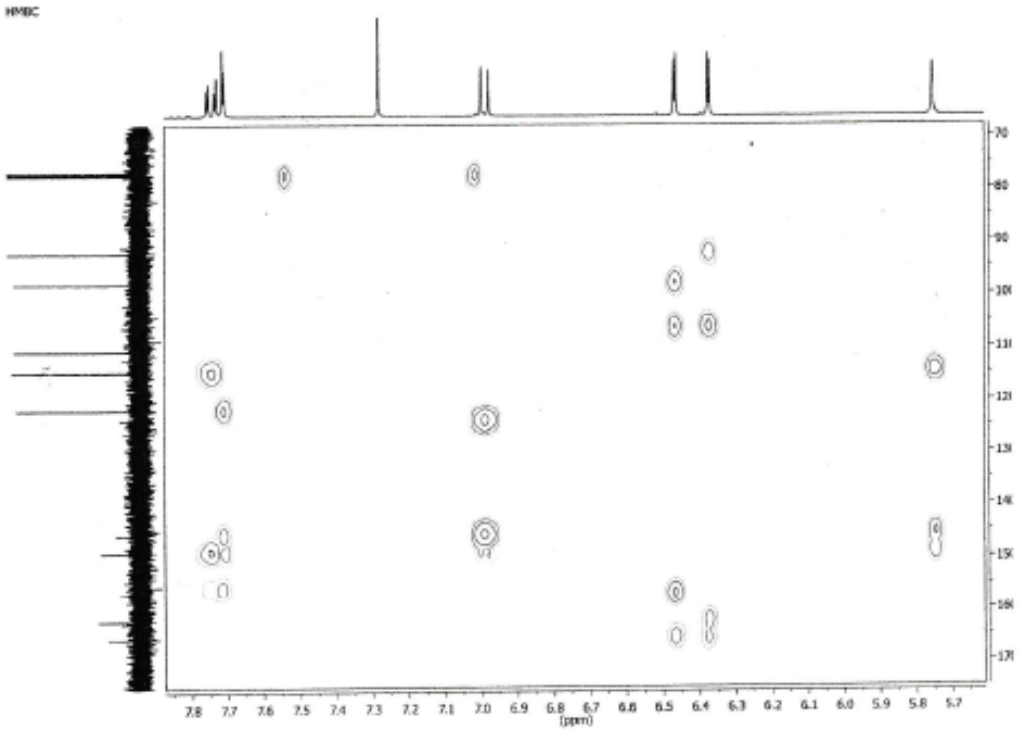

13C
OH
OCH3
H3CO
O
OCH3
OH O
215

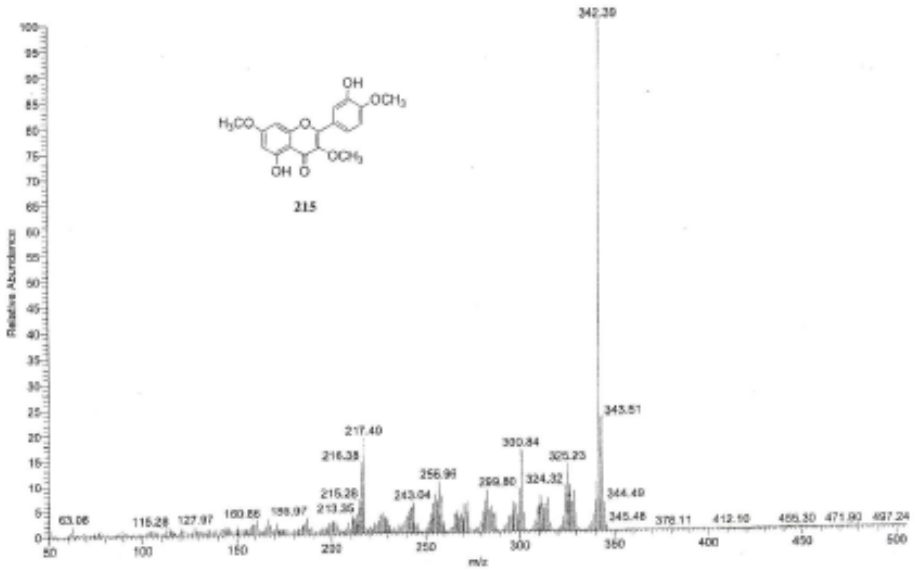

DEPT90
DEPT135
13C
(ppm)

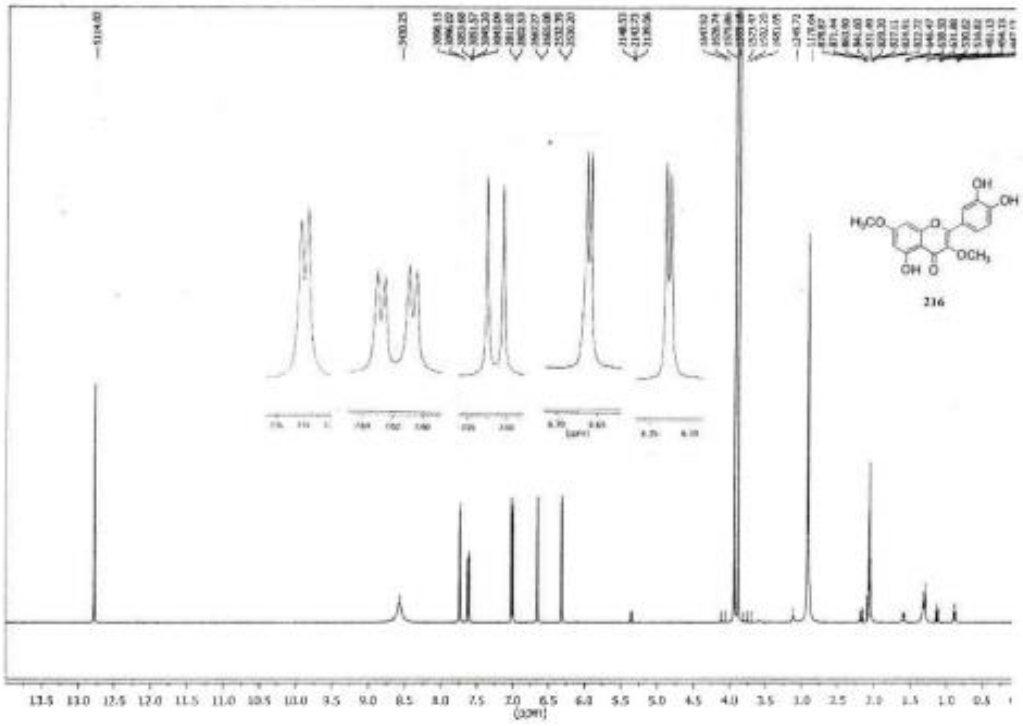

COSY
(ppm)
OH
OCH3
H3CO
OCH3
OH O
215

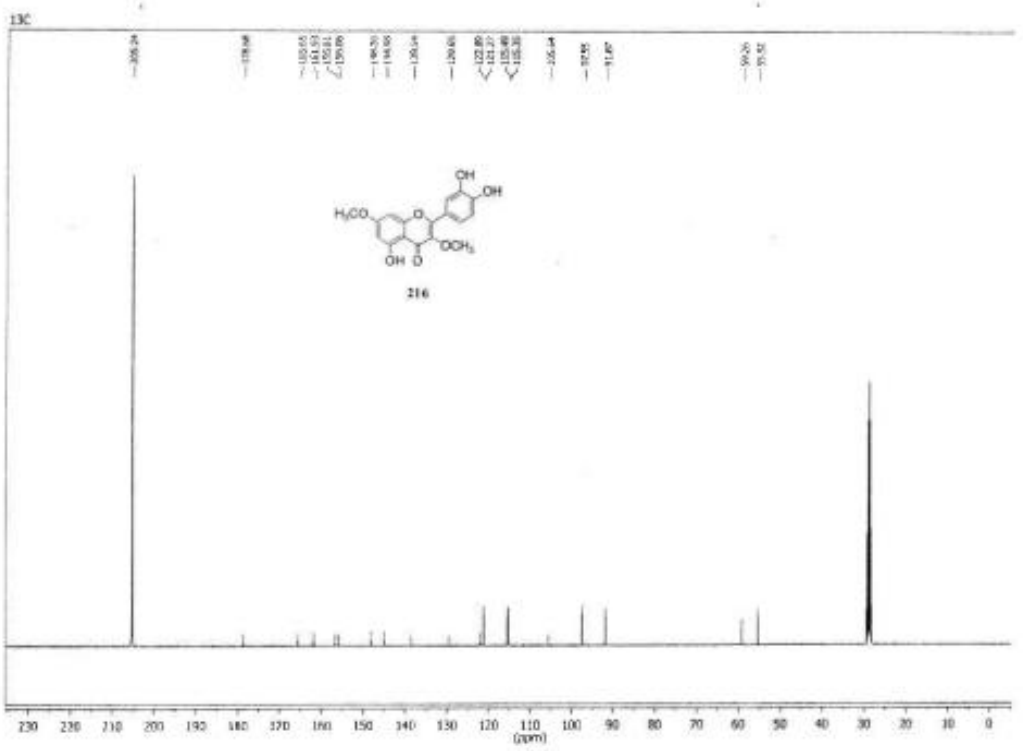

HMQC
OH
OCH3
H3CO
OCH3
OH O
215
8.2 8.0 7.8 7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6
(ppm)

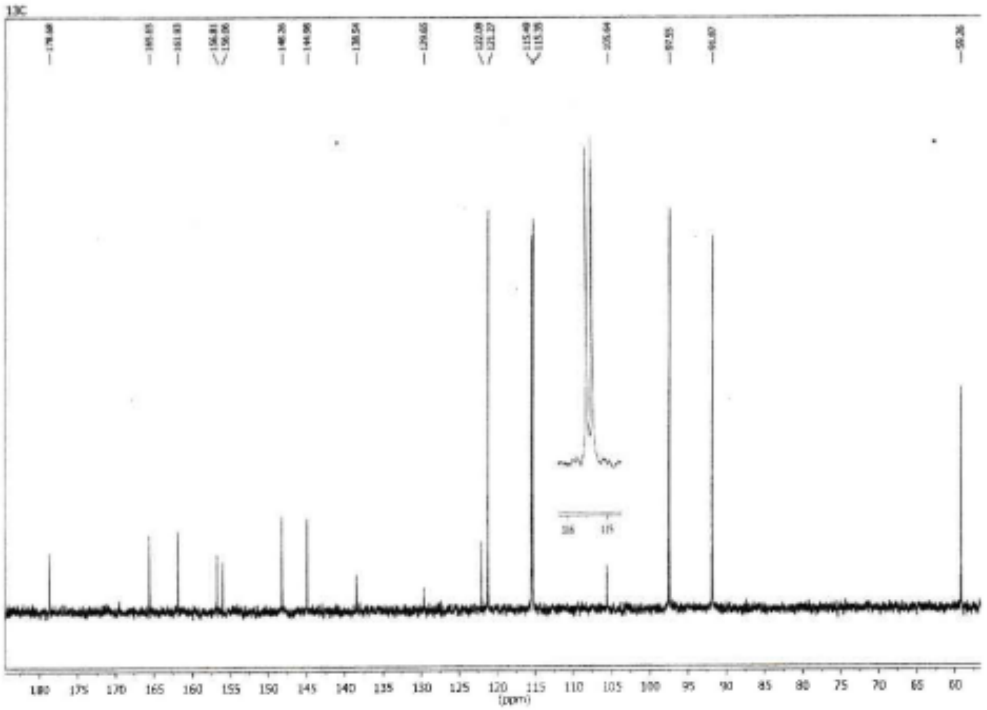

HMBC
OH
OCH3
H3CO
OCH3
OH O
215
13 12 11 10 9 8 7 6 5 4 3 2 1
(ppm)

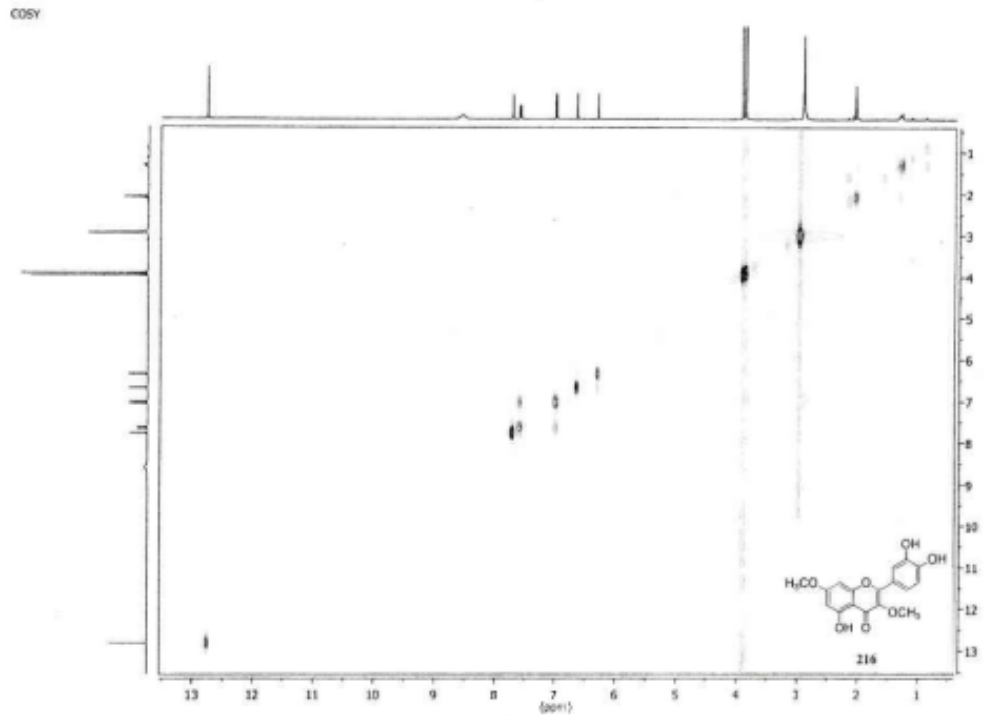

HMBC
7.8 7.7 7.6 7.5 7.4 7.3 7.2 7.1 7.0 6.9 6.8 6.7 6.6 6.5 6.4 6.3 6.2 6.1 6.0 5.9 5.8 5.7
(ppm)


OH
OCH3
H3CO
OCH3
OH O
215
Relative Abundance
342.39
343.51
217.40
216.38
330.84
325.23
256.96
324.32
243.04
344.49
63.06
115.28
127.97
160.86
158.97
213.35
215.26
345.48
376.11
412.10
455.30
471.90
497.24
m/z

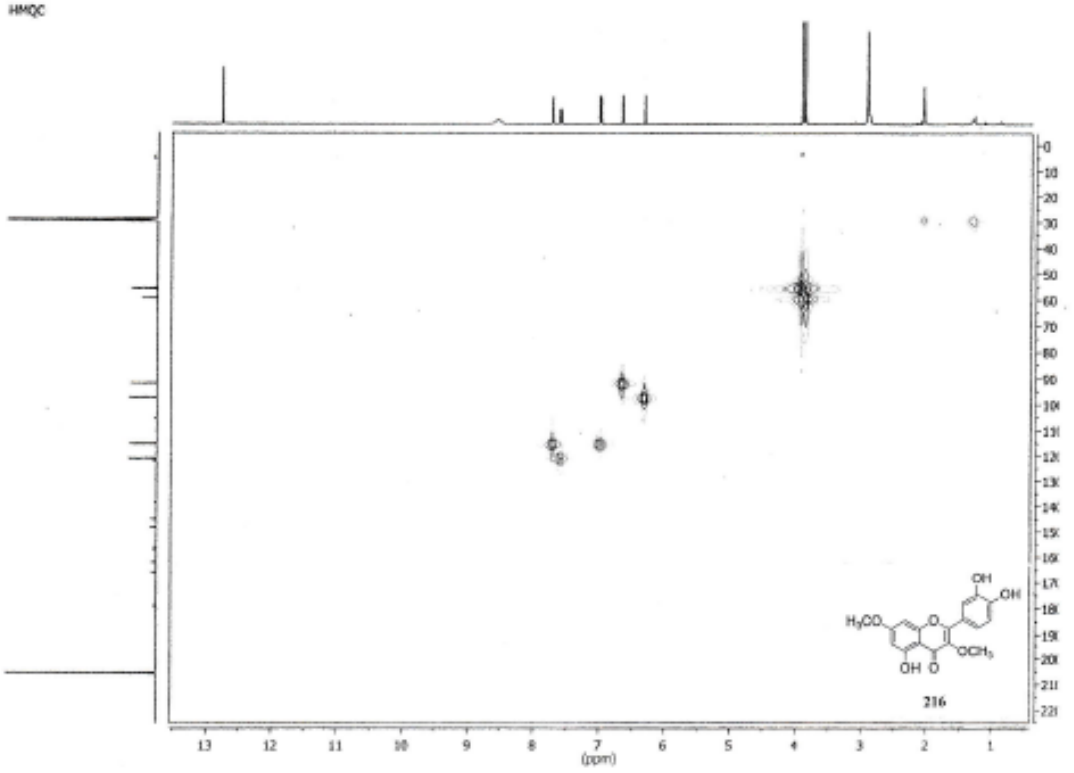

OH
OH
H3CO
OCH3
OH O
216


13C
OH
OH
H3CO
OCH3
OH O
216

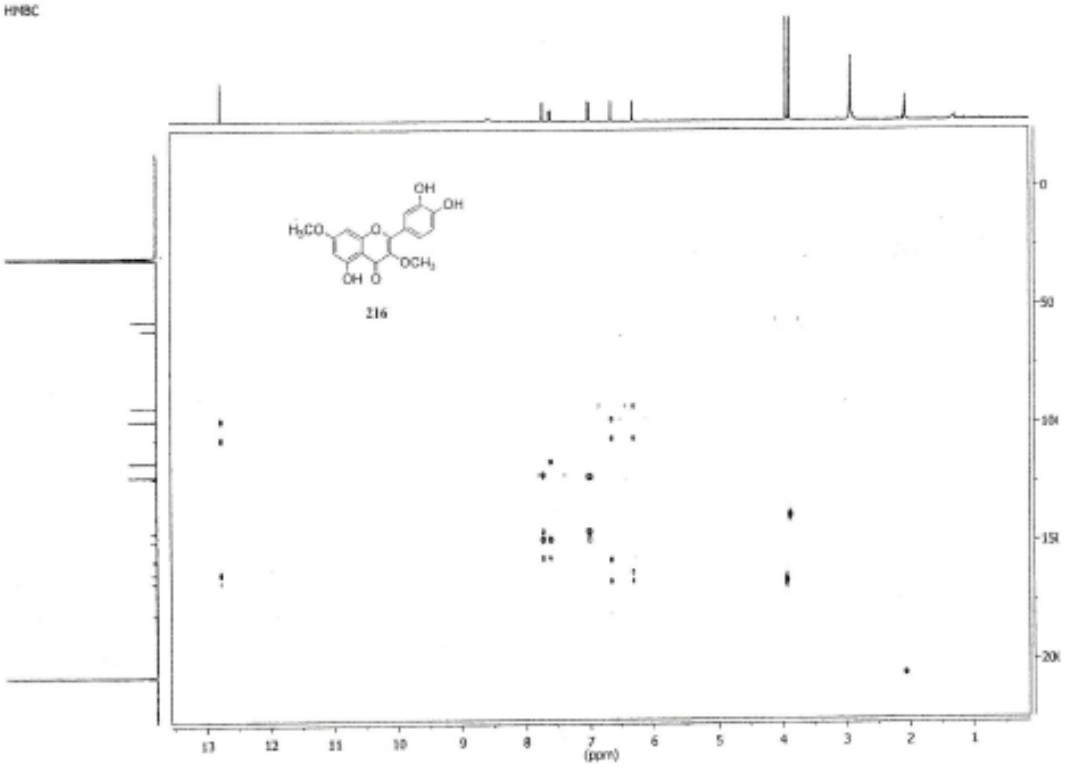

13C
(ppm)

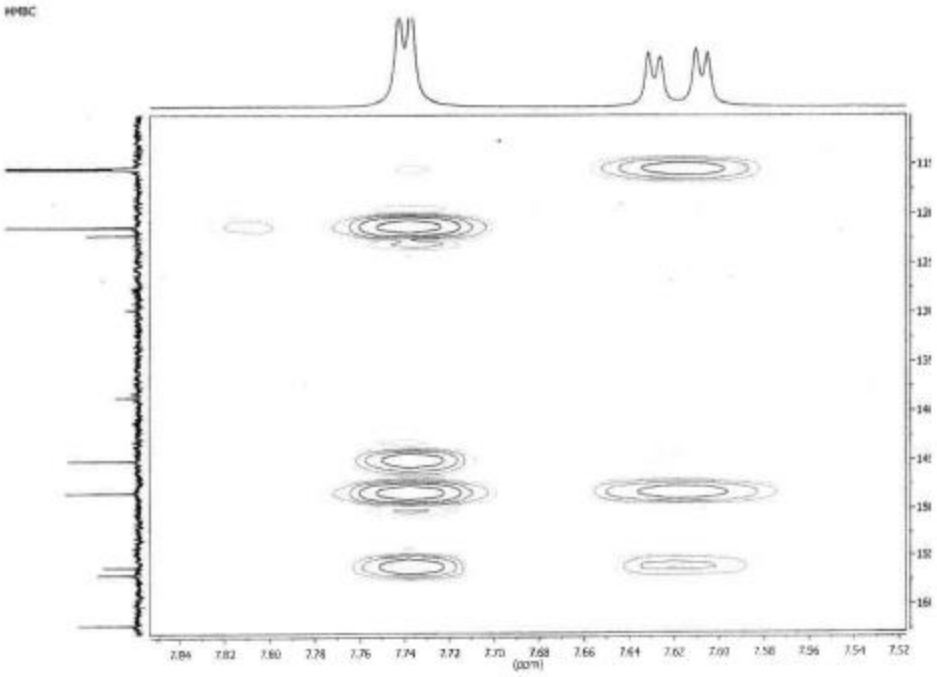

COSY
OH
OH
H3CO
O
OCH3
OH
O
216
(ppm)

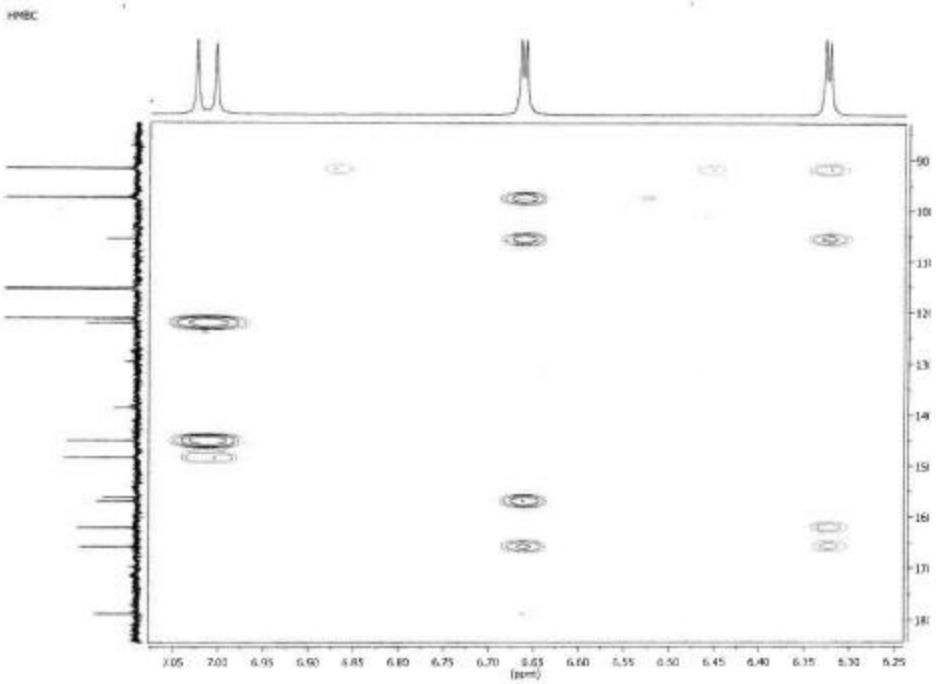
HMQC

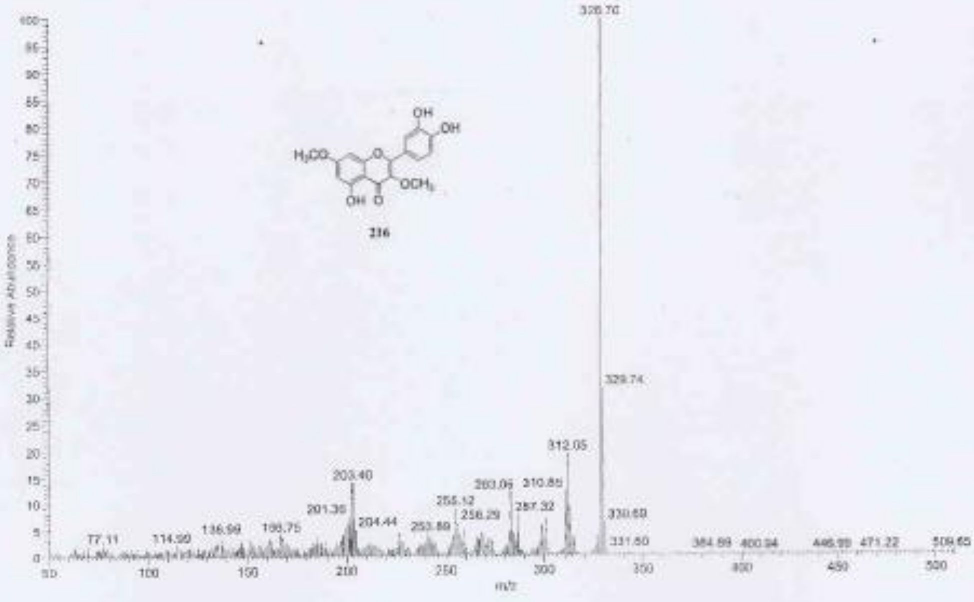

OH
OH
H3CO
OCH3
OH O
216
13 12 11 10 9 8 7 6 5 4 3 2 1
(ppm)

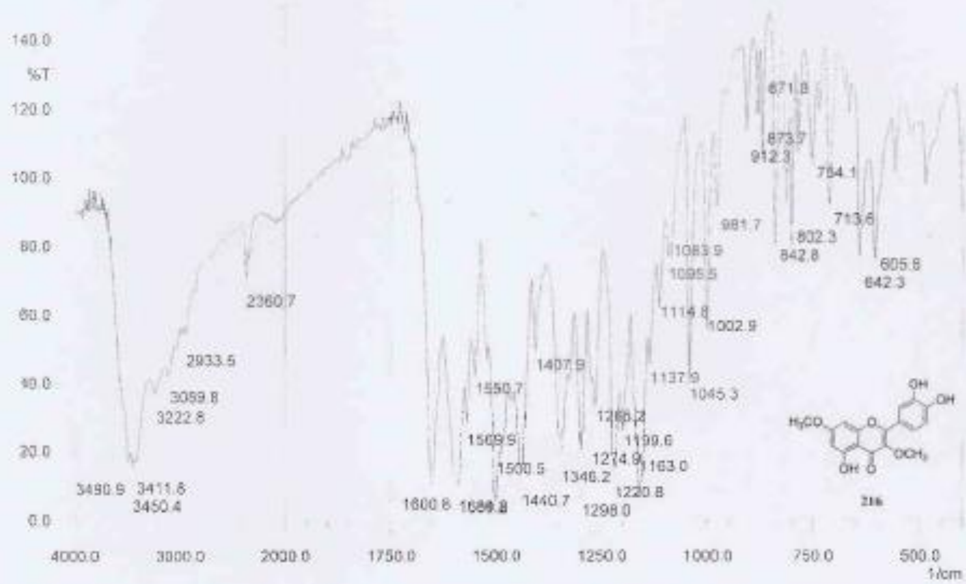
HMBC


OH
OH
H3CO
OCH3
OH O
216
13 12 11 10 9 8 7 6 5 4 3 2 1
(ppm)


HMBC
(ppm)


HMBC
(ppm)


Relative Abundance
m/z
H₃CO
OH
OCH₃
216


%T
1/cm
2360.7
2933.5
3222.8
3450.4
1407.9
1550.7
1045.3
1002.9
1114.8
605.8
642.3
842.8
754.1
216

APPENDIX C

Publications

Int. J. Chem. Sci.: 13(4), 2015, 1705-1712
ISSN 0972-768X
www.sadgurupublications.com

CHEMICAL CONSTITUENTS FROM AERIAL PARTS OF *POLYALTHIA EVECTA* (PIERRE) FINET & GAGNEP. VAR. *ATTOPEUENSIS*

SIRINAPA NANTAPAP[a], KALLAYA SANGRUENG[b], NARONG NUNTASAEN[c], PUTTINAN MEEPOWPAN[a,*] and WILART POMPIMON[d,*]

[a]Department of Chemistry and Center for Innovation in Chemistry,
Faculty of Science, Chiang Mai University, 50200 CHIANG MAI, THAILAND
[b]Department of Chemistry, Faculty of Science and Technology,
Phranakhon Rajabhat University, 10220 BANGKOK, THAILAND
[c]The Forest Herbarium, Department of National Park, Wildlife and Plant Conservation,
Ministry of Natural Resources and Environment, 10900 BANGKOK, THAILAND
[d]Laboratory of Natural Products, Center for Innovation in Chemistry,
Faculty of Science, Lampang Rajabhat University, 52100 LAMPANG, THAILAND

ABSTRACT

The first phytochemical investigation from aerial parts of *Polyalthia evecta* (Pierre) Finet & Gagnep. var. *attopeuensis* led to the isolation of six compounds, including two triterpenoids; stigmasterol (**1**) and β-sitosterol (**2**), two aporphine alkaloids; oxostephanine (**3**) and dicentrinone (**4**), one diureide of glyoxylic acid; allantoin (**5**) and one styryl lactone; goniothalamin (**6**). Characterization of all compounds was carried out by extensive spectroscopic analysis and comparison with literature. All compounds were previously isolated from *Polyalthia*, excepting dicentrinone (**4**) and goniothalamin (**6**) that are being reported for the first time from this genus.

Key words: Annonaceae, *Polyalthia evecta* var. *attopeuensis*, Triterpenoids, Aporphine alkaloids, Diureide of glyoxylic acid.

INTRODUCTION

Polyalthia is a genus of flowering plants in family annonaceae, which consists about 120 species of shrubs and trees. This genus is extensively distributed in tropical and subtropical areas[1]. Previous chemical investigations of this genus showed that some

*Author for correspondence; E-mail: wilart_p@hotmail.com, pmeepowpan@gmail.com

aporphines[2-7], tetrahydroprotoberberines[4,8], sesquiterpenylindoles[9,10], isoquinolines[7,11], flavonoids[12], azafluorenes[13], terpenes[14], clerodane diterpenoids[15-18], furans[19], acetogenins[20], prenylated benzopyrans[21-22], polyacetylenes[23], styryl-lactones[24], chalcones[1], polyphenolics[25] and other chemical constituents reported from *Polyalthia* genus review[26]. Many constituents have cytotoxic[27,28], antifungal, antiviral[19,29], antimicrobial[19,30,31], antimalarial[6,7], anti-HIV[32], antibacterial[33], anticancer activity[19,25,34,35] from various *Polyalthia* species. In addition, it is widely used in traditional medicine in many tropical countries such as in India[26], Thailand[23] and Malaysia[1]. Due to the tremendous uses in medicinal applications of *Polyalthia*, this research proposal aims to investigate the chemical constituents of *P. evecta* var. *attopeuensis*, as no research work has been published on this variety till now.

EXPERIMENTAL

Materials and methods

Plant material

P. evecta var. *attopeuensis* is called "Khamhom" in Thai. The aerial parts of this plant were collected in Sakon Nakhon province and identified by Narong Nuntasaen. A voucher specimen (BKF No. 137701) has been deposited at the Forest Herbarium, Department of National Park, Wildlife and Plant Conservation, Ministry of Natural Resources and Environment, Bangkok, Thailand.

Extraction and isolation

The air-dried powdered of *P. evecta* var. *attopeuensis* (1.5 kg) were successively percolated with hexane (3 L x 3 days x 4 times) and then extracted with EtOAc (3 L x 3 days x 4 times) and MeOH (3 L x 3 days x 4 times) at room temperature, respectively and followed by filtration. The filtrates were combined and evaporated to dryness under reduced pressure to afford hexane, ethyl acetate and methanol extracts as 32.23, 29.83 and 69.95 g, respectively.

The hexane extract was separated by column chromatography (CC) over silica gel, eluted with various proportions of EtOAc:*n*-hexane (0:100 to 100:0), followed by the increasing amount of MeOH in EtOAc (0:100 to 100:0). Fractions were collected and combined on the basis of TLC behavior. The solvents were evaporated to dryness to afford eight fractions (F_1–F_3). Fraction F_2 (7.62 g) was eluted by EtOAc:*n*-hexane (5:95). Fractions were collected and combined, then solvent were removed under reduced pressure to afford subfraction A_1–A_3. Further, the subfraction A_3 (3.59 g) was separated by CC over silica gel

(*n*-hexane) and then recrystallized with 95% EtOH to give a mixture of compounds **1** and **2** (317.10 mg).

The EtOAc extract was separated by CC over silica gel. Gradient elution was conducted initially with *n*-hexane, gradually enriched with EtOAc (0:100 to 100:0), followed by increasing amount of MeOH in EtOAc (0:100 to 100:0). Fractions were collected and combined on the basis of TLC characteristic. The solvents were evaporated to dryness to afford five fractions (F_1'–F_5'). Fraction F_2' (6.63 g) on elution by EtOAc:*n*-hexane (30:70) afford subfractions I_1–I_6. Further, the subfraction I_4 was chromatographed over silica gel eluted with MeOH:CH_2Cl_2 (5:95 to 10:90) to afford subfraction M_1–M_5. Subfraction M_3 (16.90 mg) and M_4 (1.90 mg) were purified by Sephadex LH-20 column (MeOH) to yield compounds **6** (6.00 mg) and **3** (5.10 mg), respectively.

The methanol extract was fractionated to CC on silica gel eluted with gradient EtOAc:*n*-hexane (80:20 to 100:0) and gradually enriched with MeOH:EtOAc (0:100 to 100:0) to afford four fractions (F_1''–F_4''). Fraction F_3'' (12.60 g) was recrystallized with 95% EtOH to yield compound **5** (19.80 mg). The residue of fraction F_3'' was subjected to silica gel CC eluted with gradient MeOH:CH_2Cl_2 (0:100 to 100:0) to obtain subfractions L_1–L_4. Subfractions L_2 (69.20 mg) was separated by Sephadex LH-20 column (MeOH) to afford subfractions P_1–P_2. Then the subfractions P_2 (37.30 mg) was separated by Sephadex LH-20 column (MeOH) to afford subfractions Q_1–Q_3. Subfraction Q_2 (12.60 mg) was rechromatographed over Sephadex LH-20 column (MeOH) to obtain subfractions R_1–R_3. Finally R_3 (12.00 mg) was recrystallized with MeOH: CH_2Cl_2 (80:20) to yield compound **4** (15.60 mg). Subfractions L_3 (115.00 mg) was purified by preparative TLC on silica gel plate, eluted with MeOH:CH_2Cl_2 (2:98) to give compound **3** (9.30 mg).

RESULTS AND DISCUSSION

Phytochemical study

P. evecta var. *attopeuensis* were extracted, isolated, purified and identified to obtain two triterpenoids **(1-2)**, two aporphine alkaloids **(3-4)**, one diureide of glyoxylic acid **(5)** and one styryl lactone **(6)**. The structures of compounds were elucidated by spectroscopic techniques (^{1}H, ^{13}C, 2D-NMR and MS) and comparison with the previously literature data[36-40] (Fig. 1).

In this investigation, all the compounds except for compound **4** and **6** were isolated from *Polyalthia* genus. The steroid mixtures of compound **1** and **2** were revealed recently from *P. rumphii*[41]. Compound **3** was previously reported from *P. cauliflora* var. *beccarii*, *P.*

suaveolens, *P. stenopetala*, *P. suberosa*, *P. insignis*[2,8,26], *P. rumphii*[42]. Compound **5** was displayed from *P. longifolia* var. *pendula*[18,30], *P. sclerophylla*[41].

Structure elucidation and identification

Mixture of stigmasterol (1) and β-sitosterol (2)

White plates. **(1)** $C_{29}H_{48}O$ (*m/z* 412), ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.36 (1H, m, H-6), 5.15 (1H, dd, *J* = 15.1, 8.6 Hz, H-23), 5.02 (1H, dd, *J* = 15.1, 8.7 Hz, H-22), 3.53 (1H, m, H-3), 1.02 (3H, d, *J* = 6.5 Hz, H-21), 1.01 (3H, s, H-19), 0.86 (3H, d, *J* = 6.8 Hz, H-26), 0.81 (3H, d, *J* = 7.6 Hz, H-29), 0.80 (3H, d, *J* = 6.2 Hz, H-27), 0.70 (3H, s, H-18). **(2)** $C_{29}H_{50}O$ (*m/z* 414), ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.36 (1H, m, H-6), 3.53 (1H, m, H-3), 1.01 (3H, s, H-19), 0.92 (3H, d, *J* = 6.4 Hz, H-21), 0.85 (3H, d, *J* = 7.6 Hz, H-29), 0.84 (3H, d, *J* = 6.5 Hz, H-26), 0.81 (3H, d, *J* = 6.2 Hz, H-27), 0.68 (3H, s, H-18) (Lit.[36]).

Oxostephanine (3)

Yellow powder. $C_{18}H_{11}NO_4$ (*m/z* 305), ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.76 (1H, d, *J* = 5.2 Hz, H-5), 8.21 (1H, d, *J* = 8.1 Hz, H-11), 7.62 (1H, d, *J* = 5.2 Hz, H-4), 7.56 (1H, t, *J* = 8.3 Hz, H-10), 7.08 (1H, s, H-3), 7.02 (1H, d, *J* = 8.4 Hz, H-9), 6.27 (2H, s, H-12), 3.98 (3H, s, 8-OCH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 181.74 (C-7), 161.91 (C-8), 151.71 (C-2), 147.58 (C-1), 146.72 (C-3a), 144.84 (C-5), 135.33 (C-6a), 135.23 (C-7a), 134.59 (C-10), 123.29 (C-4), 122.13 (C-1b), 120.88 (C-1a), 119.74 (C-11), 112.35 (C-9), 108.76 (C-11a), 103.11 (C-3), 102.19 (C-12), 56.44 (8-OCH_3) (Lit.[37]).

Dicentrinone (4)

Orange needles. $C_{19}H_{13}NO_5$ (*m/z* 335), ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.87 (1H, d, *J* = 5.2 Hz, H-5), 7.96 (1H, s, H-11), 7.95 (1H, s, H-8), 7.73 (1H, d, *J* = 5.2 Hz, H-4), 7.11 (1H, s, H-3), 6.36 (2H, s, H-12), 4.08 (3H, s, 10-OCH_3), 3.99 (3H, s, 9-OCH_3). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 181.23 (C-7), 153.87 (C-10), 151.55 (C-1), 149.53 (C-9), 147.02 (C-2), 145.48 (C-6a), 144.79 (C-5), 135.55 (C-3a), 127.75 (C-7a), 125.93 (C-11a), 123.96 (C-4), 122.64 (C-1b), 109.61 (C-11), 108.85 (C-8), 108.34 (C-1a), 102.76 (C-3), 102.37 (C-12), 56.27 (10-OCH_3), 56.14 (9-OCH_3) (Lit.[38]).

Allantoin (5)

White crystals. $C_4H_6N_4O_3$ (*m/z* 158), ^{1}H NMR (DMSO-d_6, 500 MHz): δ 10.54 (1H, s, NH-1), 8.05 (1H, s, NH-3), 6.88 (1H, d, *J* = 8.2 Hz, NH-6), 5.78 (2H, s, NH-8), 5.23 (1H, d, *J* = 8.2 Hz, H-4). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 173.63 (C-5), 157.39 (C-7), 156.80 (C-2), 62.44 (C-4) (Lit.[39]).

Goniothalamin (6)

White crystals. $C_{13}H_{12}O_2$ (*m/z* 200), ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.40 (1H, d, *J* = 7.4 Hz, H-11, 13), 7.34 (1H, t, *J* = 7.5 Hz, H-10, 14), 7.29 (1H, d, *J* = 7.2 Hz, H-12), 6.93 (1H, dt, *J* = 9.8, 4.3 Hz, H-4), 6.74 (1H, d, *J* = 16.0 Hz, H-8), 6.28 (1H, dd, *J* = 16.0, 6.4 Hz, H-7), 6.10 (1H, dt, *J* = 9.8, 1.7 Hz, H-3), 5.11 (1H, dd, *J* = 14.2, 6.6 Hz, H-6), 2.55 (2H, m, H-5). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 163.79 (C-2), 144.44 (C-4), 135.81 (C-9), 133.17 (C-8), 128.69 (C-11, 13), 128.36 (C-12), 126.71 (C-10, 14), 125.70 (C-7), 121.77 (C-3), 77.91 (C-6), 29.92 (C-5) (Lit.[40]).

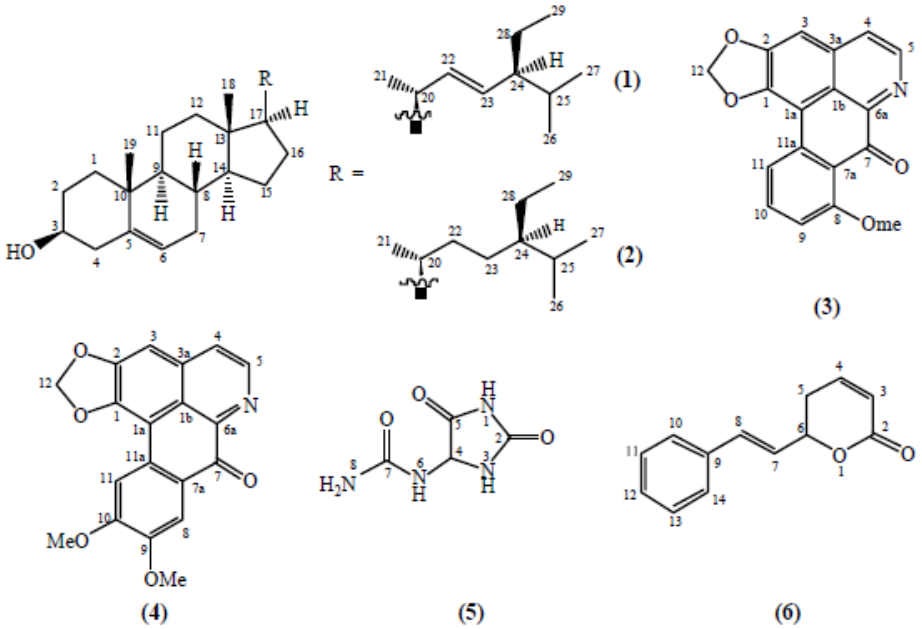


Fig. 1: Chemical structure of the isolated compounds from *P. evecta* var. *attopeuensis*

To the best of our knowledge, this is the first report of oxostephanine **(3)**, dicentrinone **(4)**, allantoin **(5)** and goniothalamin **(6)** from *P. evecta*. In addition, this is the first isolation of dicentrinone **(4)** and goniothalamin **(6)** from *Polyalthia* genus. Consequently, these compounds perhaps serve as potential chemotaxonomic markers for *P. evecta* var. *attopeuensis* and may be used to discriminate among varieties of *P. evecta*.

ACKNOWLEDGEMENT

The authors thank the Center for Innovation in Chemistry (PERCH-CIC), Commission on Higher Education, Ministry of Education and the grant of Thailand's Office

of Higher Education Commission for the Project on Higher Education and Research Promotion (HERP) 002/2014 and the Graduate School, Chiang Mai University for their generous financial support. In addition, we thank Department of Chemistry, Faculty of Science, Chiang Mai University for facilities supporting this research.

REFERENCES

1. N. A. Ghani, N. Ahmat, N. H. Ismail, I. Zakaria and N. K. N. A. Zawawi, Res. J. Med. Plant, **6**, 74-82 (2012).
2. A. Cave, H. Guinaudeau, M. Leboeuf, A. Ramahatra and J. Razafindrazaka, Planta Med., **33**, 243-250 (1978).
3. H. Guinaudeau, A. Ramahatra, M. Leboeuf and A. Cave, Plant Med. Phytother., **12**, 166-168 (1978).
4. M. H. Abu Zarga and M. Shamma, J. Nat. Prod., **45**, 471-475 (1982).
5. J. D. Connolly, M. E. Haque and A. A. Kadir, Phytochemistry, **43**, 295-297 (1996).
6. S. Kanokmedhakul, K. Kanokmedhakul, D. Yodbuddee and N. Phonkerd, J. Nat. Prod., **66**, 616-619 (2003).
7. S. Kanokmedhakul, K. Kanokmedhakul and R. Lekphrom, J. Nat. Prod., **70**, 1536-1538 (2007).
8. M. Lavault, H. Guinaudeau, J. Bruneton, T. Sevenet and H. A. Hadi, Phytochemistry, **29**, 3845-3847 (1990).
9. C. M. Hasan, T. M. Healey, P. G. Waterman and C. H. Schwalbe, J. Chem. Soc. Perkin Trans. I, 2807-2812 (1982).
10. R. Hocquemiller, G. Dubois, N. Kunesch, M. Leboeuf and A. Cave, Tetrahedron Lett., **26**, 4937-4940 (1985).
11. A. Jossang, M. Leboeuf, P. Cabalion and A. Cave, Planta Med., **49**, 20-24 (1983).
12. T. R. Seetharaman, Fitoterapia, **57**, 198-199 (1986).
13. Y. C. Wu, C. Y. Duh, S. K. Wang, K. S. Chen and T. H. Yang, **53**, 1327-1331 (1990).
14. H. Y. Li, N. J. Sun, Y. Kashiwada, L. Sun, J. V. Snider, L. M. Cosentino and K. H. Lee, J. Nat. Prod., **56**, 1130-1133 (1993).
15. A. Kijjoa, M. M. M. Pinto, P. M. M. Pinho, B. Tantisewie and W. Herz, Phytochemistry, **29**, 653-655 (1990).

16. C. M. Hasan, M. A. Hossain, M. A. Rashid and J. D. Connolly, Fitoterapia, **65**, 283-284 (1994).

17. A. Islam, A. Sayeed, G. Sadik, M. M. Rahman and G. R. M. A. M. Khan, The Sciences, **1**, 320-323 (2001).

18. S. Faizi, R. A. Khan, N. R. Mughal, M. S. Malik, K. S. Sajjadi and A. Ahmad, Phytother. Res., **22**, 907-912 (2008).

19. S. Kanokmedhakul, K. Kanokmedhakul, I. Kantikeaw and N. Phonkerd, J. Nat. Prod., **69**, 68-72 (2006).

20. X. Zheng, R. Yang, R. Xu and G. Qin, Zhiwa Xuebao, **36**, 557-560 (1994).

21. M. C. Gonzales, A. Serrano, M. C. Zafra-Polo, D. Cortes and K. S. J. Rao, J. Nat. Prod., **58**, 1278-1284 (1995).

22. M. C. Gonzalez, M. A. Sentandreu, K. S. Rao, M. C. Zafra-Polo and D. Cortes, Phytochemistry, **43**, 1361-1364 (1996).

23. S. Kanokmedhakul, K. Kanokmedhakul, I. I. Ohtani and M. Isobe, Phytochemistry, **47**, 131-133 (1998).

24. P. Tuchinda, B. Munyoo, M. Pohmakotr, P. Thinapong, S. Sophasan, T. Santisuk and V. Reutrakul, J. Nat. Prod., **69**, 1728-1733 (2006).

25. S. Machana, N. Weerapreeyakul and S. Barusrux, Asian Pac. J. Trop. Biomed., 368-374 (2012).

26. M. P. Padmaa and R. L. Khosa, J. Pharm. Res., **2**, 594-605 (2009).

27. X. Ma, I. S. Lee, H. B. Chai, K. Zaw, N. R. Farnsworth, D. D. Soejarto, G. A. Cordell, J. M. Pezzuto and A. D. Kinghorn, Phytochemistry, **37**, 1659-1662 (1994).

28. C. Y. Chen, F. R. Chang, Y. C. Shih, T. J. Hsieh, Y. C. Chia, H. Y. Tseng, H. C. Chen, S. J. Chen, M. C. Hsu and Y. C. Wu, J. Nat. Prod., **63**, 1475-1478 (2000).

29. M. A. Rashid, K. R. Gustafson, J. H. Cardellina and M. R. Boyd, Nat. Prod. Lett., **15**, 21 (2001).

30. S. Faizi, R. A. Khan, S. Azher, S. A. Khan, S. Tauseef and A. Ahmad, Planta Med., **69**, 350-355 (2003).

31. M. M. Murthy, M. Subramanyam, M. H. Bindu and J. Annapurna, Fitoterapia, **76**, 336-339 (2005).

32. P. Tuchinda, M. Pohmakotr, V. Reutrakul, W. Thanyachareon, S. Sophasan, C. Yoosook, T. Santisuk and J. M. Pezzuto, Planta Med., **67**, 572-575 (2001).

33. K. Young, H. Jayasuriya, J. G. Ondeyka, K. Herath, C. Zhang, S. Kodali, A. Galgoci, R. Painter, V. Brown-Driver, R. Yamamoto, L. L. Silver, Y. Zheng, J. I. Ventura, J. Sigmund, S. Ha, A. Basilio, F. Vicente, J. R. Tormo, F. Pelaez, P. Youngman, D. Cully, J. F. Barrett, D. Schmatz, S. B. Singh and J. Wang, Antimicrob. Agents Chemother., **50**, 519-526 (2006).

34. P. Prayong, S. Barusrux and N. Weerapreeyakul, Fitoterapia, **79**, 598-601 (2008).

35. S. Machana, N. Weerapreeyakul, S. Barusrux, K. Thumanu and W. Tanthanuch, Asian Pac. J. Trop. Biomed., 589-596 (2012).

36. W. De-Eknamkul and B. Potduang, Phytochemistry, **62**, 389-398 (2003).

37. T. T. Thuy, T. V. Sung, K. Franke and L. Wessjohann, J. Chem., **44**, 110-114 (2006).

38. S. Hongthong, C. Kuhakarn, V. Reutrakul, S. Jariyawat, P. Piyachaturawat, N. Nuntasaen and T. Jaipetch, Nat. Prod. Commun., **9**, 929-932 (2014).

39. S. Sang, A. Lao, H. Wang, Z. Chen, J. Uzawa and Y. Fujimoto, Phytochemistry, **48**, 569-571 (1998).

40. C. Wattanapiromsakul, B. Wangsintaweekul, P. Sangprapan, A. Itharat and N. Keawpradub, Songklanakarin J. Sci. Technol., **27**, 479-487 (2005).

41. S. Saepou, M. Pohmakotr, V. Reutrakul, C. Yoosook, J. Kasisit, C. Napaswad and P. Tuchinda, Planta Med., **76**, 721-725 (2010).

42. T. S. Wang, Y. P. Luo, J. Wang, M. X. He, M. G. Zhong, Y. Li and X. P. Song, Nat. Prod. Commun., **8**, 1427-1429 (2013).

Revised : 04.08.2015 *Accepted : 05.08.2015*

Int. J. Chem. Sci.: 13(3), 2015, 1149-1156
ISSN 0972-768X
www.sadgurupublications.com

STRUCTURE AND CRYSTALLOGRAPHIC CHARACTERIZATION OF POLYOXYGENATED CYCLOHEXANE DERIVATIVE FROM *ACER CHIANGDAOENSE* SUNTISUK

SIRINAPA NANTAPAP[a], SAKSRI SANYACHARERNKUL[a], SAMROENG NARAKAEW[b], KALLAYAS SANGRUENG[c], WITTAWAT PHONJAROEN[b], SUNISA YAYONG[b], NARONG NUNTASAEN[d], PUTTINAN MEEPOWPAN[a] and WILART POMPIMON[b,*]

[a]Department of Chemistry and Center for Innovation in Chemistry, Faculty of Science, Chiang Mai University, 53000 CHIANG MAI, THAILAND
[b]Laboratory of Natural Products, Center for Innovation in Chemistry, Faculty of Science, Lampang Rajabhat University, 52100 LAMPANG, THAILAND
[c]Department of Chemistry, Faculty of Science and Technology, Phranakhon Rajabhat University, 10220 BANGKOK, THAILAND
[d]The Forest Herbarium, Department of National Park, Wildlife and Plant Conservation, Ministry of Natural Resources and Environment, 10900 BANGKOK, THAILAND

ABSTRACT

A polyoxygenated cyclohexane derivative, (-)-quebrachitol [(1*R*, 3*R*, 4*S*, 6*S*)-2-methoxy cyclohexane-1,3,4,5,6-pentol] **(1)** was isolated from the stems of *Acer chiangdaoense*. Additionally, this is the first report of phytochemical from this plant. The structure was recognised by spectral methods, principally 2D NMR spectroscopic techniques, which complicated pooled applications of COSY, HMQC and HMBC. The relative configurations of the molecular structure of **1** were similarly confirmed by single crystal X-ray diffraction.

Key words: *Acer chiangdaoense*, Sapindaceae, Crystallographic, Polyoxygenated cyclohexane.

INTRODUCTION

Acer, the genus which belongs to the Sapindaceae family, which consists of more than 200 species wildly distributed in the temperate zones of the northern hemisphere,

*Author for correspondence; E-mail: wilart_p@hotmail.com, pmeepowpan@gmail.com

including Asia, North America and Europe[1]. In Thailand, there are six species of Acer genus including *A. oblongum* Wall. ex DC., *A. calcaratum* Gagnep., *A. laurinum* Hassk., *A. chiangdaoense* Santisuk, *A. thomsonii* Miq. and *A. pseudowilsonii* Y. S. Chen[2,3].

A. chiangdaoense is confined to the open habitats along the edges of lower montane rain forests at an altitude of about 1300-2200 m. This includes the deep shade of lower montane rain forest in Doi Chiangdao in Chiangdao District, Chiang Mai, and Doi Tung in Mae Fa Luang District, Chiang Rai, Thailand[4].

Previous phytochemical investigations of the Acer genus have reported on the isolation of phenolic glycosides such as gallotannins from *A. rubrum*[5-7], acertannin from *A. saccharum*[8], salidroside from *A. tegmentosum*[9], and another phenolic compounds such as cathechins from *A. Rubrum* and *A. nikoense*[10,11], cyanidins from *A. platanoides* and *A. rubrum*[10,12], chalcone from *A. rubrum*[10], tyrosol from *A. tegmentosum*[9]. In addition, triterpenes have been also isolated from *A. mandshuricum*[13]. Here, we report the first isolation and identification of polyoxygenated cyclohexane derivative from *A. chiangdaoense*. The compound **1** was comprehensively elucidated by NMR and X-rays crystallographic techniques.

EXPERIMENTAL

General experiment procedure

The IR spectra in KBr disk were recorded on a Shimadzu 8900 FTIR spectrophotometer. ^{1}H and ^{13}C NMR, ^{1}H-^{1}H COSY, HMQC and HMBC spectra were recorded with a Unity *plus* 500 spectrometer (Varian Inc., USA) operating at 500 MHz for ^{1}H, and 125 MHz for ^{13}C-NMR, respectively. Melting points were recorded in degree Celsius (°C) and were measured on a B-540 melting point apparatus (Büchi, Flawil, Switzerland). Low resolution mass spectra were recorded on a Thermo Finnegan Polaris Q mass spectrometer at 70 eV (probe) for EIMS. The X-ray data set was collected on a Bruker SMART APEX II diffractometer, using the Mo-Kα radiation, at 100 (2) K. Column chromatography was conducted on silica gel 60 (70-230 mesh, Merck KGaA, Darmstadt, Germany). TLC was performed on aluminium backed pre-coated silica gel 60 PF_{254} sheets and detection carried out with UV detector.

Plant material

The stems of *A. chiangdaoense* were collected at an altitude of about 1350 m in Doi Tung in Mae Fa Luang District, Chiang Rai, Thailand, and identified by Mr. Narong Nantasean. A voucher specimen (BKF 150554) has been deposited at The Forest Herbarium,

Department of National Park, Wildlife and Plant Conservation, Ministry of Natural Resources and Environment, Bangkok, Thailand.

Extraction and isolation

The air dried powdered stems of *A. chiangdaoense* (3.8 Kg) were successively percolated with hexane (10 L × 3 days × 4 times) and then extracted with ethyl acetate (10 L × 3 days × 4 times) and methanol (10 L × 3 days × 4 times) at room temperature, respectively and followed by filtration. The filtrates were combined and evaporated to dryness under reduced pressure to afford hexane, ethyl acetate and methanol extracts were 7.04, 21.56, and 229.5 g, respectively.

The methanol extract was separated by column chromatography, over of silica gel (Merck Art 7734, 700 g) with gradient systems of ethyl acetate-hexane, followed by the increasing amount of methanol in ethyl acetate and finally with methanol. Fractions (1000 mL each) were collected and combined on the basis of TLC behavior. The solvents were evaporated to dryness to give 7 fractions (F1-F7). Evaporation of F6 eluted with methanol : ethyl acetate (1:9 to 2:8) gave a colorless solid (450 mg) and it was repeatedly recrystallized from water (H_2O) to afford (-)-quebrachitol (**1**) (300 mg).

X-ray crystallographic analysis

Molecular formula $C_7H_{14}O_6$, Mr = 194.18, monoclinic, $P2_1$, a = 6.674 (2), b = 7.187 (2), c = 8.720 (3) Å, β = 90.226 (10)°, V = 418.3 (2) Å^3, Z = 2, Dc = 1.542 Mg/m^3, μ = 0.136 mm^{-1}, T = 100 (2) K. One thousand eight hundred and thirty one reflections (1819 independent, R_{int} = 0.0174) were collected in θ range from 2.34 to 25.14°. Largest electron density residue: 0.146 e.Å^{-3}, R_1 (for $I > 2\sigma(I)$) = 0.0320 and wR_2 = 0.0895 (all data) with $R_1 = \Sigma||F_o| - |F_c||/\Sigma|F_o|$ and $wR_2 = \Sigma w(F_o^2 - F_c^2)^2/\Sigma w(F_o^2)^2)^{0.5}$. All the data for this structure were collected at 100 (2) K on a Bruker SMART APEX II diffractometer equipped with a graphite-monochromator Mo $K\alpha$ radiation (λ = 0.71073 Å). The structure was solved by direct methods using *SHELXS-97*[14] and all non-hydrogen atoms were refined anisotropically using the least-squares method on F^2 using *SHELXL-2013*[15]. All the H atoms in this compound was calculated geometrically with isotropic displacement parameters set to 1.2 (1.5 for hydroxyl and methyl groups) times the equivalent isotropic U values of the parent carbon atoms. Crystal data and refinement were listed in Table 1. The molecular graph was developed using *ORTEP*[16]. The CIF format crystallographic data of compound **1** (CCDC No. 1036774) is available free of charge *via* www. ccdc. cam. ac. uk/services/structure deposit/(or from Cambridge crystallographic data centre, 12, Union Road, Cambridge, CB2 1EZ, UK; fax: + 44 1223 336033).

RESULTS AND DISCUSSION

Phytochemical investigation of the methanol extracted from the stems of *A. chiangdaoense* led to the isolation of the compound, (-)-quebrachitol (**1**), which was obtained as colorless crystals, exhibited a molecular formular of $C_7H_{14}O_6$, m.p. 191.0-191.2°C and specific rotation $[a]_{589}^{25}$ – 33.38° (*c* 0.5, H_2O). The EIMS showed an ion peak $[M]^+$ at *m/z* 194. The IR spectrum showed the bands corresponding to hydroxyl groups at 3377 cm^{-1} and C-O stretching and O-H deformation of methoxyl and hydroxyl groups at 1138, 1101, 1051, 1013 cm^{-1}, respectively. The ^{1}H-NMR spectrum showed (Table 2) six oxymethine protons at δ_H 4.16 (*dd*, 3.6, 3.6; H-1), 3.95 (*dd*, 3.7, 3.7; H-6), 3.63 (*dd*, 9.6, 3.5; H-5), 3.51 (*m*; H-4), 3.48 (*m*; H-3), 3.29 (*dd*, 9.6, 3.5; H-2) and one methoxyl group at δ_H 3.34 (*s*; 2-OCH_3). The relationship between the dihedral angle and vicinal coupling constant (^3J) is given theoretically by the Karplus equation (1).

$$^3Jab = J^0\cos^2 f - 0.28\ (0° < f < 90°) \text{ and } ^3Jab = J^{180}\cos^2 f - 0.28\ (90° < f < 180°) \quad ...(1)$$

So, the relative configuration at H-1 and H-2, H-1 and H-6, H-5 and H-6 could be determined by the ^3J H-C-C-H (3.5-3.7 Hz) coupling constant which indicated the two protons were located on the same side with dihedral angle 60°. In addition, proton H-2 and H-3, H-3 and H-4, H-4 and H-5 could be purposed coupling constant by the ^3J H-C-C-H (9.6 Hz), which exhibited the two protons were located on the opposite side with dihedral angle 180°[17]. The ^{13}C-NMR and DEPT spectrums displayed seven carbon signals, which six carbon signals were assigned to oxygenated CH groups at δ_C 80.20 (C-2), 72.88 (C-3), 71.96 (C-4), 71.40 (C-6), 70.40 (C-5), 67.20 (C-1) and one OCH_3 group at δ_C 56.94 (2-OCH_3). The ^{1}H-^{1}H COSY spectrum suggested connectivities of proton H-1 to H-2 and H-6; H-2 to H-1 and H-3; H-3 to H-2 and H-4; H-4 to H-3 and H-5; H-5 to H-4 and H-6; H-6 to H-1 and H-5. In addition, the assignments of protons were supported by the HMBC correlations from H-1 to C-2, C-4, C-5, C-6; H-2 to C-1, C-3, C-4, 2-OCH_3; H-3 to C-2, C-4, C-5; H-4 to C-2, C-3, C-5; H-5 to C-3, C-4; H-6 to C-1, C-2, C-3, C-5 and proton of methoxyl group by correlation with C-2. The ^{1}H, ^{13}C and 2D-NMR spectra were compared to the previously reported for (-)-quebrachitol[18]. However, the structure was confirmed by the present evidence of the single crystal X-ray (Fig. 1).

Compound 1 presented one crystallographically independent molecule in the asymmetric unit as shown in Fig. 1. The cyclohexane ring adopted in the chair conformation has an average torsion angle of 56.48 (4)° and has ring puckering parameters (Ω, θ, φ) of 0.5799 Å, 2.60° and 123.38°, respectively[19]. The O atoms at C-1 and C-6 were in the expected axial position with an average torsion angle of 62.32 (3)° and the other four O

atoms were in equatorial positions with an average torsion angle of closed to 180° on the ring, due to the strength and directions of intermolecular hydrogen bond interactions.

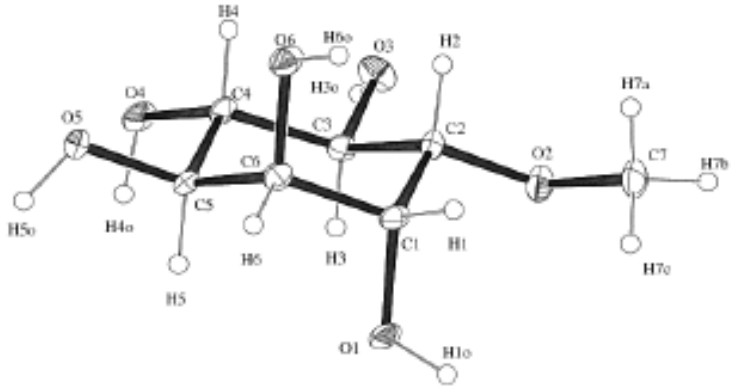


Fig. 1: ORTEP view of the asymmetric unit of 1 with the atom-labelling scheme, showing 25% probability displacement ellipsoids

The molecule has the four chiral centers at C(1,*R*), C(3,*R*), C(4,*S*), C(6,*S*) with an absolute structure parameter of –0.3 (17). The bond distances and angles are in normal range. The crystal structure is stabilized by the intermolecular hydrogen bond interactions. The molecule and adjacent molecules are held together to form one dimensional chains *via* strong O(1)–H(1o)···O(4)^{I} interaction; symmetric code (i) x, y+1, z along the *b* axis as illustrated in Fig. 2 and listed in Table 1.

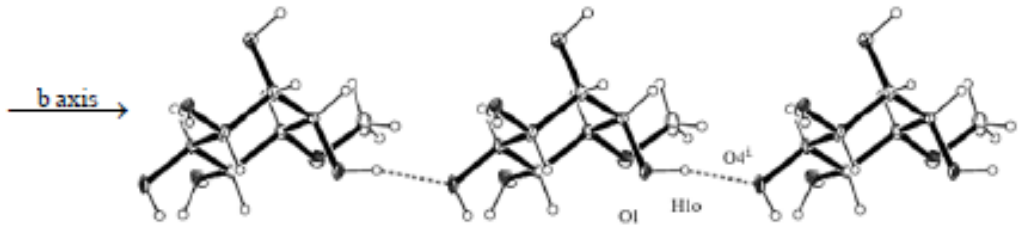


Fig. 2: ORTEP drawing of a chain *via* strong intermolecular O(1)–H(1o)···O(4)^{i} interactions along to [010]

The chains are linked together to generate two dimensional supramolecular layers *via* strong O (3, 4, 6)-H (3o, 4o, 6o)···O((5)^{ii}, (1)^{iv}, (2)^{v}) interactions of other hydroxyl groups and weak C(1)–H (1)···O (6)^{ii} interaction and the layered suparmolecular interactions

are held to produce the three dimensional supramolecular network *via* strong O(5)–H(5o)···O(3)^{iii} interactions as illustrated in Fig. 3 and listed in Table 1.

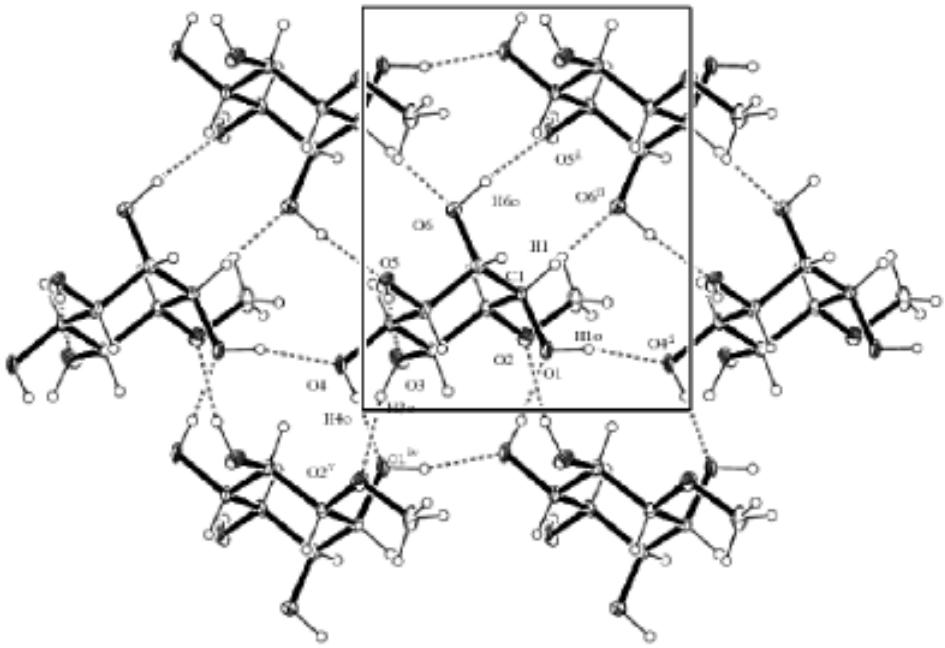


Fig. 3: ORTEP drawing of the three dimensional supramolecular network *via* strong intermolecular O–H···O and weak C–H···O interactions perpendicular to [100]

Table 1: The selected hydrogen bond interactions in 1

D–H···A	d[D–H] (Å)	d[H···A] (Å)	d[D···A] (Å)	∠[D–H···A] (°)
O(1) –H(1o)···O(4)^{i}	0.920(19)	1.80(2)	2.703(3)	166(4)
O(6) –H(6o) ···O(5)^{ii}	0.934(19)	1.83(2)	2.754(3)	168(4)
O(5) –H(5o) ···O(3)^{iii}	0.931(18)	1.85(2)	2.725(3)	155(4)
O(4) –H(4o) ···O(1)^{iv}	0.928(18)	1.83(2)	2.756(3)	172(4)
O(3) –H(3o) ···O(2)^{v}	0.92(2)	1.90(2)	2.791(4)	162(3)
C(1) –H(1) ···O(6)^{ii}	1.00	2.55	3.236(4)	125

Symmetry codes: (i) x,y+1,z; (ii) -x+2,y+1/2,-z+1; (iii) x+1,y,z; (iv) -x+2,y-1/2,-z; (v) -x+1,y-1/2,-z

Table 2: ^{1}H-NMR (500 MHz), ^{13}C-NMR (125 MHz), ^{1}H-^{1}Hand ^{1}H-^{13}C correlations in D_2O data for the isolated compound 1

Position	δ^{13}C, ppm (DEPT)	δ^{1}H, ppm(*mult*, J Hz)	COSY	HMBC
1	67.20 (CH)	4.16 (*dd*, 3.6, 3.6)	H-2, H-6	C-2, C-4, C-5, C-6
2	80.20 (CH)	3.29 (*dd*, 9.6, 3.5)	H-1, H-3	C-1, C-3, C-4, 2-OCH_3
3	72.88 (CH)	3.48 (*m*)	H-2, H-4	C-2, C-4, C-5
4	71.96 (CH)	3.51 (*m*)	H-3, H-5	C-2, C-3, C-5
5	70.40 (CH)	3.63 (*dd*, 9.6, 3.5)	H-4, H-6	C-3, C-4
6	71.40 (CH)	3.95 (*dd*, 3.7, 3.7)	H-1, H-5	C-1, C-2, C-3, C-5
2-OCH_3	56.94 (CH_3)	3.34 (*s*)	-	C-2, C-3

Note: δ in ppm from TMS [coupling constants (*J*) in Hz are given in parentheses]

(-)-quebrachitol (1)

MP: 191.0-191.2°C.

$[\alpha]_D$: - 33.38° (*c* 0.5, H_2O).

IR (KBr): 3377, 2941, 2928, 1138, 1101, 1051, 1013 cm^{-1}.

EIMS (EI, 70 eV): *m/z* (%) = 194 [M^+] (5), 180 (5), 179 (4), 164 (9), 162 (9), 126 (100), 109 (51), 92 (46), 75 (11).

Supplementary data

NMR spectra (^{1}H-NMR (500 MHz, D_2O), ^{13}C-NMR (125 MHz, D_2O), DEPT, COSY and HMBC) for compound 1 are also available.

ACKNOWLEDGMENT

The authors thank the Center for Innovation in Chemistry (PERCH-CIC), Commission on Higher Education, Ministry of Education and the grant of Thailand's Office of Higher Education Commission for the Project on Higher Education and Research Promotion (HERP) 002/2014 and the Graduate school, Chiang Mai University for their generous financial support. In addition, we thank Department of Chemistry, Faculty of Science, Chiang Mai University for facilities supporting this research.

REFERENCES

1. K. Ogata, Bulletin of the Tokyo University Forests, **60**, 1-100 (1967).
2. Y. S. Chen, Blumea, **55**, 242-245 (2010).
3. T. Santisuk, Nord. J. Bot., **12**, 695-698 (1992).
4. T. Santisuk, Nat. Hist. Bull. Siam Soc., **46**, 93-104 (1998).
5. A. González-Sarrías, T. Yuan and N. P. Seeram, Food Chem. Toxicol., **50**, 1369-1376 (2012).
6. C. Wan, T. Yuan, L. Li, V. Kandhi, N. B. Cech, M. Xie and N. P. Seeram, Bioorg. Med. Chem. Lett., **22**, 597-600 (2012).
7. T. Yuan, C. Wan, K. Liu and N. P. Seeram, Tetrahedron, **68**, 959-964 (2012).
8. A. Honma, T. Koyama and K. Yazawa, Food Chem., **123**, 390-394 (2010).
9. Y. H. Hwang, H. Park and J. Y. Ma, J. Ethnopharmacol., **148**, 99-105 (2013).
10. C. Wan, T. Yuan, M. Xie and N. P. Seeram, Biochem. Syst. Ecol., **44**, 1-3 (2012).
11. T. Yonezawa, J. W. Lee, H. Akazawa, M. Inagaki, B. Y. Cha, K. Nagai, K. Yagasaki, T. Akihisa and J. T. Woo, Bioorg. Med. Chem. Lett., **21**, 3248-3251 (2011).
12. T. Fossen and Ø. M. Andersen, Phytochemistry, **52**, 1697-1700 (1999).
13. Y. Ding, C. Liang, J. H. Kim, Y. M. Lee, J. H. Hyun, H. K. Kang, J. A. Kim, B. S. Min and Y. H. Kim, Bioorg. Med. Chem. Lett., **20**, 1528-1531 (2010).
14. G. M. Sheldrick, Acta Crystallogr. A, **64**, 112-122 (2008).
15. G. M. Sheldrick, SHELXL2013. University of Göttingen, Germany (2013).
16. M. N. Burnett and C. K. Johnson, Oak Ridge National Laboratory: Oak Ridge, TN, USA (1996).
17. Y. H. Lan, C. Fang-Rong, Y. Jin-Hsien, Y. Yu-Liang and C. Yi-Ling, J. Nat. Prod., **66**, 487-490 (2003).
18. M. Díaz, A. González, I. Castro-Gamboa, D. Gonzalez and C. Rossini, Carbohyd. Res., **343**, 2699-2700 (2008).
19. D. Cremer, J. A. Pople, J. Am. Chem. Soc., **97**, 1354-1358 (1975).

Revised : 17.05.2015 *Accepted : 19.05.2015*

Appl Biol Chem
DOI 10.1007/s13765-016-0178-3

Online ISSN 2468-0842
Print ISSN 2468-0834

ARTICLE

Antifungal of modified neolignans from *Mitrephora wangii* Hu

Saksri Sanyacharernkul[1] · Sirinapa Nantapap[1] · Kallaya Sangrueng[2] · Narong Nuntasaen[3] · Wilart Pompimon[4] · Puttinan Meepowpan[1]

Received: 24 August 2015 / Accepted: 28 January 2016

Abstract Chromatographic separation of leaves and twigs from *M. wangii* had led to the isolation of conocarpan (**1**) and 3′-methoxyconocarpan (**2**). These structures were assigned on the basis of spectroscopic methods. Compound **1** was modified by organic reactions and the modified compound **5** exhibited potent antifungal, *Pyricularia oryzae*. The method used poisoned food technique with mycelial growth inhibition for 14 days, 57 % (100 ppm). Compound 5 was stronger than mancozeb which the fungi toxicity drug used as a positive control.

Keywords Annonaceae · Conocarpan · *Mitrephora wangii* · Neolignans · *Pyricularia oryzae*

✉ Wilart Pompimon
wilart_p@hotmail.com

✉ Puttinan Meepowpan
pmeepowpan@gmail.com

[1] Department of Chemistry and Center for Innovation in Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand

[2] Department of Chemistry, Faculty of Science and Technology, Phranakhon Rajabhat University, Bangkok 10220, Thailand

[3] The Forest Herbarium, Department of National Park, Wildlife and Plant Conservation, Ministry of Natural Resources and Environment, Bangkok 10900, Thailand

[4] Laboratory of Natural Products, Department of Chemistry, Faculty of Science, Center for Innovation in Chemistry, Lampang Rajabhat University, Lampang 52100, Thailand

Introduction

The genus Mitrephora belongs to the Annonaceae family and comprises approximately 48 species distributed in Asia (Deepralard et al. 2007; Rayanil et al. 2013) such as Philippines, Indonesia, Malaysia, Thailand, China, and Southern India. *M. wangii* is a small to medium size tree. It has a smooth bark, devoid of patterns. The oblong leaves are glossy from above, with the undersides covered by fine hairs. The flowers possess a creamy yellow shade. The leaves and twigs of *M. wangii* were collected from Chiang Mai province, Thailand, in 2012. BKF no. 99291. It was identified by Mr. Narong Nuntasaen, The Forest Herbarium, Department of National Park Wildlife and Plant Conservation, Ministry of Natural Resources and Environment. The previous phytochemical investigations of Mitrephora species discovered diterpenoids (Zgoda-Pols et al. 2002; Deepralard et al. 2007; Meng et al. 2007; Rayanil et al. 2013), polyacetylene carboxylic acids/esters (Zgoda-Pols et al. 2001; Li et al. 2009), lignans (Deepralard et al. 2007; Ge et al. 2008), sesquiterpenes (Deepralard et al. 2007; Ge et al. 2008), and alkaloids (Deepralard et al. 2007). Among these compounds, the diterpenoids and alkaloids showed significant antimicrobial, antimalarial, and cytotoxic activities (Zgoda-Pols et al. 2002; Li et al. 2005; Mueller et al. 2009). *M. wangii* has revealed the presence of several alkaloids, phenolic amide, steroids, neolignans, and allantoin. The neolignan derivative compounds, namely conocarpan (**1**) and 3′-methoxyconocarpan (**2**), exhibited significant cytotoxicities (Tanamatayara, 2011). However, there has been no reported either phytochemistry or biological activities of *M. wangii* prior to our work.

Published online: 15 February 2016

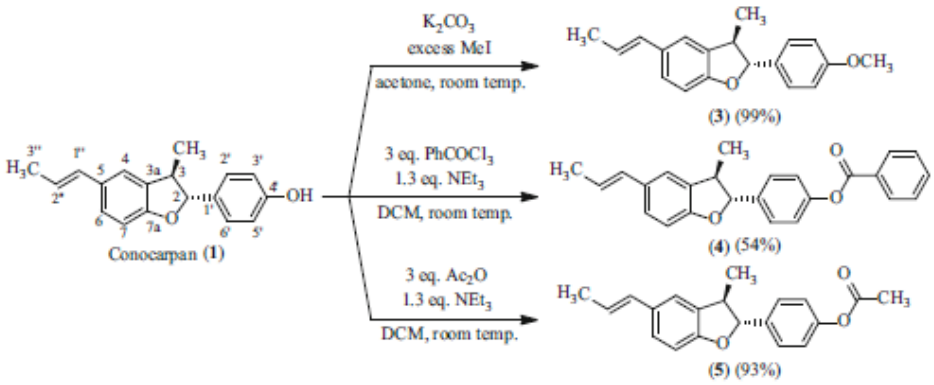


Fig. 1 Structural modification products of compounds **3–5**

Materials and methods

Extraction and isolation

Air-dried leaves and twigs of *M. wangii* (3 kg) (BKF no. 99291) were successively macerated with three organic solvents such as hexane (7 L × 5 time), ethyl acetate (7 L × 7 time), and methanol (6 L × 4 time), followed by filtration. The filtrates were evaporated to dryness under reduced pressure to afford 307.15, 296.83, and 190.00 g of crude extracts, respectively. The hexane extract was separated by silica gel column (No. 7734, 600 g), eluting with gradient hexane and ethyl acetate (100:0 to 0: 100). Fractions with similar pattern on TLC (10 % ethyl acetate in hexane) were combined to yield 5 fractions (HA_1-HA_5). The fraction HA_3 was rechromatographed (silica gel) followed by crystallization with EtOH to obtain a compound **1** viz. conocarpan (**1**) (0.4959 g). Ethyl acetate extract was isolated by column chromatography (CC) technique to give 8 fractions (EA_1-EA_8). The fraction EA_2 was rechromatographed 3 times to achieve compound **2**, to be precise 3′-methoxyconocarpan (**2**) (0.0042 g), and fraction EA_5 was also rechromatographed 3 times to afford compound **1** (0.9700 g). Identification of the isolated compounds was done by means of spectroscopic methods (^1H, ^{13}C NMR and 2D NMR, IR, EI-MS) and comparison with those reports.

Structural modification

Compound **3** (94.1 mg, 99 % yield) was prepared by methylation of conocarpan (**1**) (101.1 mg) which was dissolved in acetone (20 mL) and adding K_2CO_3 to the solution mixture. Then, the excess MeI was added to the reaction mixture and then stirred at room temperature for 24 h. The resulting mixture was filtrated, evaporated to dryness, and purified to methylation product by preparative thin-layer chromatography (EtOAc:hexane (2:8) as a developing solvent).

Compound **4** (77.6 mg, 54 % yield) was prepared by benzoylation of conocarpan (**1**) (99.9 mg) which was dissolved in CH_2Cl_2 and adding benzoyl chloride to the solution mixture. Then, Et_3N was added to the reaction mixture and then stirred at room temperature for 24 h. The resulting mixture was quenched with an aqueous saturated solution of $NaHCO_3$ (10 mL × 3 times) and extracted with CH_2Cl_2 (10 mL × 3 times). The combined organic layer was dried ($MgSO_4$), filtered, and concentrated in vacuo. Purification of the residue by preparative thin-layer chromatography (EtOAc:hexane (2:8) as a developing solvent) gave benzoylation product.

Compound **5** (113.7 mg, in 93 % yield) was obtained by acetylation of conocarpan (**1**) (104.5 mg) when anhydrous Ac_2O was employed in the benzoylation mentioned above.

Antifungal activity assay

The antifungal activity of pure and modified compounds was evaluated against *P. oryzae* separately by the poisoned food technique (Grover and Moore 1962). Treatment sets comprise the concentration at 100, 200, and 300 ppm. Each concentration was aseptically poured into the petri plate followed by the addition of melted PDA and was swirled gently to achieve thorough mixing of the contents. In control sets, requisite amounts of sterile water were added to the medium. After the solidification of the media, the mycelia of 10 mm diameter of fungus (taken from a 7-day-old culture) were put into the center of each plate. The treatment and control sets were incubated at 25 ± 2 °C for

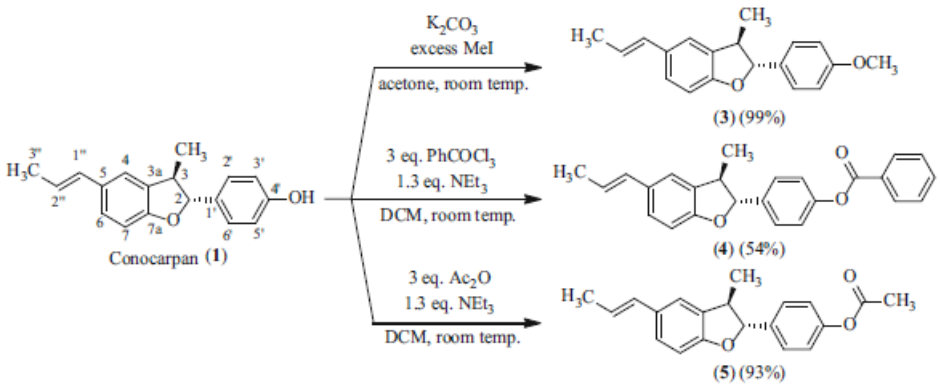


Fig. 1 Structural modification products of compounds **3–5**

Materials and methods

Extraction and isolation

Air-dried leaves and twigs of *M. wangii* (3 kg) (BKF no. 99291) were successively macerated with three organic solvents such as hexane (7 L × 5 time), ethyl acetate (7 L × 7 time), and methanol (6 L × 4 time), followed by filtration. The filtrates were evaporated to dryness under reduced pressure to afford 307.15, 296.83, and 190.00 g of crude extracts, respectively. The hexane extract was separated by silica gel column (No. 7734, 600 g), eluting with gradient hexane and ethyl acetate (100:0 to 0: 100). Fractions with similar pattern on TLC (10 % ethyl acetate in hexane) were combined to yield 5 fractions (HA_1-HA_5). The fraction HA_3 was rechromatographed (silica gel) followed by crystallization with EtOH to obtain a compound **1** viz. conocarpan (**1**) (0.4959 g). Ethyl acetate extract was isolated by column chromatography (CC) technique to give 8 fractions (EA_1-EA_8). The fraction EA_2 was rechromatographed 3 times to achieve compound **2**, to be precise 3′-methoxyconocarpan (**2**) (0.0042 g), and fraction EA_5 was also rechromatographed 3 times to afford compound **1** (0.9700 g). Identification of the isolated compounds was done by means of spectroscopic methods (^1H, ^{13}C NMR and 2D NMR, IR, EI-MS) and comparison with those reports.

Structural modification

Compound **3** (94.1 mg, 99 % yield) was prepared by methylation of conocarpan (**1**) (101.1 mg) which was dissolved in acetone (20 mL) and adding K_2CO_3 to the solution mixture. Then, the excess MeI was added to the reaction mixture and then stirred at room temperature for 24 h. The resulting mixture was filtrated, evaporated to dryness, and purified to methylation product by preparative thin-layer chromatography (EtOAc:hexane (2:8) as a developing solvent).

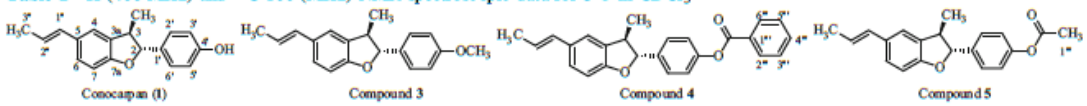
Compound **4** (77.6 mg, 54 % yield) was prepared by benzoylation of conocarpan (**1**) (99.9 mg) which was dissolved in CH_2Cl_2 and adding benzoyl chloride to the solution mixture. Then, Et_3N was added to the reaction mixture and then stirred at room temperature for 24 h. The resulting mixture was quenched with an aqueous saturated solution of $NaHCO_3$ (10 mL × 3 times) and extracted with CH_2Cl_2 (10 mL × 3 times). The combined organic layer was dried ($MgSO_4$), filtered, and concentrated in vacuo. Purification of the residue by preparative thin-layer chromatography (EtOAc:hexane (2:8) as a developing solvent) gave benzoylation product.

Compound **5** (113.7 mg, in 93 % yield) was obtained by acetylation of conocarpan (**1**) (104.5 mg) when anhydrous Ac_2O was employed in the benzoylation mentioned above.

Antifungal activity assay

The antifungal activity of pure and modified compounds was evaluated against *P. oryzae* separately by the poisoned food technique (Grover and Moore 1962). Treatment sets comprise the concentration at 100, 200, and 300 ppm. Each concentration was aseptically poured into the petri plate followed by the addition of melted PDA and was swirled gently to achieve thorough mixing of the contents. In control sets, requisite amounts of sterile water were added to the medium. After the solidification of the media, the mycelia of 10 mm diameter of fungus (taken from a 7-day-old culture) were put into the center of each plate. The treatment and control sets were incubated at 25 ± 2 °C for

Table 1 ^{1}H (400 MHz) and ^{13}C 100 (MHz) NMR spectroscopic data for **3–5** in $CDCl_3$



Conocarpan (**1**) Compound 3 Compound 4 Compound 5

Position	Conocarpan (**1**)		Compound (**3**)		Compound (**4**)		Compound (5)	
	$\delta^{13}C$ (DEPT)	$\delta^{1}H$ (*mult*, J Hz)	$\delta^{13}C$ (DEPT)	$\delta^{1}H$ (*mult*, J Hz)	$\delta^{13}C$ (DEPT)	$\delta^{1}H$ (*mult*, J Hz)	$\delta^{13}C$ (DEPT)	$\delta^{1}H$ (*mult*, J Hz)
1	–	–	–	–	–	–	–	–
2	92.70 (CH)	5.11 (1H, *d*, 8.9)	92.82(CH)	5.08 (1H, *d*, 8.9)	92.13 (CH)	5.17 (1H, *d*, 8.6)	92.07 (CH)	5.12 (1H, *d*, 8.6)
3	45.01 (CH)	3.43 (1H, *m*)	45.15(CH)	3.39 (1H, *m*)	45.57 (CH)	3.41 (1H, *m*)	45.49 (CH)	3.36 (1H, *m*)
3a	132.31 (C)	–	132.53 (C)	–	133.66 (C)	–	133.47 (C)	–
4	120.73 (CH)	7.17 (1H, *s*)	120.73 (CH)	7.12 (1H, *obsc.*)	120.81 (CH)	7.22 (1H, *obsc.*)	120.79 (CH)	7.09 (1H, *obsc.*)
5	131.34 (C)	–	131.25 (C)	–	131.44 (C)	–	131.42 (C)	–
6	126.26 (CH)	7.16 (1H, *d*, 8.2)	126.28 (CH)	7.12 (1H, *obsc.*)	126.37 (CH)	7.22 (1H, *obsc.*)	126.35 (CH)	7.09 (1H, *obsc*)
7	109.24 (CH)	6.81 (1H)	109.21 (CH)	6.76 (1H)	109.33 (CH)	6.79 (1H)	109.30 (CH)	6.76 (1H)
7a	157.96 (C)	–	159.73 (C)	–	158.25 (C)	–	158.21 (C)	–
8	17.68 (CH_3)	1.41 (3H, *d*, 6.8)	17.92(CH_3)	1.38 (3H, *d*, 6.8)	18.13 (CH_3)	1.43 (3H, *d*, 6.8)	18.10 (CH_3)	1.39 (3H, *d*, 6.8)
1′	132.43 (C)	–	132.53 (C)	–	132.09 (C)	–	132.09(C)	–
2′, 6′	127.86 (2CH)	7.29 (2H, *d*, 8.5)	127.60 (2CH)	7.34 (2H, *obsc.*)	127.14 (2CH)	7.49 (2H, *obsc.*)	127.14 (2CH)	7.40 (2H, *obsc.*)
3′, 5′	115.49 (2CH)	6.81 (2H, *d*, 6.6)	114.06 (2CH)	6.90 (2H, *obsc.*)	121.76 (2CH)	7.13 (2H, *obsc.*)	121.76 (2CH)	7.09 (2H, *obsc.*)
4′	155.66 (C)	–	158.35(C)	–	150.51 (C)	–	150.51 (C)	–
4′-O-Sub	- (OH)	5.85 (1H, *s*)	55.32 (C)	3.80 (3H, *s*)	165.15 (C)	–	169.48 (C)	–
1‴	–	–	–	–	138.52 (C)	–	21.16 (CH_3)	2.26 (3H, *s*)
2‴, 6‴	–	–	–	–	130.20 (CH)	8.20 (1H, *obsc.*)	–	–
3‴, 5‴	–	–	–	–	128.59 (CH)	7.49 (1H, *obsc.*)	–	–
4‴,	–	–	–	–	129.44 (CH)	7.62 (1H, *obsc.*)	–	–
1″	130.68 (CH)	6.41 (1H, *dd*, 15.7, 1.5)	130.79 (CH)	6.36 (1H, *dd*, 15.7, 1.6)	130.69 (CH)	6.36 (1H, *dd*, 15.7, 1.6)	130.67(CH)	6.35 (1H, *dd*, 15.7, 1.6)
2″	123.08 (CH)	6.13 (1H, *dq*, 15.7, 6.6)	124.56 (CH)	6.09 (1H, *m*)	123.17 (CH)	6.09 (1H, *m*)	123.17 (CH)	6.08 (1H, *m*)
3″	18.34 (CH_3)	1.90 (3H, *dd*, 6,6, 1.6)	18.88 (CH_3)	1.85 (3H, *dd*, 6.6, 1.6)	18.43 (CH_3)	1.85 (3H, *dd*, 6.6, 1.6)	18.43 (CH_3)	1.84 (3H, *dd*, 6.6, 1.6)

Sub substituent group, *obsc* obscure signal

Table 2 Mycelial growth inhibition (*P. oryzae*) of conocarpan (**1**) and modified compounds **3-5** at different concentrations in comparison with mancozeb for 7 and 14 periods

Compounds	Concentrations (ppm)	Percentage inhibition of mycelial growth	
		7 days	14 days
1	100	00.00 ± 0.00	00.00 ± 0.00
	200	3.95 ± 0.14	2.32 ± 0.06
	300	23.03 ± 0.09	23.84 ± 0.78
3	100	1.50 ± 0.14	6.30 ± 0.08
	200	8.95 ± 0.56	15.83 ± 0.60
	300	18.95 ± 0.44	30.79 ± 0.20
4	100	7.50 ± 0.14	13.50 ± 0.96
	200	11.00 ± 0.13	26.05 ± 0.52
	300	22.75 ± 0.55	37.69 ± 0.09
5	100	37.50 ± 0.37	57.75 ± 0.32
	200	45.41 ± 0.18	59.20 ± 0.21
	300	51.82 ± 0.13	67.54 ± 0.10
Mancozeb (positive control)	100	10.53 ± 0.25	18.54 ± 0.13
	200	53.95 ± 0.29	63.91 ± 0.90
	300	60.53 ± 0.01	81.46 ± 0.03

The values are means of three replicates ± standard error. All compounds were dissolved in 5 % acetone

7 and 14 days. Assessment of the fungitoxicity was carried out in terms of the percentage of mycelial growth inhibition (Dixit et al. 1978).

$$\text{Mycelial growth inhibition } (\%) = \frac{d_c - d_t}{d_c} \times 100,$$

where d_c is the average increase in mycelial growth in control set (mm) and d_t is the average increase in mycelial growth in treatment set (mm).

Results and discussion

Chemical structure elucidation

The present study reported the isolation of conocarpan (**1**) and 3′-methoxyconocarpan (**2**) from leaves and twigs of *M. wangii*. Compounds **1** and **2** have previously been isolated from *Krameria cytisoides* (Achenbach et al. 1987). Conocarpan (**1**) was shown to exhibit anticytotoxic (Rimando et al. 1994), antibacterial (Arnone et al. 1988), and antiyeast (Freixa et al. 2001) properties. Moreover, the conocarpan (**1**) was partially synthesized by methylation, benzoylation, and acetylation (Fig. 1).

Compound **3** was obtained as a white colorless solid. The molecular formula was valid as $C_{19}H_{20}O_2$ from the EI-MS ion at *m/z* 280.02, while the molecular weight of **1** was 266.34 *m/z*, suggesting that one methyl group was substituent on the molecule of precursor. Its spectroscopic data (^{1}H NMR and ^{13}CMNR) were closely related to those of **1** (Table 1). However, the ^{1}H NMR spectrum showed the methyl singlet at δ 3.80 (*s*, 3H) and ^{13}CNMR spectrum appeared at δ 55.32 ppm (CH_3), which was the chemical shift value of methoxy group. On the other hand, hydroxyl δ at 5.85 ppm disappeared after the methylation reaction occured. Compound **4** was produced from the benzoylation reaction of conocarpan, which was a yellow solid. The molecular formula was established as $C_{25}H_{22}O_3$ consistent with molecular ion peak at *m/z* 370.16 (EI-MS). The ^{1}H NMR and ^{13}C NMR spectra of **4** were closely related to those of conocarpa. However, the hydroxyl group was substituted by the benzoyl group, the which has the ^{1}H NMR spectrum appeared at δ 8.20 ppm (*obsc.*,1H), 7.49 ppm (*obsc.*, 1H), and 7.62 ppm (*obsc.*, 1H) and ^{13}C NMR spectrum appeared at δ 165.15 ppm (C=O), 138.52 ppm (C), 130.20 ppm (2CH), 128.59 ppm (2CH), and 129.44 ppm (CH). Compound **5** was obtained as a white colorless solid and its molecular formula was determined to be $C_{20}H_{20}O_3$ from its molecular ion peak at *m/z* 308.12 (EI-MS). In addition, similar to the compounds **3** and **4**, the compound **5** also has the ^{1}H NMR and ^{13}C NMR spectra closely related to those of conocarpan, including the hydroxyl substitution at 4′-O-sub position. ^{1}H NMR spectrum of the substituent appeared at δ 2.36 (*s*, 3H) and ^{13}C NMR spectrum appeared at δ 169.48 ppm (C=O) and 21.16 ppm (CH_3), which was the chemical shift value of acetyl group.

Conocarpan (**1**) and its modified compounds **3–5** were tested for rice blast disease. The modified compound **5**, one of the important agricultural fungicides against *P. oryzae*, is a fungal pathogen that causes rice blast disease (Tanaka et al. 2009).

Antifungal activity test

The neolignan, conocarpan (**1**), and its derivatives revealed several biological activities such as anti-inflammatory (Baumgartner et al. 2011a, b), antifungal (Pessini et al. 2005; Lemos et al. 2013), antinociceptive (Silva et al. 2010) and anti-tuberculosis activities (Scodro et al. 2013). Therefore, we modified structure **1** to the active compounds **3–5** for antifungal test. Compound **1** and modified compounds **3–5** induced inhibition of the mycelial growth of the test fungus *P. oryzae* in Table 1. Fungicide (mancozeb) was used as a positive control. Compound **5** showed highly significant fungitoxicity of about three-fold at 100 ppm for 14 days (57.75 %) in comparison with mancozeb (18.54 %), while other concentrations showed lower inhibitions than positive control. Alternatively, conocarpan (**1**) and compounds **3** and **4** showed less inhibitory potency compared with compound **5** and fungicide at the same concentrations (Table 2).

Acknowledgments The authors thank the Center for Innovation in Chemistry (PERCH-CIC) and Commission on Higher Education, Ministry of Education, and the grant of Thailand's Office of Higher Education Commission for the Project on Higher Education and Research Promotion (HERP) 002/2014 for generous financial support. In addition, we thank the Department of Chemistry, Faculty of Science, Chiang Mai University, for facilities supporting this research.

References

Achenbach H, Grob J, Dominguez XA, Cano G, Star JV, BrussoloLDC Munoz G, Salgado F, Lopez L (1987) Lignans neolignans and norneolignans from *Krameria cystisoides*. Phytochemistry 26:1159–1166

Arnone A, Modugno DV, Nasini G, Venturini I (1988) Isolation and structure determination of neiv active neolignans and norneolignans from Ratanhia. Gazz Chim Ital 118:675–682

Baumgartner L, Sosa S, Atanasov AG, Bodensieck A, Fakhrudin N, Bauer J, Favero GD, Ponti C, Heiss EH, Schwaiger S, Ladurner A, Widowitz U, Logoa RD, Rollinger JM, Werz O, Bauer R, Dirsch VM, Tubaro A, Stuppner H (2011a) Lignan derivatives from *Krameria lappacea* roots inhibit acute inflammation in vivo and pro-inflammatory mediators in *vitro*. J Nat Prod 74:1779–1786

Baumgartner L, Schwaiger S, Stuppner H (2011b) Quantitative analysis of anti-inflammatory lignan derivatives in *Ratanhiae radix* and its tincture by HPLC–PDA and HPLC–MS. J Pharmaceut Biomed 56:546–552

Deepralard K, Pengsuparp T, Moriyasu M, Kawanishi K, Suttisri R (2007) Chemical constituents of *Mitrephora maingayi*. Biochem Syst Ecol 35:696–699

Dixit SN, Tripathi NN, Tripathi SC (1978) Fungitoxicity of some seed extract. Natl Acad Sci Letters 1:287–288

Freixa B, Vila R, Ferro E, Adzet T, Cangueral S (2001) Antifungal principles from *Piper fulvens*. Planta Medica 67:873–875

Ge F, Tang CP, Ye Y (2008) Lignanamides and sesquiterpenoids from stems of *Mitrephora thorelii*. Helv Chim Acta 91:1023–1030

Grover RK, Moore JD (1962) Toximetric study of fungicides against brown rot organism *Sclerotinia fructicola* and S. laxa. Phytopathology 52:876–880

Lemos COT, Svidzinsk TIE, Baeza LC, Miranda N, Nakamura CV, Cortez DAG, Cardozo-Filho L, Cabral VF (2013) Characterization and anti-inflammatory effects of iodinated acetylenic acids isolated from the Marine Sponges Suberites mammilaris and Suberites japonicas. Nat Prod Res 27:2355–2359

Li C, Lee D, Graf TN, Phifer SS, Nakanishi Y, Burgess JP, Riswan S, Setyowati FM, Saribi AM, Soejarto DD, Farnsworth NR, Falkinham JO III, Kroll DJ, Kinghorn AD, Wani MC, Oberlies NH (2005) A hexacyclic ent-trachylobane diterpenoids possessing an oxetane ring from *Mitrephora glabra*. Org Lett 7:5709–5712

Li C, Lee D, Graf TN, Phifer SS, Nakanishi Y, RiswanS Setyowati FM, Saribi AM, Soejarto DD, Farnsworth NR, Falkinham JO III, Kroll DJ, Kinghorn AD, Wani MC, Oberlies NH (2009) Bioactive constituents of the stem bark of *Mitrephora glabra*. J Nat Prod 72:1949–1953

Meng DH, Xu YP, Chen WL, Zou J, Lou LG, Zhao WM (2007) Antitumor clerodane-type diterpenes from *Mitrephora thorelii*. J Asian Nat Prod Res 9:679–684

Mueller D, Davis RA, Duffy S, Avery VM, Camp D, Quinn RJ (2009) Antimalarial activity of azafluorenone Alkaloids from the Australian Tree *Mitrephora diversifolia*. J Nat Prod 72:1538–1540

Pessini GL, Dias-Filho BP, Nakamura CVN, Cortez DAG (2005) Antifungal activity of the extracts and neolignans from *Piper regnellii* (Miq.) C. DC. var. pallescens (C. DC.) Yunck. J Braz Chem Soc 16:1130–1133

Rayanil KO, Limpanawisut S, Tuntiwachwuttikul P (2013) Ent-pimarane and ent-trachylobane diterpenoids from *Mitrephora alba* and their cytotoxicity against three human cancer cell lines. Phytochemistry 89:125–130

Rimando A, Pezzuto J, Farnsworth N, Santisuk T, Reutrakul V (1994) Revision of the NMR assignments of pterostilbene and of dihydrodehydrodiconieferyl alcohol: cytotoxic constituents from *Anogeissus acuminata*. Nat Prod Lett 4:267–272

Scodro RBL, Pires CTA, Carrara VS, Lemos COT, Cardozo-Filho L, Souza VA, Corrêa AG, Siqueira VLD, Lonardoni MVC, Cardoso RF, Cortez DAG (2013) Anti-tuberculosis neolignans from *Piper regnellii*. Phytomedicine 20:600–604

Silva RZD, Yunes RA, Souza MMD, Monache FD, Cechinel-Filho V (2010) Antinociceptive properties of conocarpan and orientin obtained from *Piper solmsianum* C. DC. var. solmsianum (Piperaceae). J Nat Med 64:402–408

Tanaka M, Nakayashiki H, Tosa Y (2009) Population structure of eleusine isolates of *Pyricularia oryzae* and its evolutionary implications. J Gen Plant Pathol 75:173–180

Tanamatayara P (2011) Bioactive compounds from *Pterospermum grande* Craib and *Mitrephora wangii* Hu. Silpakorn University, Thailand

Zgoda-Pols JR, Freyer AJ, Killmer LB, Porter JR (2001) Polyacetylene carboxylic acids from *Mitrephora celebica*. J Nat Prod 64:1348–1349

Zgoda-Pols JR, Freyer AJ, Killmer LB, Porter JR (2002) Antimicrobial diterpenes from the stem bark of *Mitrephora celebica*. Fitoterapia 73:434–438

LIST OF PUBLICATIONS

Author's Name	Ms. Sirinapa Nantapap	
Date/Year of Birth	15 May 1984	
Place of Birth	Lampang Province, Thailand	
Education	2003-2006	B. S. (Food Science and Technology) Hons, Faculty of Agro Industry, Chiang Mai University
	2008-2010	M. Ed. (Science), Faculty of Science, Lampang Rajabhat University
Scholarship	-The Center of Excellence for Innovation in Chemistry (PERCH-CIC) -The Graduate School, Chiang Mai University -The Commission on Higher Education, Ministry of Education and the Grant of Thailand's Office of Higher Education Commission for the Project on Higher Education and Research Promotion (HERP) 002/2014	
Experience	The International Foundation for Science (IFS), Department of Chemistry, Mahidol University (November 2012-June 2013)