

REFERENCES

1. Deltino, R. T., Santos-Filho, O. A., Figneroa-Villar, J. D., Type 2 antifolates in the chemotherapy of falciparum malaria. *J Braz. Chem. Soc.*, 2002, 13, 724-741.
2. Phillipson, J.D. Phytochemistry and medicinal plants. *Phytochem*, 2001, 56, 237-243.
3. Meshnick, S. R., Taylor, T.E., and Kamchonwongpaisan, S. Artemisinin and the antimalarial endoperoxides : from herbal remedy to targeted chemotherapy. *Microbiological Review*, 1996, 60(2), 301-315.
4. Newton, P., Suputtamongkol, Y., Teja-Isavadharm, P., Pukrittayakamee, S., Navaratnam, V., Bates, I., and White, N. J. Antimalarial bioavailability and disposition of artesunate in acute falciparum malaria. *Antimicrobial Agents and Chemotherapy*, 2000, 971-977.
5. Looareesuwan, S., Walairatana, P., Vanijanonta, S., Pitisuttithum, P., Viravan, C. Treatment of acute, uncomplicated falciparum malaria with oral dihydroartemisinin. *Am Trop Med Parasitol*, 1996, 90(1), 21-28.
6. Warrell, D. A., Clinical features of malaria. In: Gilles H.M., Wareell, D.A. editors. *Essential malariology*. 3rd. UK: Butler and Tanner, 1993, 39-49.
7. Klayman, D. L. Qinghaosu (artemisinin) : an antimalarial drugs from China. *Science*, 1985, 228, 1049-1055.
8. De Vries, P. J., Dein, T. K. Clinical pharmacology and therapeutic potential of artemisinin and its derivatives in the treatment of malaria. *Drugs*, 1996, 52(6), 818-836.
9. Meshnick, S. R. Artemisinin : mechanisms of action, resistance and toxicity. *Int J for Parasitology*, 2002, 32, 1655-1660.
10. Titulaer, H. A. C., Zuidema, J., Lugt, C. B. Formulations and pharmacokinetics of artemisinin and its derivatives. *Int J Pharm*, 1991, 69, 83-92.

11. Haynes, R. K., Vonwiller, S.C. Extraction of artemisinin and artemisinic acid : preparation of artemether and new analogues, *Trans R Soc Trop Med Hyg*, 1994, 88(1 suppl), 23-26.
12. Valecha, N., Tripathi, K. D. Artemisinin : current status in malaria. *Indian J of Pharmacology*, 1997, 29, 71-75.
13. Idowu, O. R. Determination of arteether in blood plasma by high performance liquid chromatography with ultraviolet detection after hydrolysis with acid. *J Chromatogr*, 1989, 493, 125-136.
14. Dhingra, V., Rao, V., and Narasu M. L. Current status of artemisinin and its derivatives as antimalarial drugs. *Life science*, 2000, 66(4), 279-300.
15. Meshnick, S. R. The mode of action of antimalarial endoperoxides. *Trans R. Soc Trop Med Hyg*. 1994, 88(1 supp) : 31-32.
16. Meshnick, S. R., Jefford, C. W., Posner, G. H., Avery, M. A., and Peters, W. Second-generation antimalarial endoperoxides. *Parasitology*, 1996, 12(2), 79-82.
17. Van Agtmael, M. A., Eggelte, T. A., and van Boxtel, C. J. Artemisinin drugs in the treatment of malaria : from medicinal herb to registered medication. *Tips*, 1999, 20, 199-205.
18. Woerdenbag, H. J., Pras, N., van Uden, W., Wallaart, T. E., Beekman, A. C., Lugt, C. B. Progress in the research of artemisinin related antimalarial : and update. *Pharm World Sci*, 1994, 16(4), 169-180.
19. Maggs, J. L., Modden, S., Bishop, L P., O'Neill, P. M., and Park, B. K. The rat biliary metabolites of dihydroartemisinin, an antimalarial endoperoxide. *The American Society for Pharmacology and Experimental Therapeutics*, 1997, 25(10), 1200-1204.
20. Woerdenbag, H. J., Lugt, C. B., Pras, N. *Artemisinin annua* L. : a source of novel antimalarial drugs. *Pharm Weekbi Sci*, 1990, 12(5), 169-181.
21. Titulaer, H.A.C., Zuidema, J., Lugt, C.B. Formulation and pharmacokinetic of Artemisinin and its derivatives. *Int J Pharm*, 1991, 69,83-92.

22. Smith, S. L., Sadler, C. J., Dodd, C. C., Edwards, G., Ward, S. A., Park, B. K., McLean, W. G. The role of glutathione in the neurotoxicity of artemisinin derivatives *in vitro*. *Biochemical Pharmacology*, 2001, 61, 409-416.
23. Smith, S. L., Fishwick, J. Enhanced *in vitro* neurotoxicity of artemisinin derivatives in the presence of haemin. *Biochemical Pharmacology*, 1997, 53, 5-10.
24. Panisko, D. M., Keystone, J. S. Treatment of malaria 1990. *Drugs*, 1990, 39 (2), 160-189.
25. Singh, N. P., Lai, H. Selective toxicity of dihydroartemisinin and holotransferrin toward human breast cancer cells. *Life Science*, 2001, 70, 49-56.
26. Lai, H., Singh, N. Selective cancer cell cytotoxicity from exposure to dihydroartemisinin and holotransferrin. *Cancer Letters*, 1995, 91, 41-46.
27. Chen, H-H., Zhou, H-J., Fang, X. Inhibition of human cancer cell line growth and human umbilical vein endothelial cell angiogenesis by artemisinin derivatives *in vitro*. *Pharmacological Research*, 2003, 48, 231-236.
28. Li, Y., Wu, J-M., Shan, F., Wu, G-S., Ding, J., Xiao, D., Han, J-X., Atassi, G., Leonce, S., Caignard, D-H., and Renard, P. Synthesis and cytotoxicity of dihydroartemisinin ethers containing cyanoarylmethyl group. *Bioorganic & Medicinal Chemistry*. 2003, 11, 977-984.
29. Werdenbag, H. J., Moskai, T. A., Pras, N., Malingre, T. M. Cytotoxicity of artemisinin-related endoperoxides to ehrlich ascites tumor cells. *J of Natural Products*. 1993, 56(6), 849-856.
30. World Health Organization (WHO). Draft monographs for antimalarial substances and dosage forms. 1998.
31. Noedl, H., Wernsodorfer, W. H., Krudsood, S., Wilairatana, P., Kollaritsch, H., Wiedermann, G., Looareesuwan, S. Antimalarial activity of azithromycin, artemisinin and dihydroartemisinin in fresh isolates of *Plasmodium falciparum* in Thailand. *Acta Tropica*, 2001, 80, 39-44.

32. Ringwald, P., Bickii, J., and Basco, L. K. *In vitro* activity of dihydroartemisinin against clinical isolates of *Plasmodium falciparum* in Yaounde, Cameroon. *Am J Trop Med Hyg*, 1999, 61(2), 187-192.
33. Wilairatana, P., Chanthavanich, P., Singhasivanon, P., Treeprasertsuk, S., Krudsook, S., Chalermrut, K., Phisalaphong, C., Kraissintu, K., Looareesuwan, S. A comparison of three different dihydroartemisinin formulations for the treatment of acute uncomplicated falciparum malaria in Thailand. *Int J for Parasitology*, 1998, 28, 1213-1218.
34. Idowu, O. R., Maggs, J. L., Ward, S. A., Edwards, G. Decomposition reactions of arteether, a semisynthetic derivative of qinghaosu (artemisinin). *Tetrahedron*, 1990, 46(6), 1871-1884.
35. Acton, N., and Roth, R. J. Acid decomposition of the antimalarial beta-artether. *Heterocycles*, 1995, 41(1), 95-102.
36. Shang, x., He, C-H., Zheng, Q-T., Yang, J-J., and Liang, X-T. Chemical transformations of qinghaosu, a peroxidic antimalarial, II. *Heterocycles*, 1989, 28(1), 421-424.
37. Sy, L-K., Hui, S-M., Cheung, K-K., and Brown, G. D. A rearranged hydroperoxide from the reduction of artemisinin. *Tetrahedron*, 1997, 53(22), 7493-7500.
38. Baker, J. K., and Chi, H. T. Novel rearrangements of the trioxane ring system of the antimalarial arteether upon treatment with acid in an aqueous methanol solvent system. *Heterocycles*, 1994, 38(7), 1497-1506.
39. Haynes, R. K., Pai, H. H-O., and Voerste, A. Ring opening of artemisinin (qinghaosu) and dihydroartemisinin and interception of the open hydroperoxides with formation of N-oxides-a chemical model for antimalarial mode of action. *Tetrahedron Letters*, 1999, 40, 4715-4718.
40. Lin, A. J., Theoharides, A. D., Klynan, D. L. Thermal decomposition products of dihydroartemisinin(dihydroqinghaosu). *Tetrahedron*, 1986, 42(8) 2181-2184.

41. Chaipakdee, S. Formulation of dihydroartemisinin suppository. M. Sc's. Thesis, Department of Pharmaceutics, Faculty of Pharmacy. Mahidol University, 1999.
42. Sirichotbundit, U. Design and fabrication of fast-release dihydroartemisinin compressed tablets. M. Sc's. Thesis, Department of Pharmaceutics, Faculty of Pharmacy. Mahidol University, 1999.
43. Government Pharmaceutical Organization (GPO). Stability of study of dihydroartemisinin. 2003.
44. Aravind, K., Krishna, R. F., Douglas, R. F. Micellar solubilization of a new antimalarial drug, β -arteether. *J pharm Sci*, 1989, 78(7), 574-576.
45. Chiablaem, U. Department of *in vitro* dissolution test for dihydroartemisinin capsules. M. Sc's Thesis, Department of Pharmaceutical, Faculty of Pharmacy. Mahidol University, 2002.
46. The Animal Cell Biotechnology Laboratory, BIOTEC Central Research Unit, NSTDA. Cytotoxicity test report: corrected version. 2004
47. Plumb, J. A., Milroy, R., and Kaye, S. B. Effects of the pH dependence of 3-(4,5-dimethylthiazol-2, 5-diphenyl-tetrazolium bromide-formazan absorption on chemosensitivity determined by a novel tetrazolium-based assay. *Cancer Research*, 1989, 49, 4435-4440.
48. กรมวิทยาศาสตร์. Report of acute toxicity study of DHA impurities in mice. 2004
49. Weil, C. S. Tables for convenient calculation of median-effective dose (LD_{50} or ED_{50}) and instructions in their use. *Biometrics*, 1952, 8, 249-263.
50. The Armed Forces Research Institute of Medical Science. Report of antimalarial activity test of DHA and DHA impurities using [3H]-hypoxanthine microdilution method. 2004

51. Teja-Isavadharm, P., Peggins, J. O., Brewer, T. G., White, N. J., Webster, H. K., and Kyle, D. E. *Plasmodium falciparum*- based bioassay for measurement of artemisinin derivatives in plasma or serum. *Antimicrobial Agents and Chemotherapy*, 2004, 954-960.
52. Junior, C.C., Margues, C., Alencar, F.E.C., Durlaeher, R.R., Alween, A., Segurado, A. AC., Pang, L. W., Zalis, M. G. Antimalarial drug susceptibility testing of *Plasmodium falciparum* in Brazil using a radioisotope method. *Mem Inst Oswaldo Cruz*, Rio de Janeiro, 1999, 94(6), 803-809.
53. Melendez, V., Peggins, J. O., Brewer, T. G., Theoharides, A. D. Determination of the antimalarial arteether and its deethylated metabolite plasma by high-performance liquid Chromatography with reductive electrochemical detection. *Journal of Pharmaceutical Sciences*, 1991, 80, 132-138.
54. Chaisuwan, P. Determination of dihydroartemisinin by high performance liquid chromatography. M. Sc's. Thesis, Department of Chemistry, Faculty of Science. Mahidol University, 2003.
55. Batty, K.T., Davis, T.M.F., Thu, L.T.A., Binh, T.Q., Anh, T. K., Ilett, K.F. Selective high-performance liquid chromatographic determination of artesunate and α - and β -dihydroartemisinin in patients with falciparum malaria. *J. Chromatogr. B*. 1996; 677: 345-350.
56. กรมวิทยาศาสตร์. Report of acute toxicity study of dihydroartemisinin and artesunate in rat and mice. 2000.

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APPENDIX A

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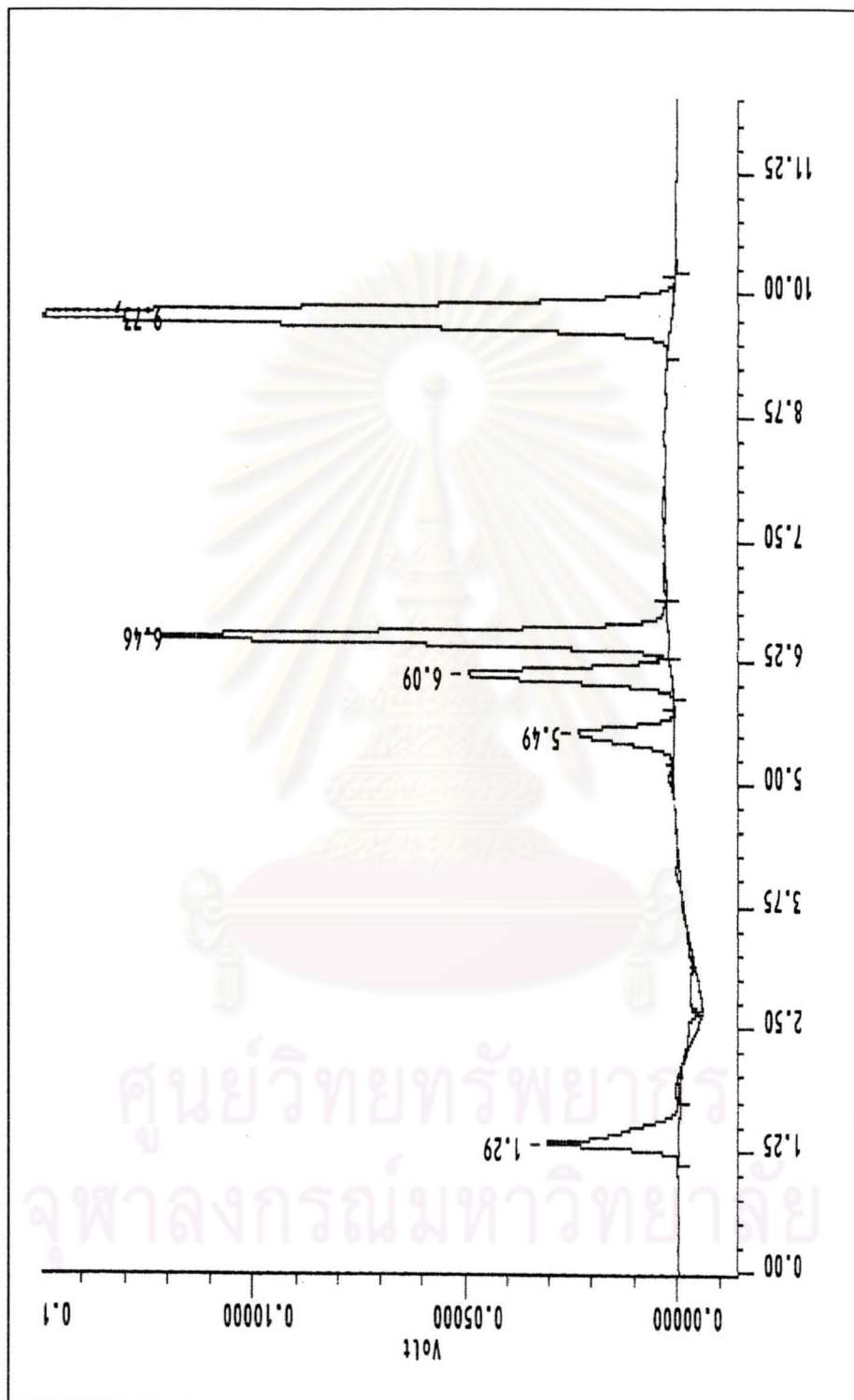


Figure 18 HPLC chromatogram of crude DHA

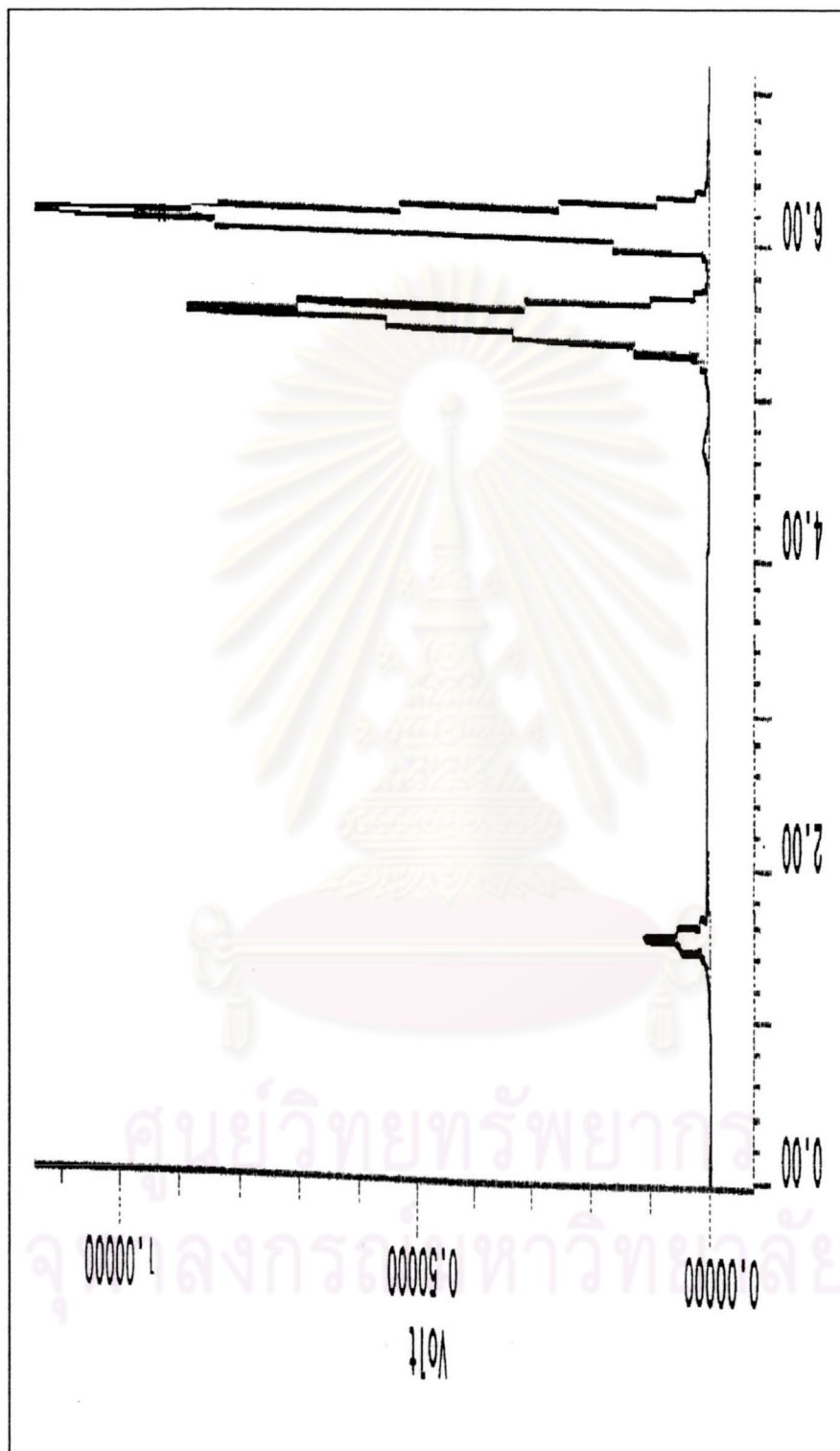


Figure 19 The HPLC chromatogram of sample for toxicity testing

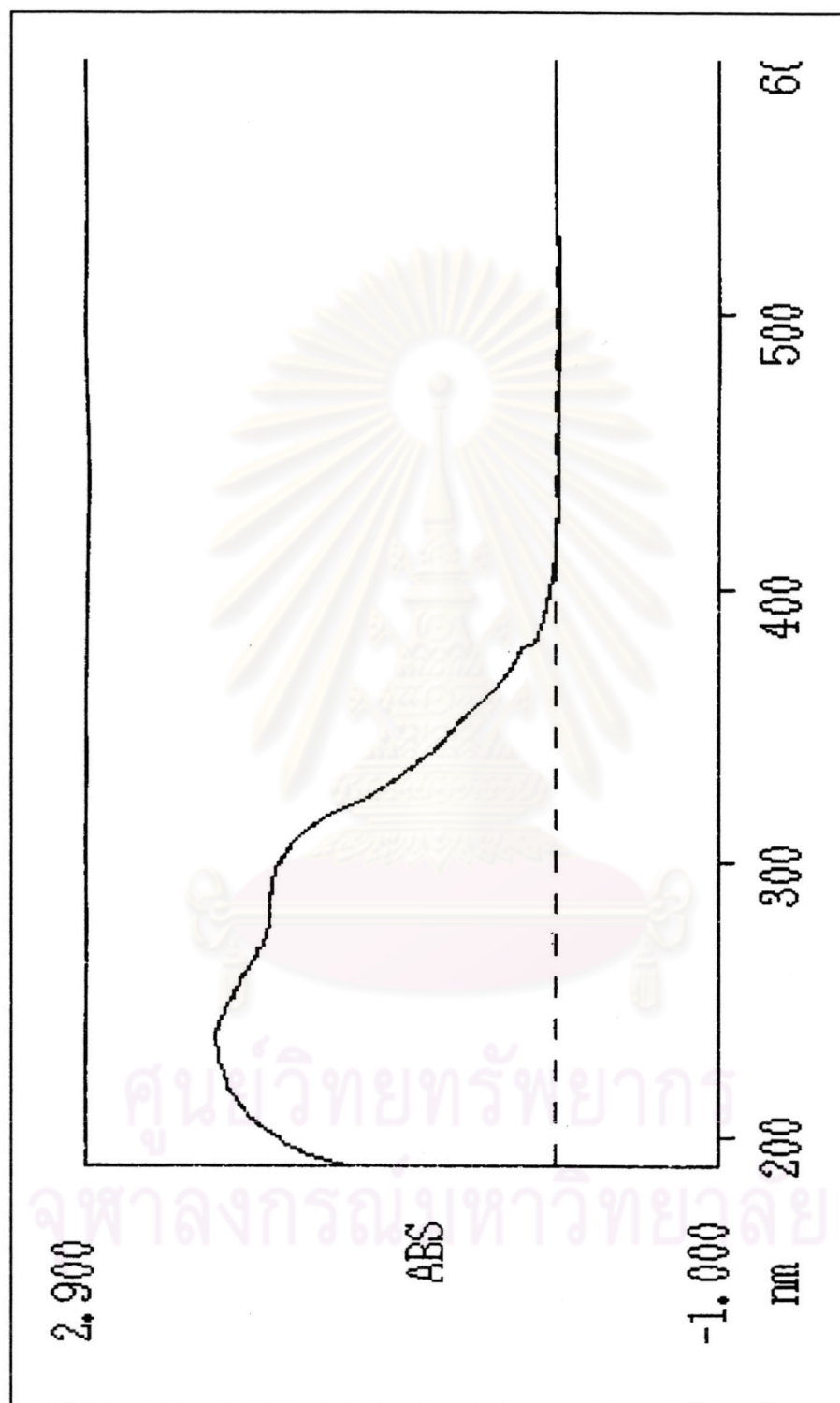


Figure 20 The UV spectrum of compound 1

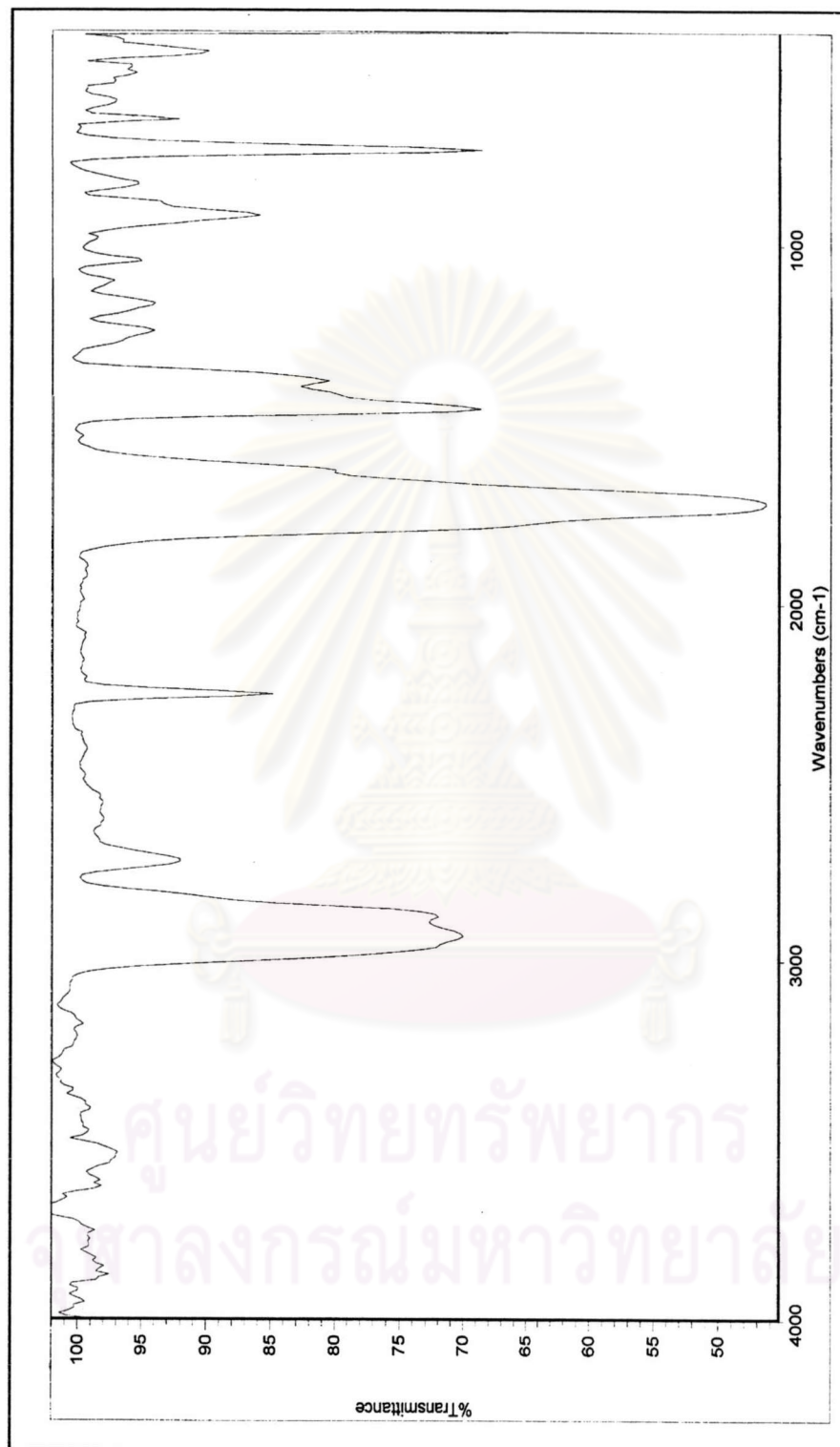


Figure 21 The IR spectrum of compound I

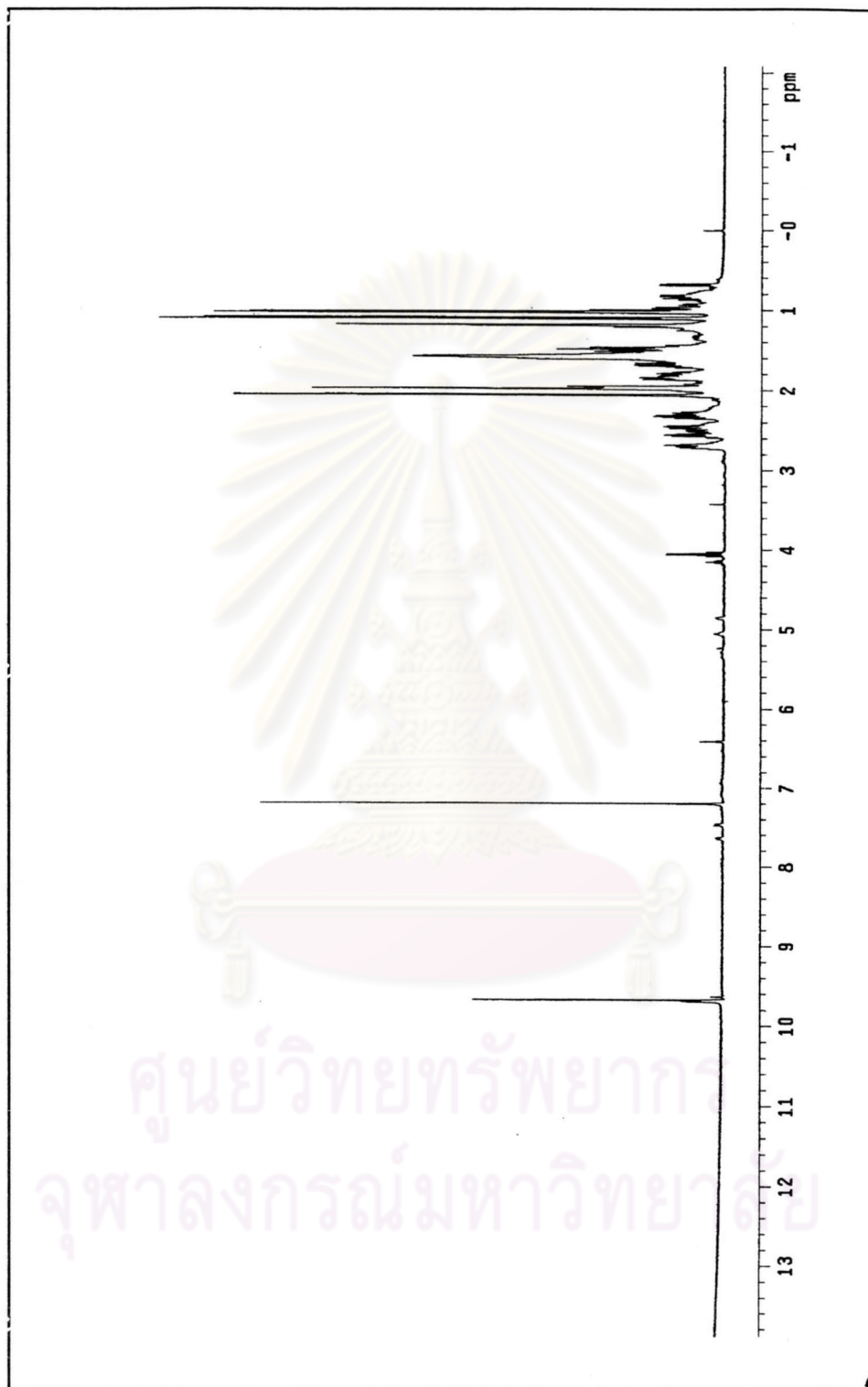


Figure 22 The $^1\text{H-NMR}$ spectrum of compound 1



Figure 23 The ^{13}C -NMR spectrum of compound 1

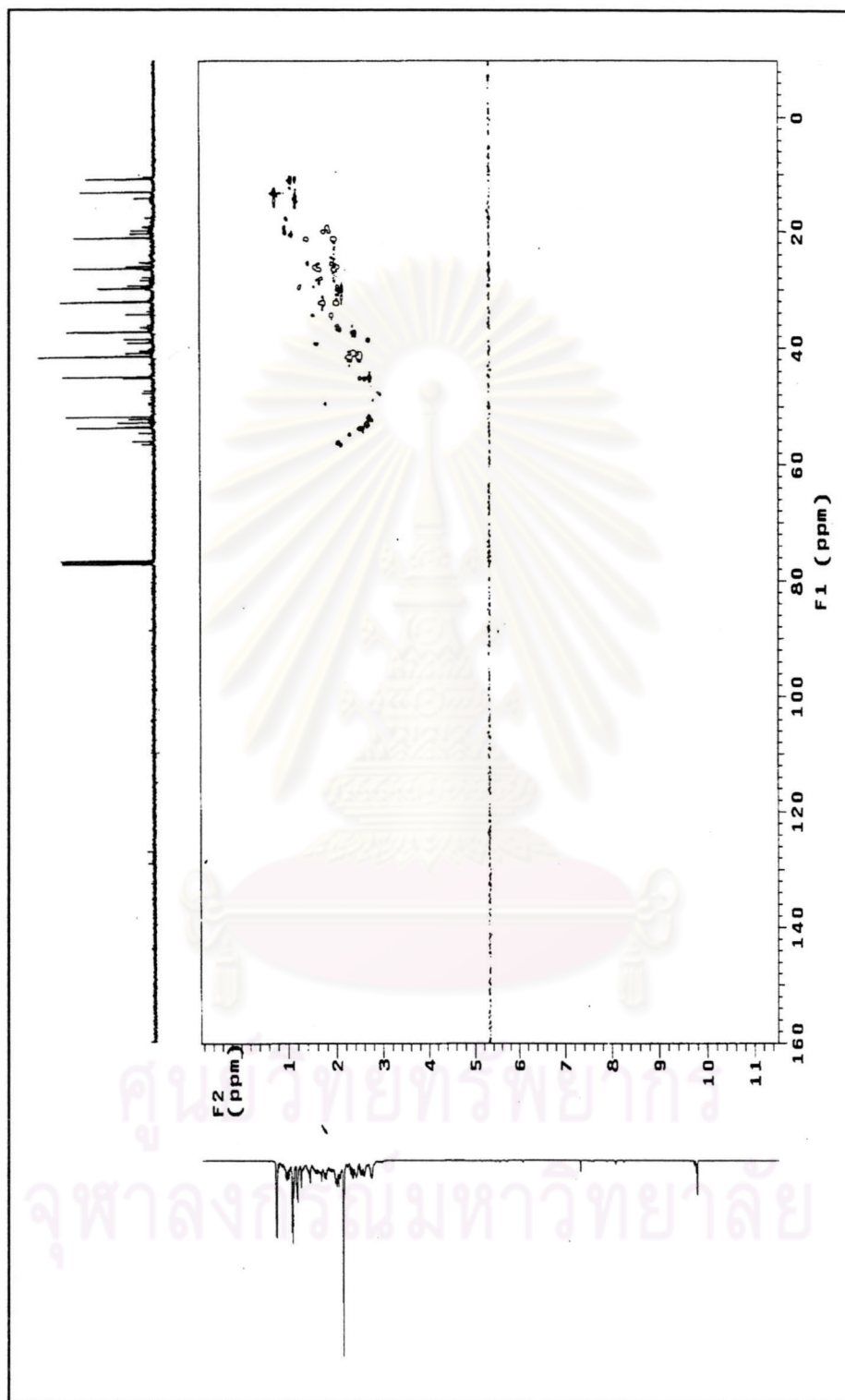


Figure 24 The HSQC-NMR spectrum of compound 1

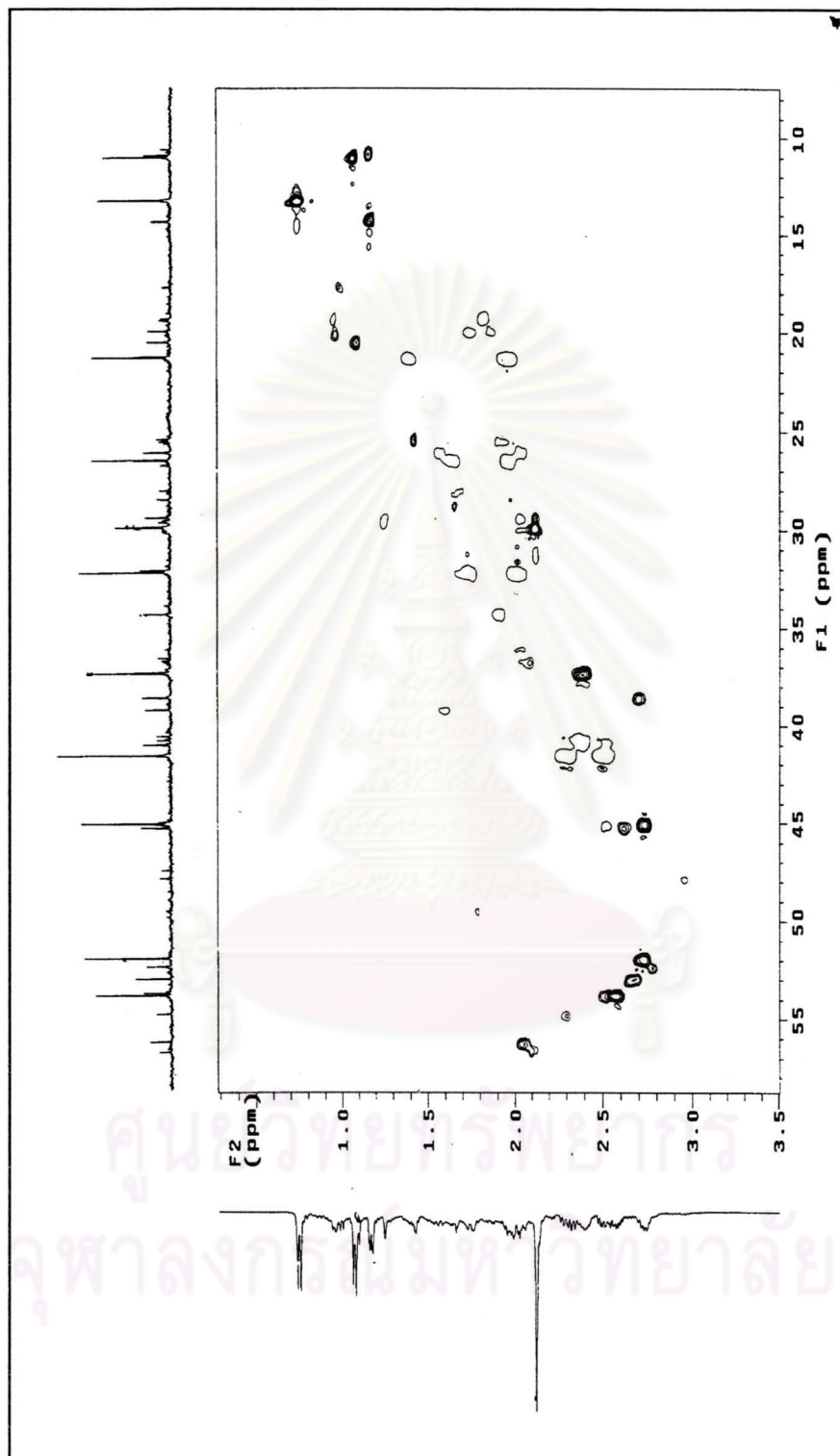


Figure 25 The HSQC-NMR spectrum of compound 1

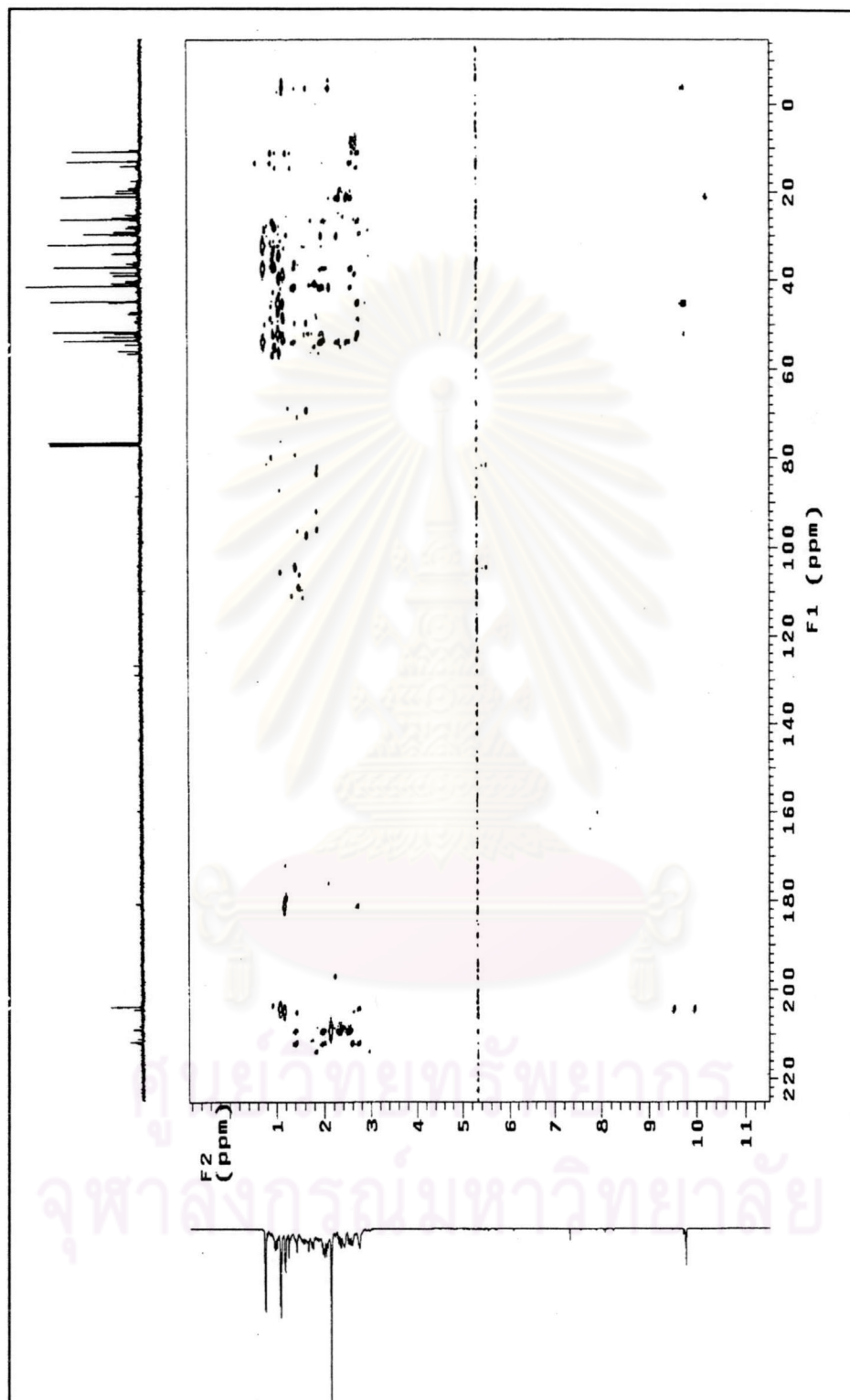


Figure 26 The HMBC-NMR spectrum of compound 1

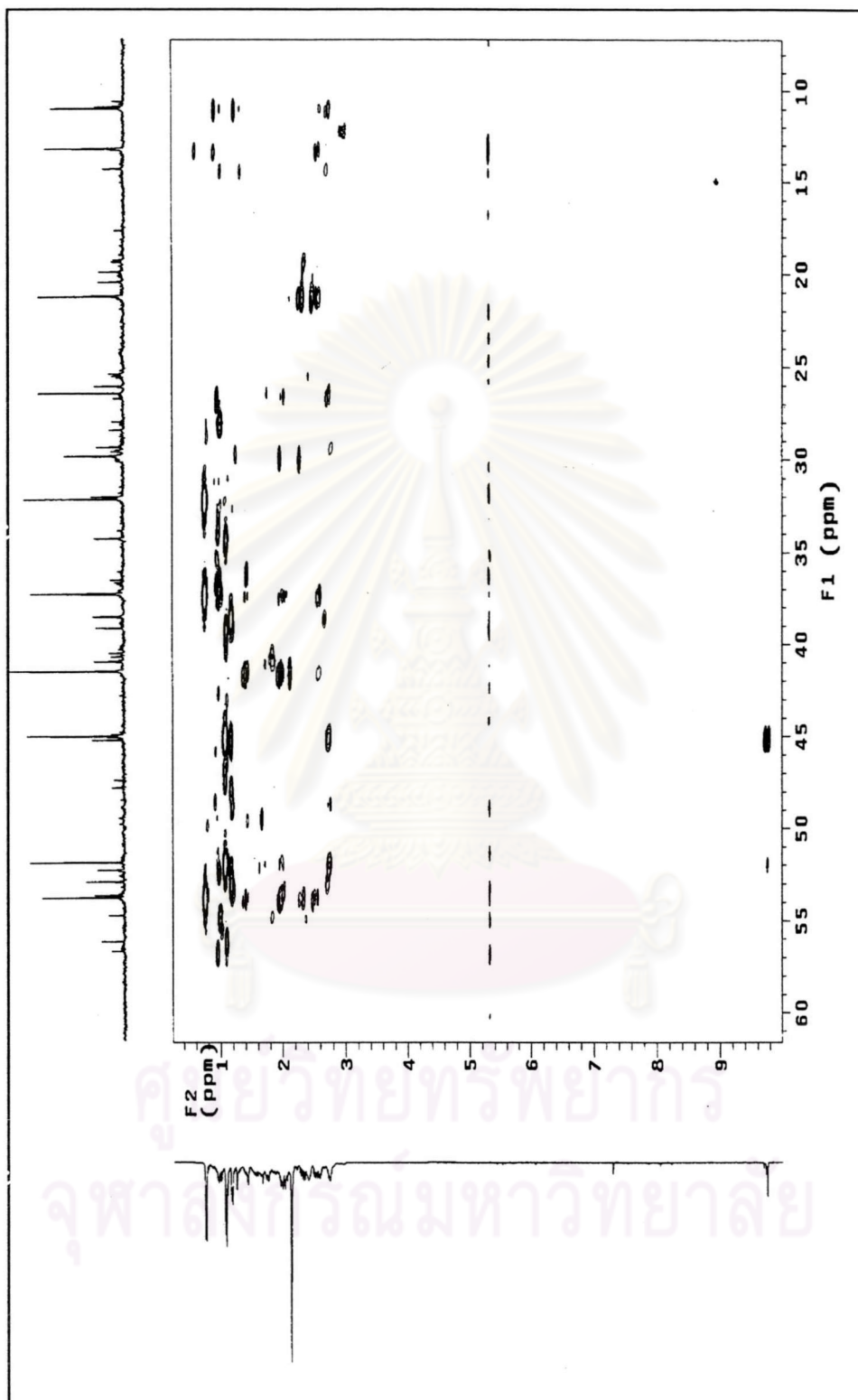


Figure 27 The HMBC-NMR spectrum of compound 1

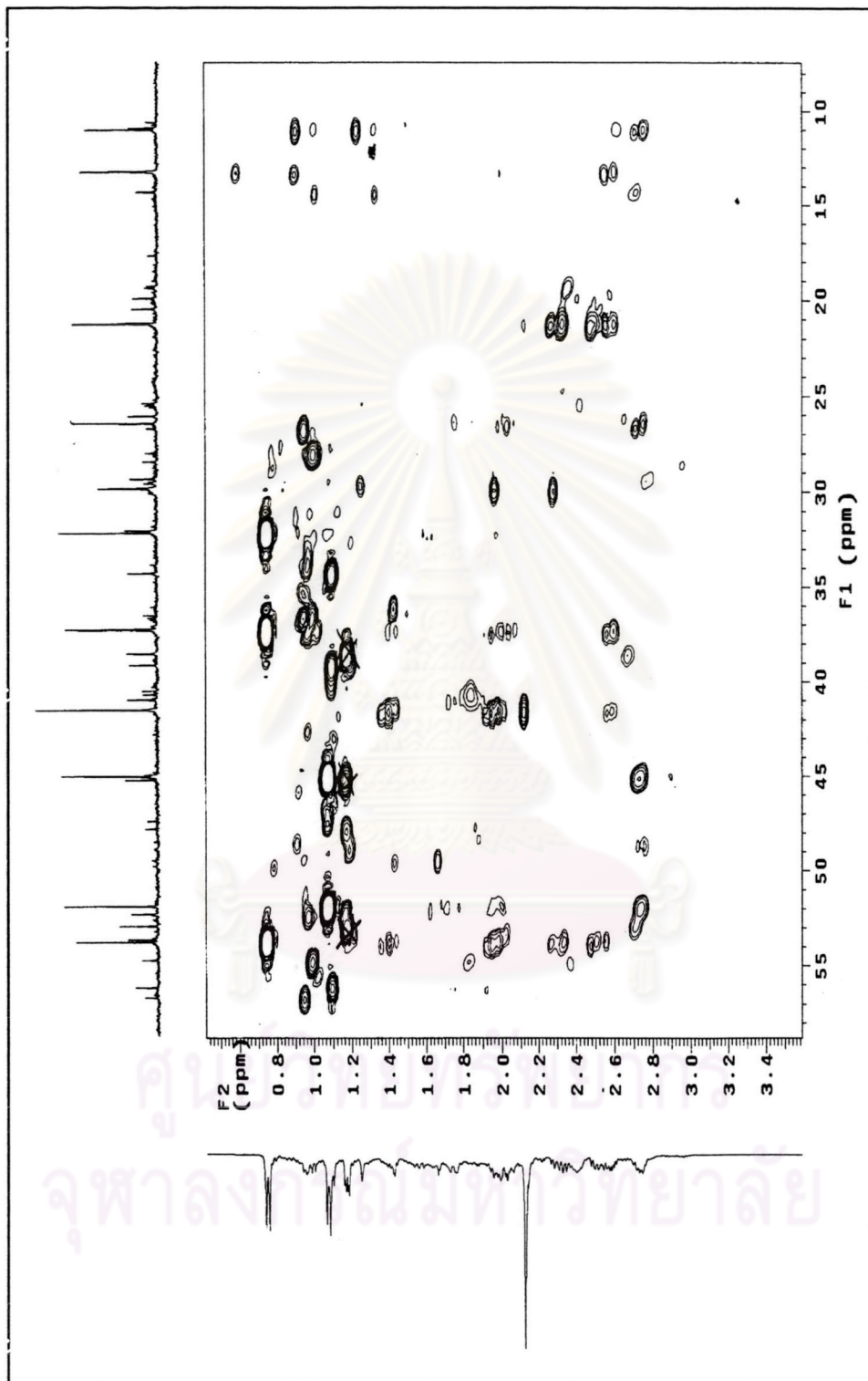


Figure 28 The HMBC-NMR spectrum of compound 1

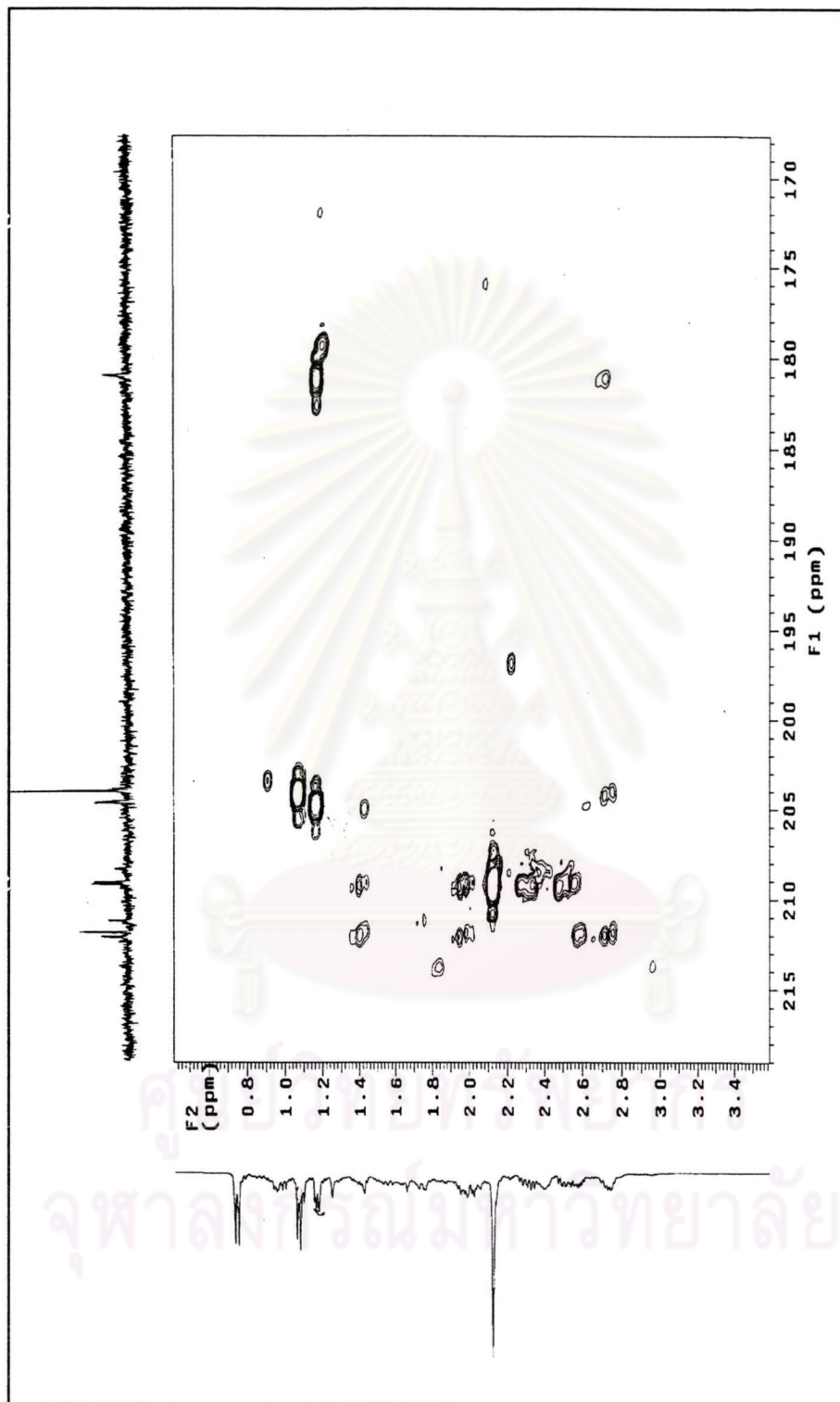


Figure 29 The HMBC-NMR spectrum of compound 1

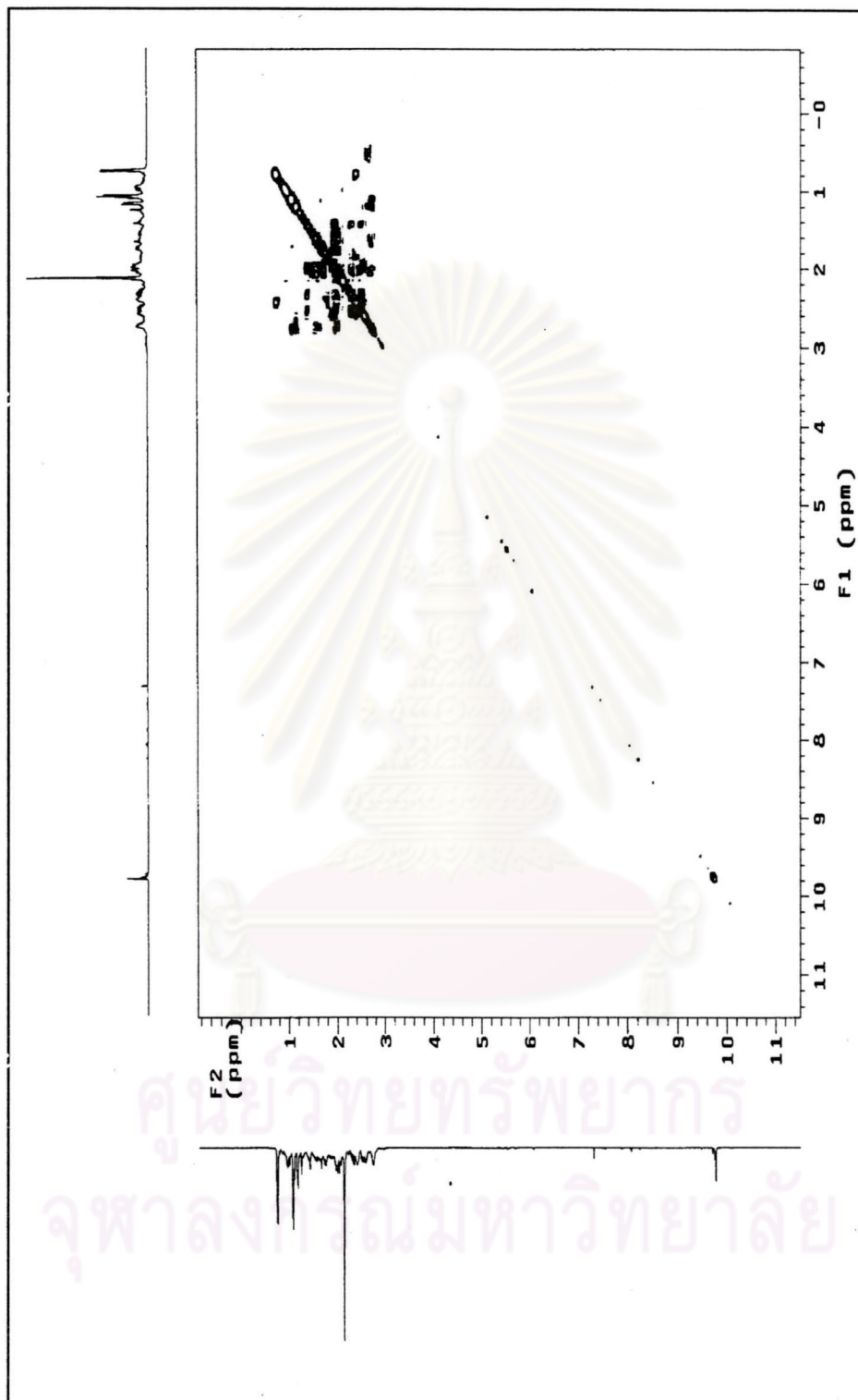


Figure 30 The COSY-NMR spectrum of compound 1

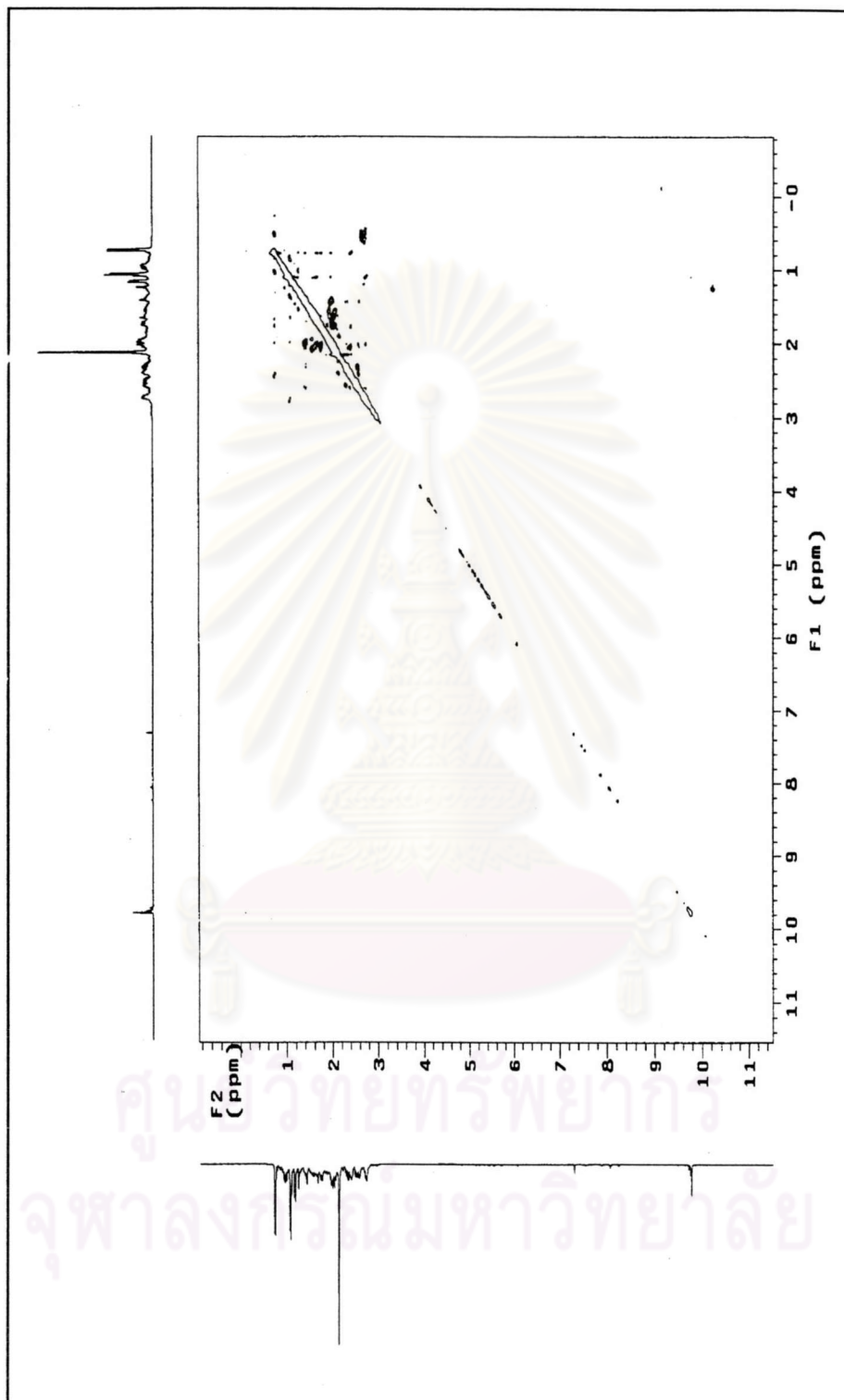


Figure 31 The NOESY-NMR spectrum of compound 1

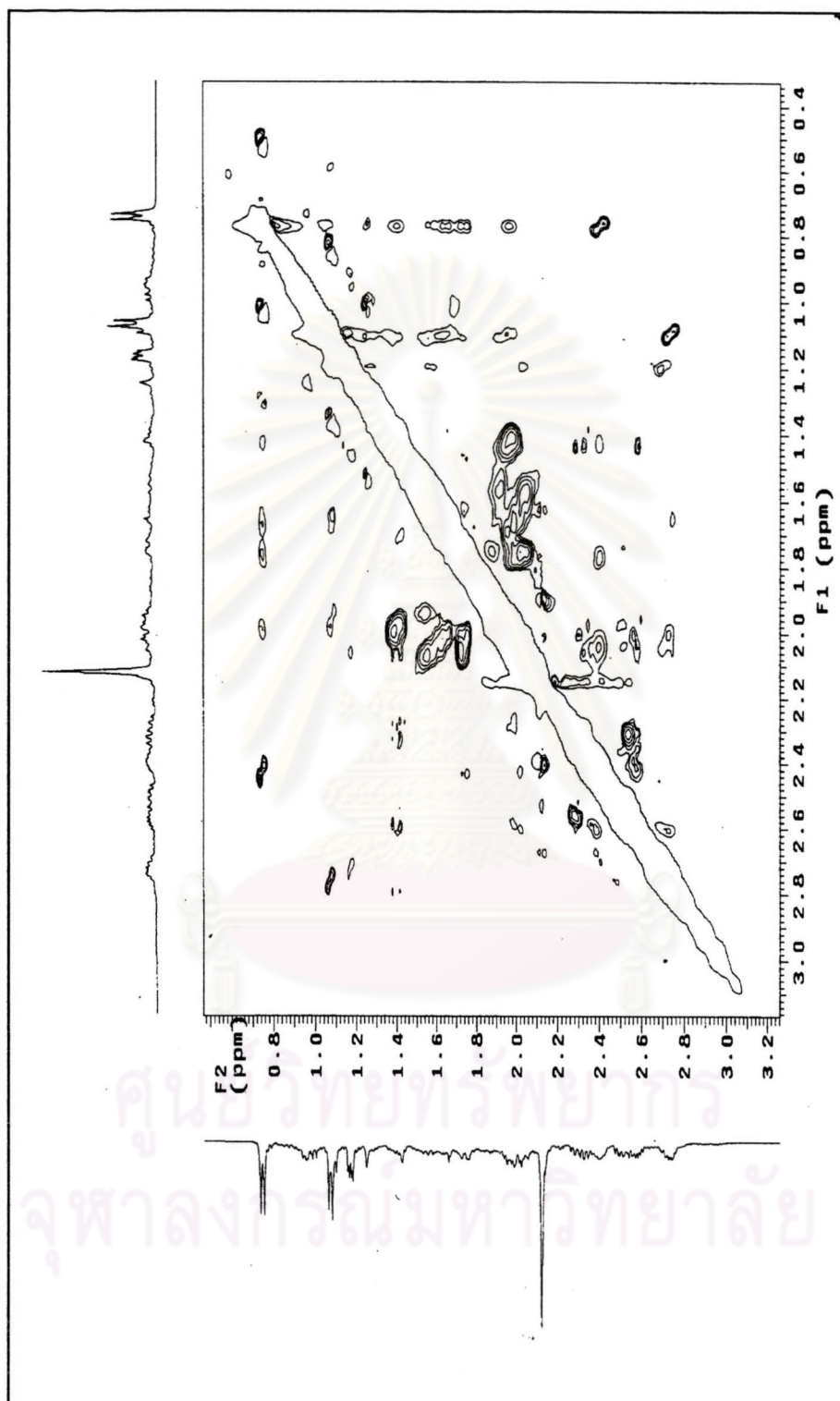


Figure 32 The NOESY-NMR spectrum of compound 1



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Figure 33 The ESI-Mass spectrum of compound 1

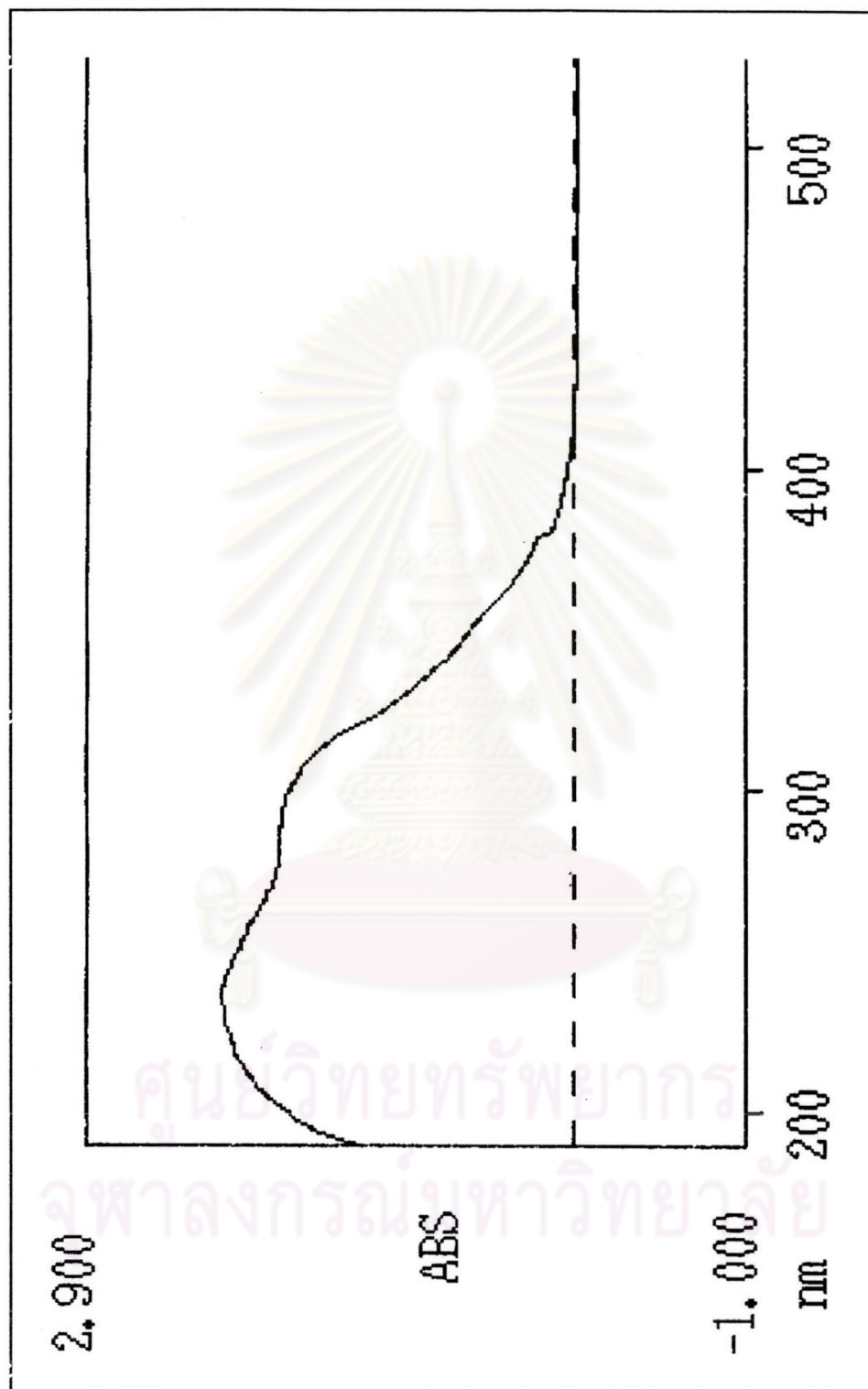


Figure 34 The UV spectrum of compound 2

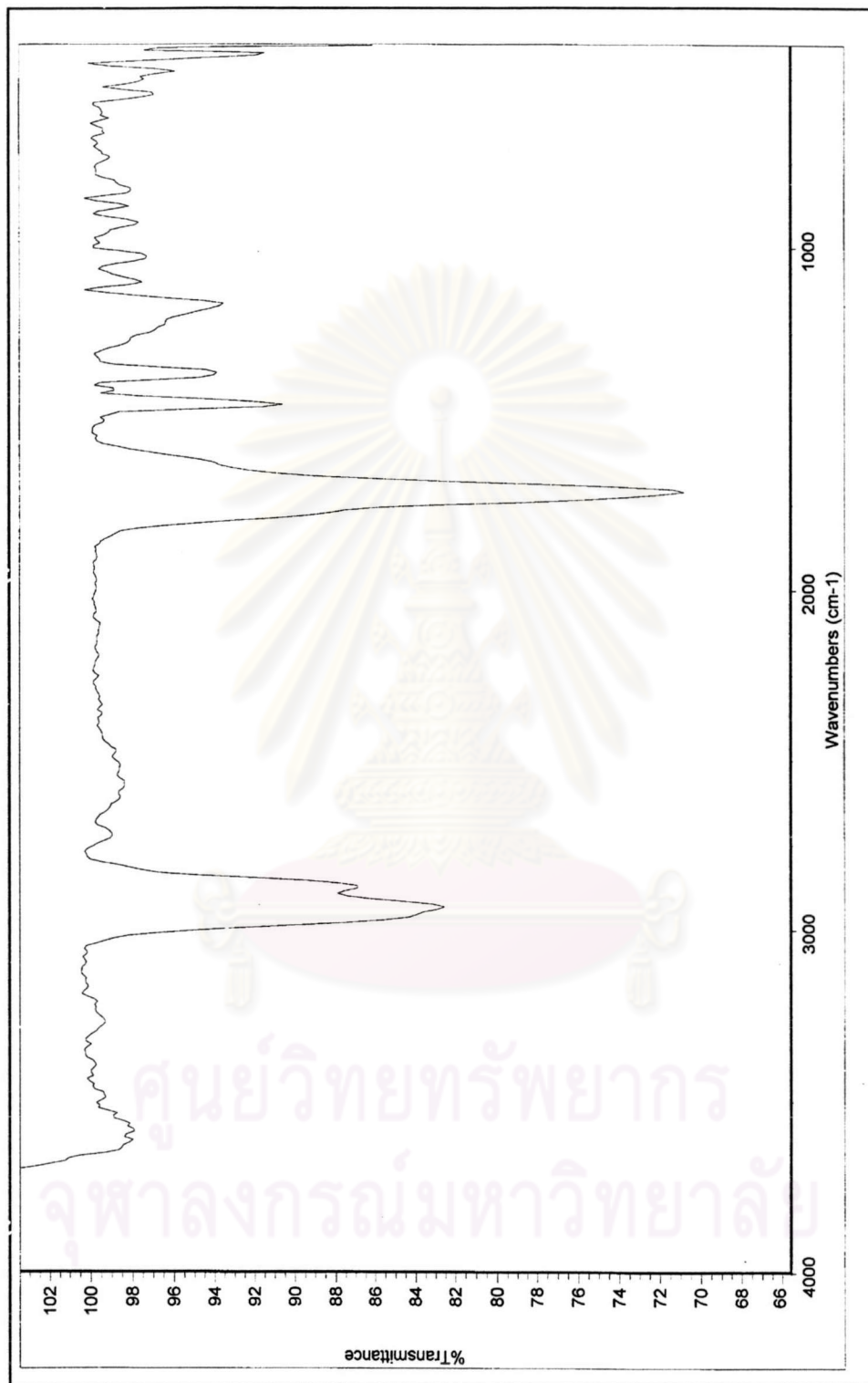


Figure 35 The IR spectrum of compound 2

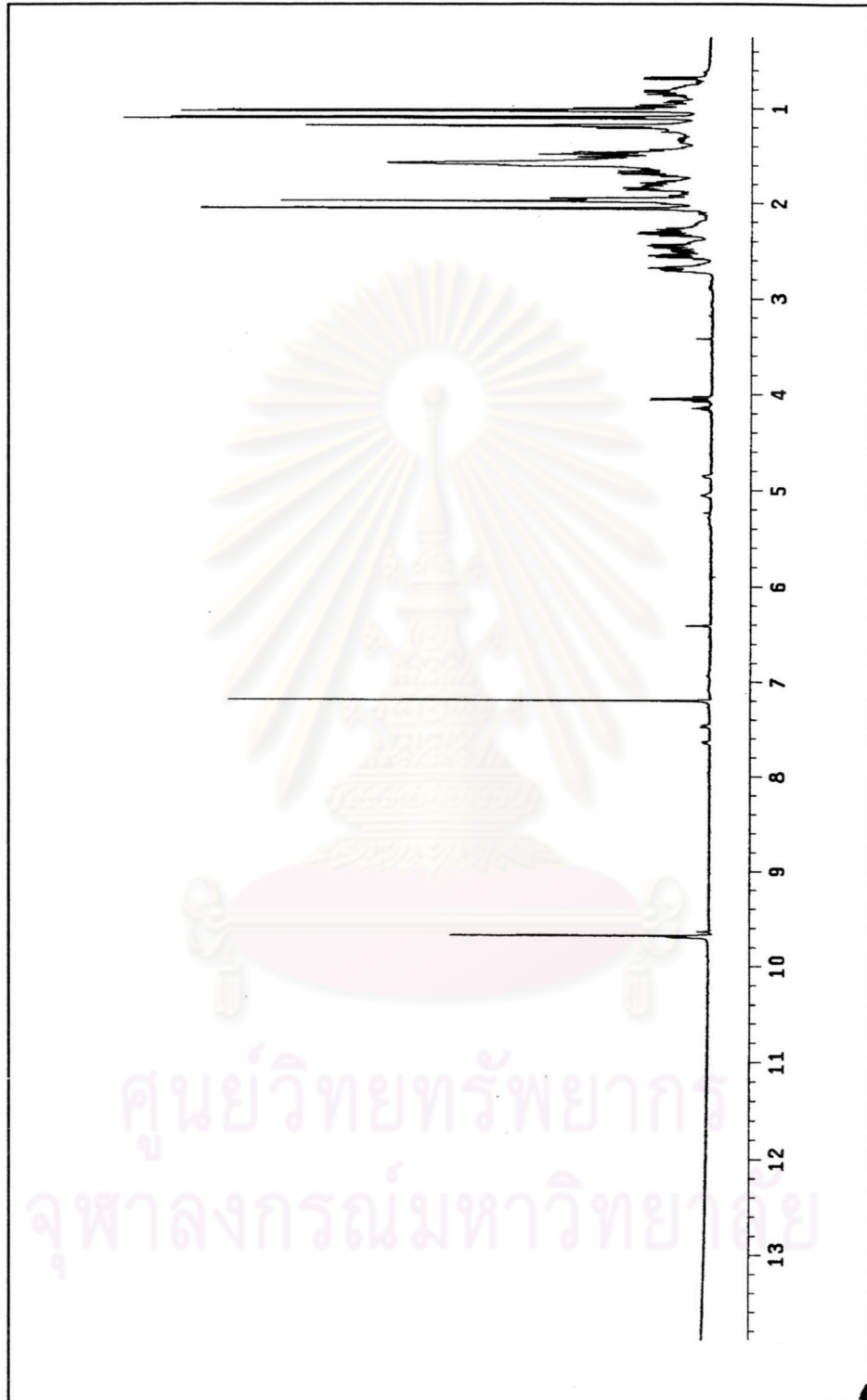


Figure 36 The $^1\text{H-NMR}$ spectrum of compound 2

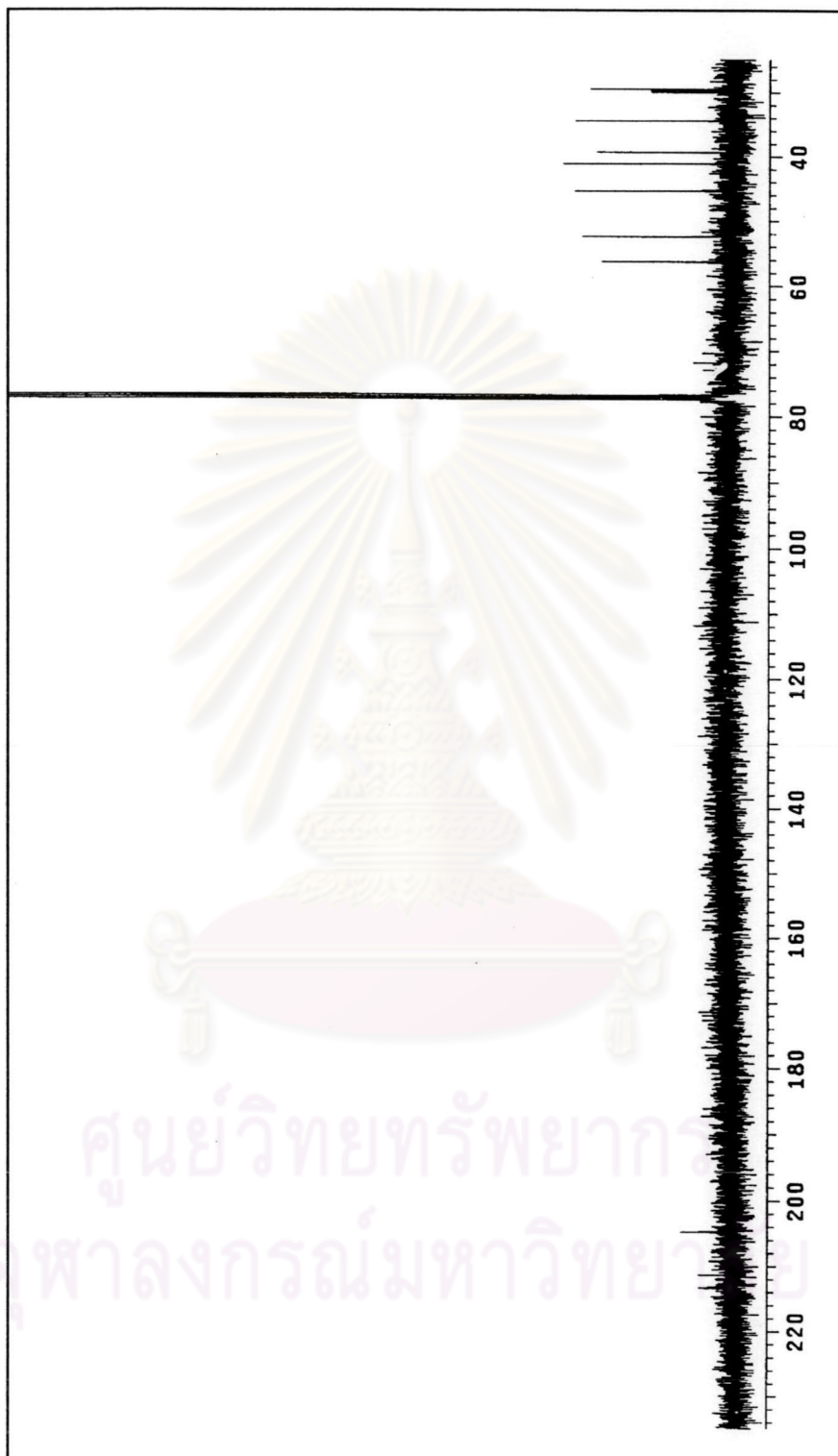


Figure 37 The $^{13}\text{C-NMR}$ spectrum of compound 2

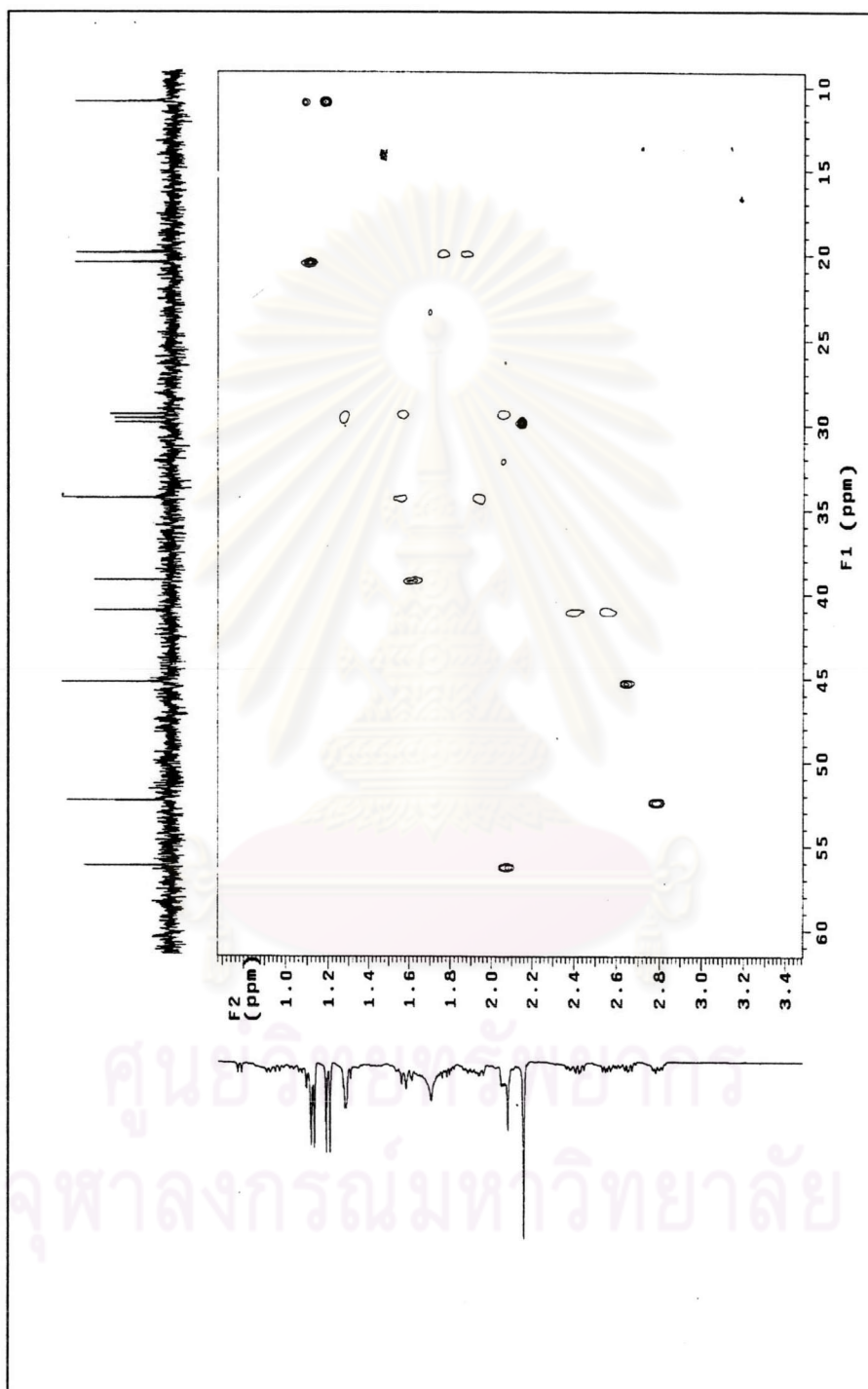


Figure 38 The HSQC-NMR spectrum of compound 2

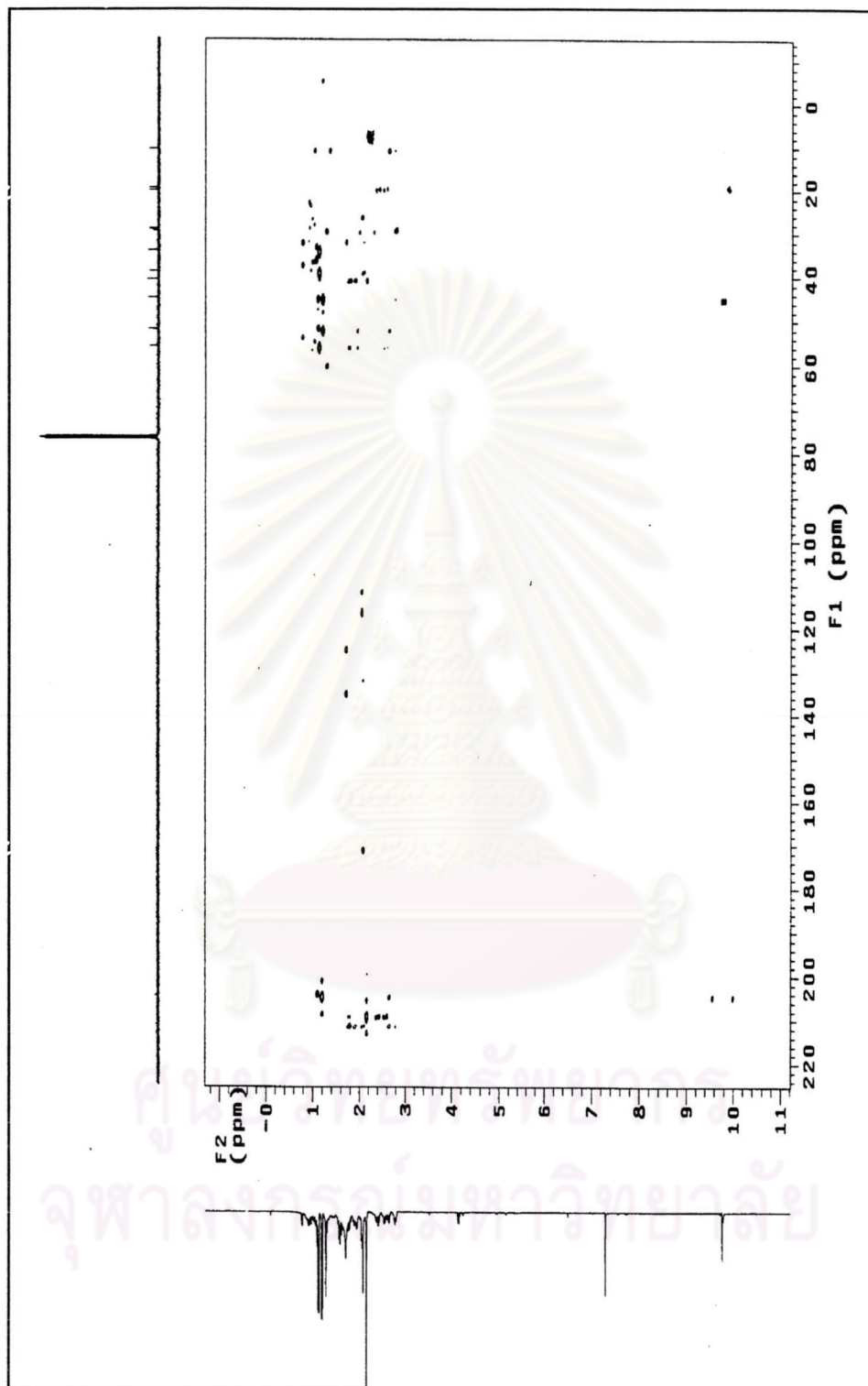


Figure 39 The HMBC-NMR spectrum of compound 2

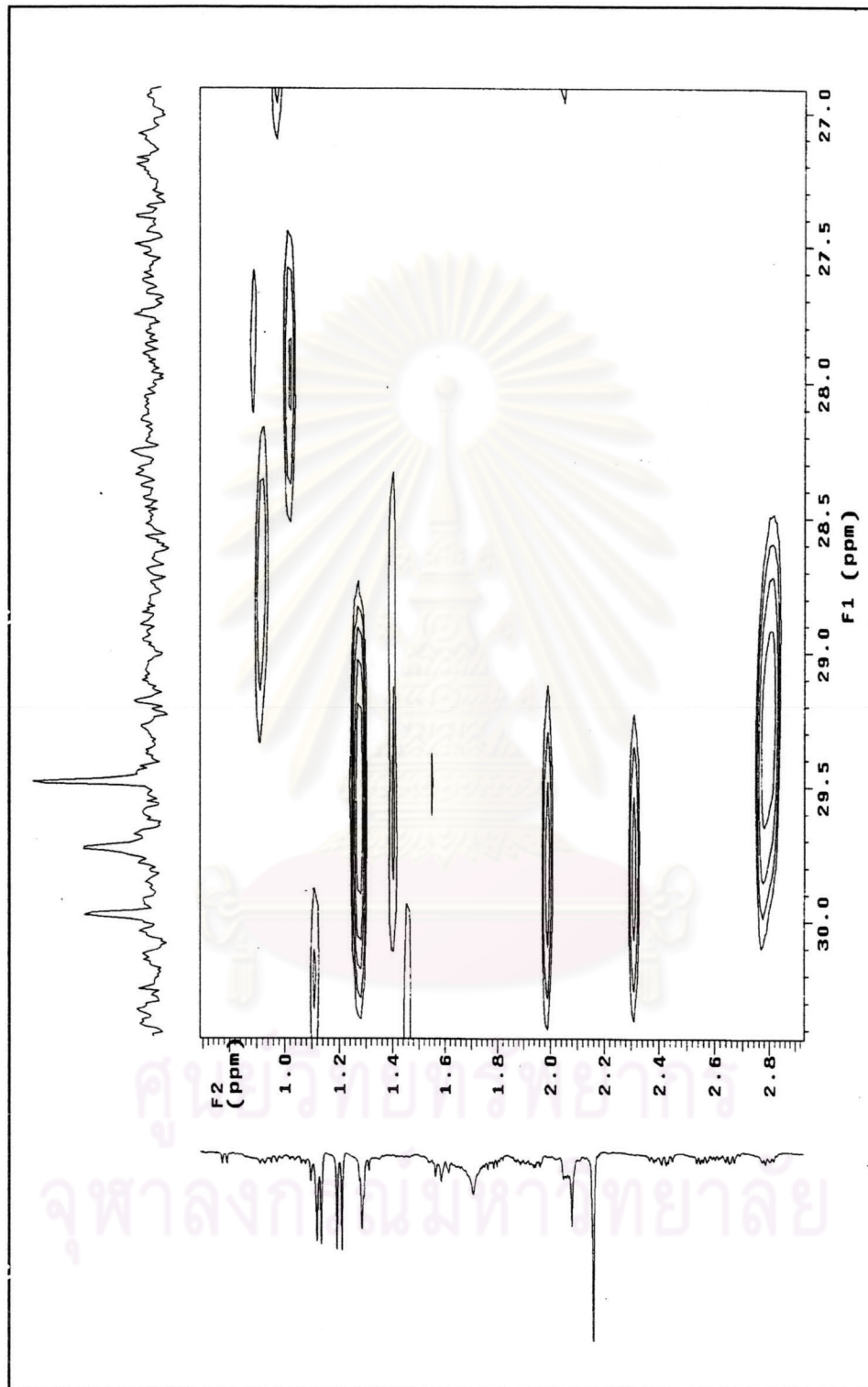


Figure 40 The HMBC-NMR spectrum of compound 2

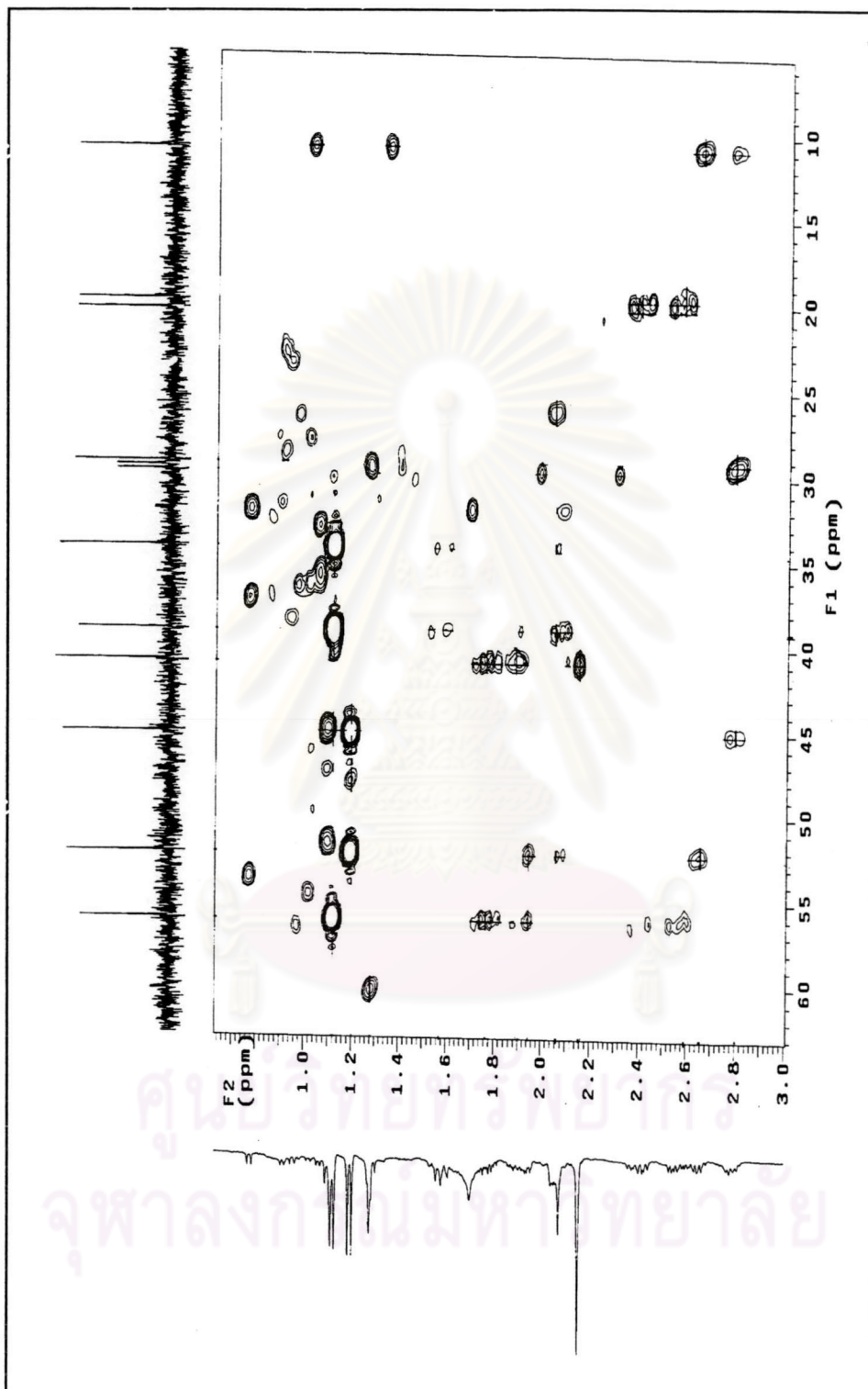


Figure 41 The HMBC-NMR spectrum of compound 2

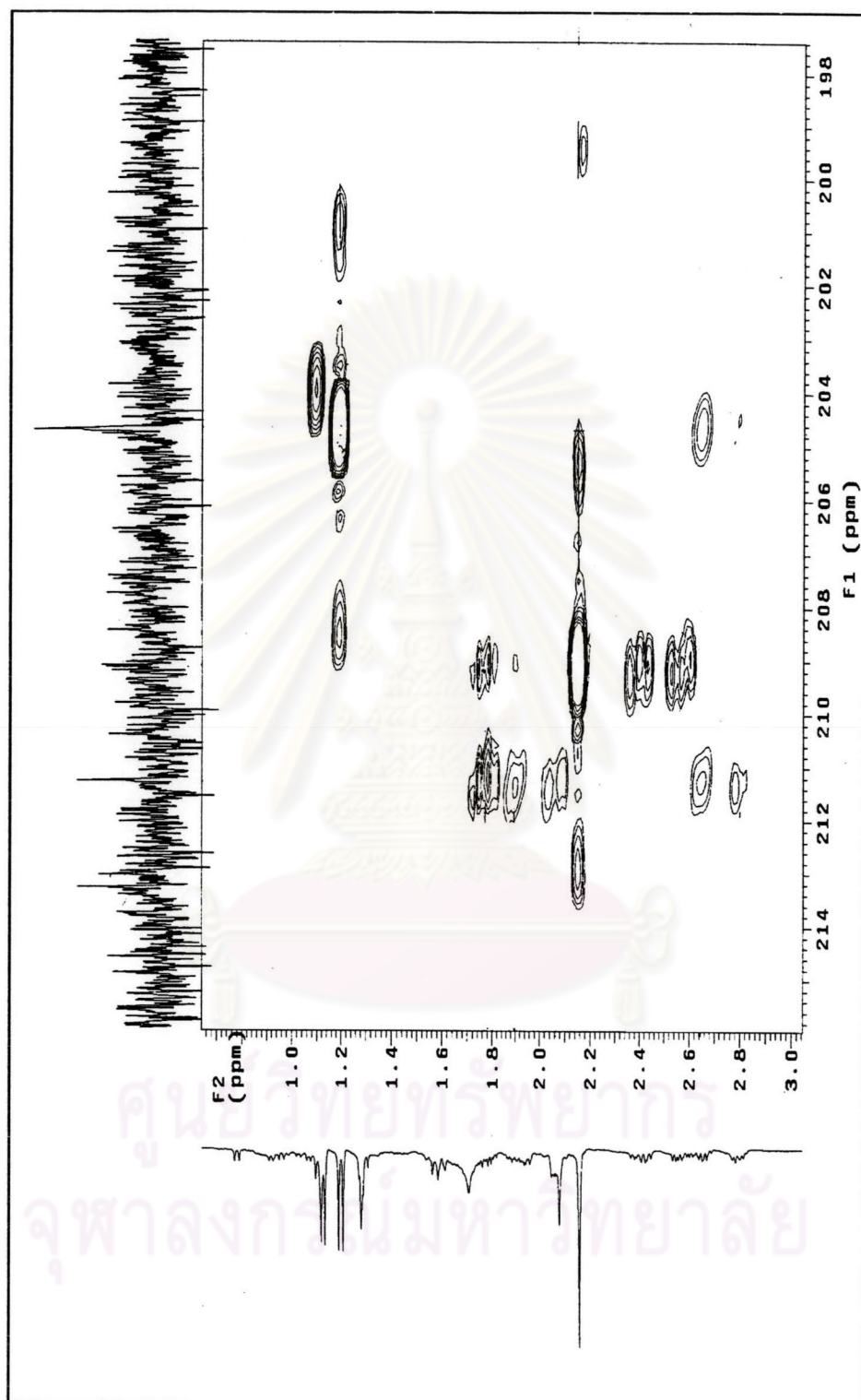


Figure 42 The HMBC-NMR spectrum of compound 2

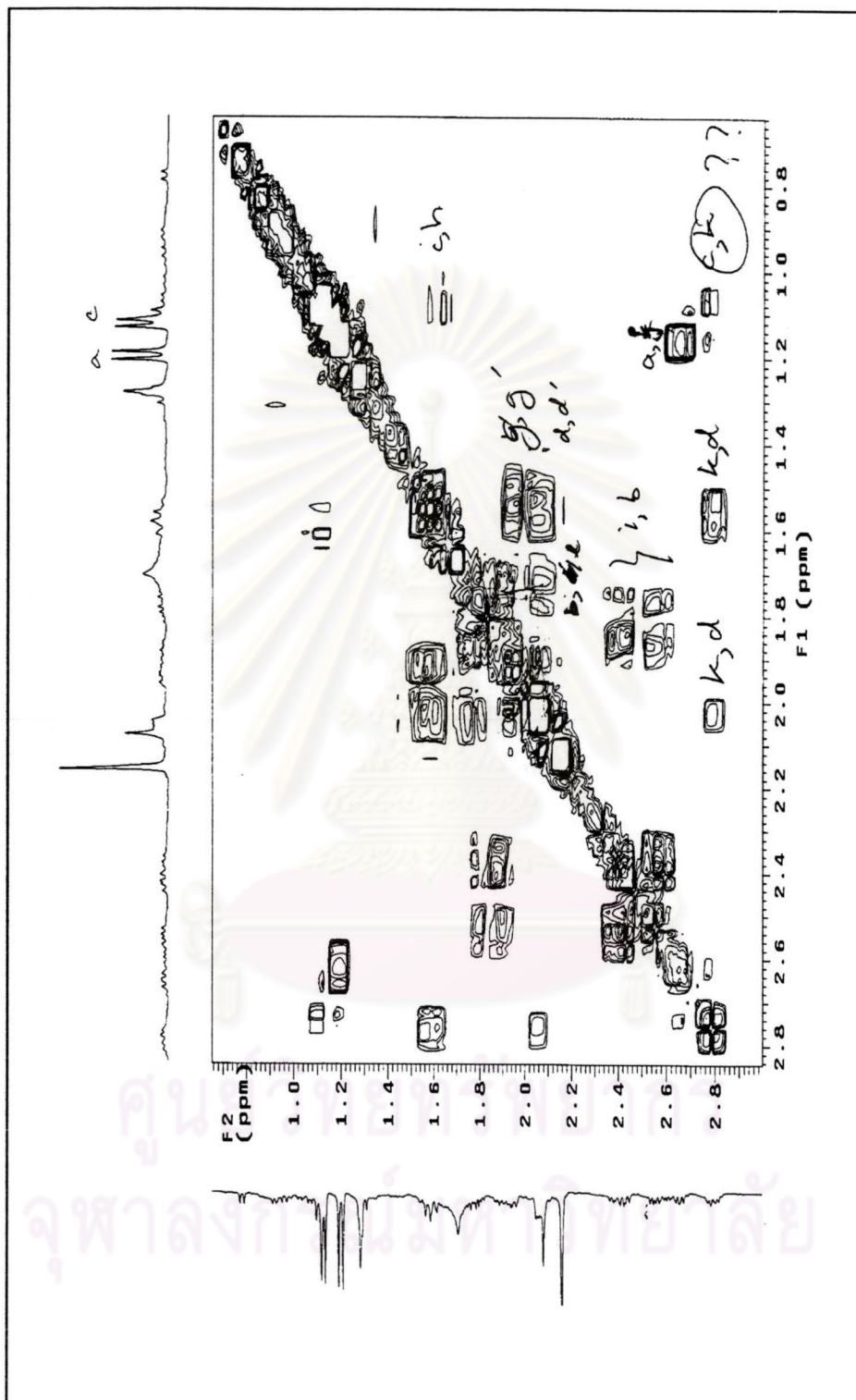
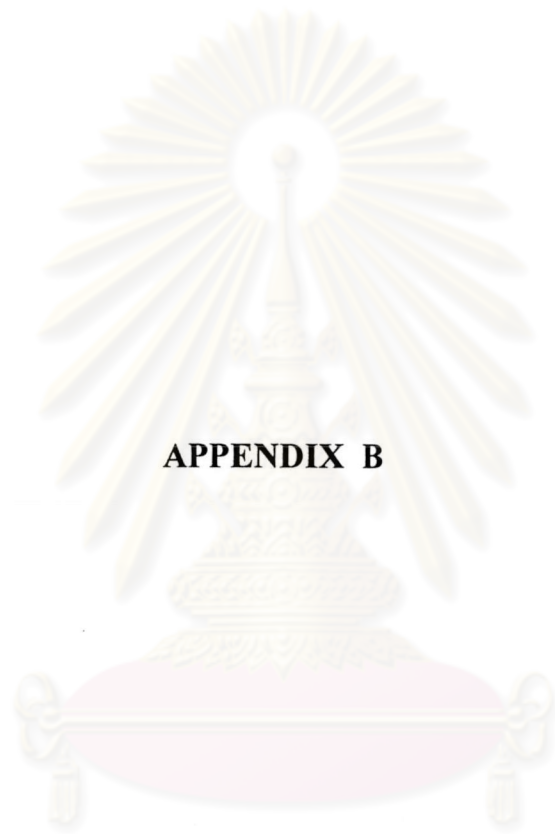


Figure 43 The COSY-NMR spectrum of compound 2



Figure 44 The ESI Mass spectrum of compound 2



APPENDIX B

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Table 10 IC₅₀'s of DHA and impurities with 8 cell lines

Cell lines	130603		300403		121101	
	IC ₅₀ (µg/ml)	STDEVP	IC ₅₀ (µg/ml)	STDEVP	IC ₅₀ (µg/ml)	STDEVP
3T3	53	17	11	3	10	1
L929	45	11	32	6	12	3
BHK	49	14	57	2	60	4
IEC-6	66	11	5	0	5	0
Vero	68	9	6	0.4	6	0
HepG2	37	13.5	21	2	26.5	5
Caco2	93	6	51	3	46.5	7
MCF7	41	9	38	3	36	5.5

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Table 11 IC₅₀'s of DHA; fresh drug (300403) with 8 cell lines

Cell lines	1 st experiment		2 nd experiment		Average	
	IC ₅₀ (µg/ml)	STDEVP	IC ₅₀ (µg/ml)	STDEVP	IC ₅₀ (µg/ml)	STDEVP
3T3	13.5	1	9	1	11	3
L929	37.5	1	26.5	1	32	6
BHK	59	2	56	0.5	57	2
IEC-6	5	0	5	0	5	0
Vero	6	0.4	6	0.4	6	0.4
HepG2	19	1	23	0.5	21	2
Caco2	50	3	52	2	51	3
MCF7	38	3	38	2.5	38	3

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Table 12 IC₅₀'s of DHA; stored drug (121101) with 8 cell lines

Cell lines	1 st experiment		2 nd experiment		Average	
	IC ₅₀ (µg/ml)	STDEVP	IC ₅₀ (µg/ml)	STDEVP	IC ₅₀ (µg/ml)	STDEVP
3T3	10	1	11	1	10	1
L929	9	1	15	2	12	3
BHK	64	1	57	2	60	4
IEC-6	4.5	0	5	0	5	0
Vero	6	0	6	0	6	0
HepG2	23	2	30	4	26.5	5
Caco2	47	0.5	46	9.5	46.5	7
MCF7	32	2	40	5	36	5.5

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Table 13 IC₅₀'s of impurities (130603) with 8 cell lines

Cell lines	1 st experiment		2 nd experiment		3 rd experiment	
	IC ₅₀ (µg/ml)	STDEVP	IC ₅₀ (µg/ml)	STDEVP	IC ₅₀ (µg/ml)	STDEVP
3T3	37.5	7	60	0.25	76.5	0.5
L929	nd	nd	56	3	34	3
BHK	37	2	66	2	nd	nd
IEC-6	nd	nd	56	5	7.65	2.5
Vero	79.5	2.5	72.5	1.5	58	2
HepG2	25.5	0.5	54	2	27	1
Caco2	94	5	93	6	nd	nd
MCF7	51	2	nd	nd	34	3

nd: no data to be valid.

Cell lines	Average	
	IC ₅₀ (µg/ml)	STDEVP
3T3	53	17
L929	45	11
BHK	49	14
IEC-6	66	11
Vero	68	9
HepG2	37	13.5
Caco2	93	6
MCF7	41	9

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Table 14 Acute toxicity test of impurities of DHA in mice

Dose (g/kg)	Number of rats died up to the present time		
	Male	Female	Total
1.25	0/5	0/5	0/10
2.5	0/5	0/5	0/10
5.0	2/5	1/5	3/10
10.0	2/5	4/5	6/10
20.0	5/5	5/5	10/10
LD ₅₀	8.06	7.07	7.54
95% CI	4.62-16.09	4.02-12.43	5.51-10.46

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Table 15 *In vitro* activity of antimalarial drug against W2

W2	MW	IC ₅₀ , ng/ml		Avg (ng/ml)	SD	% CV	n	Avg (nM)
		2/4/2004	2/11/2004					
DHA std	284.4	0.575	0.70	0.64			2	2.25
DHA T2 old	284.4	0.8297		0.83				
DHA T2 new	284.4	0.6722		0.67				
Impurities	238	48.0032	64.25	56.13			2	.235.8
CQ	515.9	20.3436	NA	20.34			1	39.4
MQ	414.8	17.4118	NA	17.41			1	42.0
Qn	648.8	284.385	NA	284.4				438.3



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Table 16 *In vitro* activity of antimalarial drug against D6

D6	MW	IC ₅₀ , ng/ml		Avg (ng/ml)	SD	% CV	n	Avg (nM)
		2/4/2004	2/11/2004					
DHA std	284.4	0.273	0.374	0.324	0.071	22.1	2	1.138
DHA T2 old	284.4	0.417		0.417	##DIV	##DIV	1	1.465
DHA T2 new	284.4	0.319		0.319	##DIV	##DIV	1	1.123
Impurities	238	23.080	23.9	23.490	0.580	2.5	2	98.0
CQ	515.9	51.580	41.664	46.622	7.011	15.0	2	90.4
MQ	414.8	1.375	3.9	4.137	0.336	8.1	2	10.0
Qn	648.8	107.029	101.338	104.183	4.024	3.9	2	160.6


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