APPENDICES

APPENDIX A
General experimental procedures

General experimental procedures

Optical rotations were measured in chloroform, using a digital polarimeter (JASCO, DIP-370). UV spectra were recorded on Cary-IE UV/VIS spectrophotometer. IR spectra were obtained from Perkin-Elmer Sustem 2000 spectrometer. NMR spectra were recorded on Bruker-AV 500 spectrometer (500 Mz) instruments using $CDCl_3$ and DMSO-d_6 as solvent with TMS as an internal standard. Mass spectra were determined using Electrospray-ionization time-of-flight mass spectrometry (ESI-TOF-MS): Micromass-LCT mass spectrometer. High performance liquid chromatography (HPLC) was carried out with a Waters system (Waters 600 HPLC pump and Waters 996 Photodiodede array detector) and HPLC column (LiChroCart 251-10 HPLC-Cartrige, 10 μm).

Bioactivity Determination

1. Cytotoxic activity

   The optical density (OD) was measured with microtiter plate reader.

2. Antimalarial activity

   Levels of incorporated radioactively labeled hypoxanthine indicating parasite growth were determined using the Topcount microplate scintillation counter (Packard, USA).

3. Antifungal activity

   The result, absorbance at 570 nm was determined with the multilabel counter Victor3 V.

4. Antitubercular activity

   The results were recorded with a fluorescence multi-well reader (CytoFlour, series 4000).

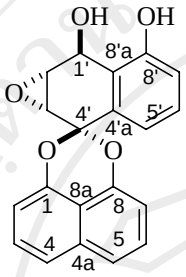
APPENDIX B

Spectral data of isolated compounds

1. Spectral data of isolated compounds from *Diospyros ehretioides* fruits

The isolated compounds were characterized by their spectroscopic data including NMR, MS and IR spectra.

Compound 70



Common name	Palmarumycin JC1
Physical appearance	White crystals
mp	208-210 °C (Lit. [25])
$[\alpha]_D^{24}$	+206.52 ° (*c* 0.1, MeOH) (Lit. [25] $[\alpha]_D^{25}$ +82.5 (*c* 0.1, MeOH))
Molecular formula	$C_{20}H_{14}O_5$
HRMS (ESI-TOF) *m/z*	357.0739 $[M+Na]^+$ (calcd. For $C_{20}H_{14}O_5Na$, 317.1236)
UV (MeOH) λmax (log ε)	204(4.76), 225(4.90), 288(4.14), 299(4.10), 314(3.95) nm
IR (KBr) νmax	3506, 3271, 3030, 2860, 1607, 1580, 1410, 1376, 1318, 1268, 1106, 1020, 992, 957 and 761 cm^{-1}

^{1}H NMR (DMSO-d_6, 500 MHz):

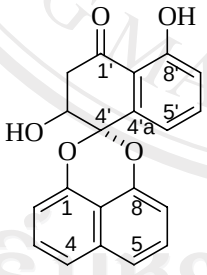
δ 3.51(1H, dd, J= 4.1, 4.1 Hz, H-2′), 3.61(1H, d, J= 4.1 Hz, H-3′),

5.34(1H, dd, J= 2.1, 5.4 Hz, H-1′), 5.56(1H, d, J= 5.6 Hz, 1′-OH), 6.97(1H, t, J= 7.9 Hz, H-2), 6.97(1H, t, J= 7.9 Hz, H-7′), 7.12(1H, d, J= 7.4 Hz, H-5′), 7.15(1H, d, J= 7.8 Hz, H-7), 7.25(1H, t, J= 7.9 Hz, H-6′), 7.48(1H, t, J= 7.9 Hz, H-5), 7.53(1H, t, J= 7.9 Hz, H-3), 7.61(1H, t, J= 8.7 Hz, H-6), 7.61(1H, t, J= 8.7 Hz, H-4) and 9.91(1H, s, 8′-OH).

^{13}C NMR (DMSO-d_6, 125 MHz):

δ 49.8(d, C-3′). 53.3(d, C-2′), 59.1(d, C-1′), 97.6(s, C-4′), 108.9(d, C-2), 109.3(d, C-7), 112.4(s, C-8a), 116.4(d, C-7′), 117.2(d, C-5′), 120.6(d, C-4), 120.7(d, C-5), 122.6(s, C-8′a) , 127.8(d, C-3), 127.9(d, C-6), 129.1(d, C-6′), 132.1(s, C-4′a), 133.8(s, C-4a), 147.2(s, C-1), 147.3(s, C-8) and 155.7(s, C-8′).

Compound 71



Common name	Palmarumycin JC2
Physical appearance	Brown solid
mp	192-194 °C
$[\alpha]_D^{24}$	115.8° (*c* 0.1, MeOH) (Lit.[25] $[\alpha]_D^{25}$ +131.9 (*c* 0.5, $CHCl_3$))
Molecular formula	$C_{20}H_{14}O_5$

HRMS (ESI-TOF) *m/z*	335.0919 $[M+H]^+$ (calcd. For $C_{20}H_{15}O_5$, 335.0919)
UV (MeOH) λmax (log ε)	223(4.69), 254(4.02), 298(3.95), 314(3.92), 327(3.95) nm
IR (KBr) νmax	3277, 2922, 1644, 1607, 1456, 1412, 1379, 1269, 1164, 1121, 1070, 975, 820 and 757 cm^{-1}

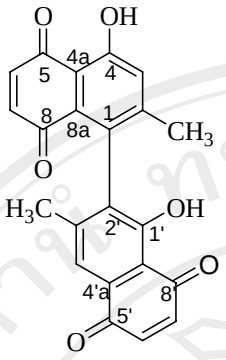
^1H NMR ($CDCl_3$, 500 MHz):

δ 2.97(1H, dd, J= 17.7, 3.9 Hz, H-2′), 3.26(1H, dd, J= 17.8, 3.4 Hz, H-2′), 4.62(1H, t, J= 3.6 Hz, H-3′), 6.93(1H, d, J= 7.6 Hz, H-2), 7.11(1H, d, J= 7.5 Hz, H-5′), 7.11(1H, d, 7.5 Hz, H-7), 7.36(1H, dd, J= 7.6, 7.6 Hz, H-7′), 7.46(1H, t, J= 7.7 Hz, H-3), 7.51(1H, t, J= 7.6 Hz, H-6), 7.57(1H, d, J= 7.6 Hz, H-4), 7.57 (1H, d, J= 7.6 Hz, H-5), 7.58(1H, t, J= 7.6 Hz, H-6′) and 12.37(1H, brs, 8′-OH).

^{13}C NMR ($CDCl_3$, 125 MHz):

δ 41.3(t, C-2′), 67.3(d, C-3′), 98.7(s, C-4′), 108.9(d, C-2), 109.6(d, C-7), 113.2(s, C-8a), 115.3(s, C-8′a), 118.0(d, C-7′), 119.9(d, C-5′), 121.1(d, C-4), 121.5(d, C-5), 127.7(d, C-3), 127.7(d, C-6), 134.2(s, C-4a), 137.1(d, C-6′), 137.9(s, C-4′a), 146.3(s, C-1), 147.1(s, C-8), 162.2(s, C-8′), and 201.0(s, C-1′).

Compound 23



Common name	Isodiospyrin
Physical appearance	Orange-red solid
mp	226-228 °C (Lit.[49] mp 229-230 °C)
Molecular formula	$C_{22}H_{14}O_6$
HRMS (ESI-TOF) *m/z*	374.0873 $[M+H]^+$ (calcd. for $C_{22}H_{15}O_6$, 375.1185)
UV (MeOH) λmax (log ε)	215 (4.88), 254 (4.57) nm
IR (KBr) νmax	2923, 1641, 1602, 1422, 1362, 1280, 1101, 851, 751, 566, 445 cm^{-1}

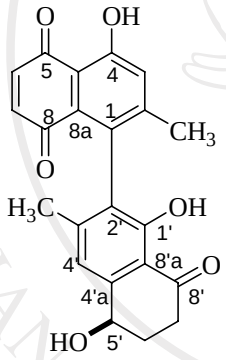
^{1}H NMR ($CDCl_3$, 500 MHz):

δ 2.03(3H, s, 2-CH_3). 2.05(3H, s, 3′-CH_3), 6.75(1H, d, J= 10.1 Hz, H-6′), 6.97 (1H, d, J= 10.2 Hz, H-7′), 6.98(1H, dd, J= 10.3, 10.3 Hz, H-6), 6.98(1H, dd, J= 10.3, 10.3 Hz, H-7), 7.32(1H, s, H-4′), 7.63(1H, s, H-3), 12.07(1H, s, 4-OH) and 12.46(1H, s, 1′-OH).

^{13}C NMR ($CDCl_3$, 125 MHz):

δ 20.5(q, 2-Me), 20.7(q, 3′-Me), 113.2(s, C-4a), 114.2(s, C-8′a), 121.4(d, C-3), 125.8(d, C-4′), 128.6(s, C-4′a), 128.8(s, C-8a), 130.3(s, C-2′), 135.1(s, C-1), 137.7(d, C-7′), 138.8(d, C-6), 139.6(d, C-6′), 140.2(d, C-7), 145.5(s, C-2), 148.2(s, C-3′), 158.6(s, C-4), 162.0(s, C-1′), 184.5(s, C-8), 185.0(d, C-5′), 190.1(s, C-8′) and 190.4(s, C-5).

Compound 72



Common name	Isodiospyrol A
Physical appearance	Brown solid
mp	101-102.8 °C
$[\alpha]_D^{24}$	-34.5 ° (*c* 0.1, $CHCl_3$)
Molecular formula	$C_{22}H_{18}O_6$
HRMS (ESI-TOF) *m/z*	401.0994 $[M+Na]^+$ (calcd. for $C_{22}H_{18}O_6Na$, 403.1005)
UV (MeOH) λmax (log ε)	215(4.51), 263(4.15), 347(3.65) nm
IR (KBr) νmax	3424, 2924, 1667, 1644, 1605, 1363, 1340, 1271, 1109, 1047, 979, 853, 753 cm^{-1}

^{1}H NMR ($CDCl_3$, 500 MHz):

δ 1.96(3H, brs, 3′-CH_3), 2.05(3H, d, J= 0.6 Hz, 2-CH_3), 2.23(1H, m, H-6′);2.43(1H, m, H-6′), 2.69(1H, m, H-7′); 3.0(1H, m, H-7′), 5.02(1H, dd, J= 7.7, 3.8 Hz, H-5′), 6.72(1H, d, J= 10.1 Hz, H-7), 6.91(1H, d, J= 10.1 Hz, H-6), 7.04(1H, brs, H-4′), 7.29(1H, d, J= 0.6 Hz, H-3), 12.52(1H, s, 4-OH) and 12.63(1H, s, 1′-OH).

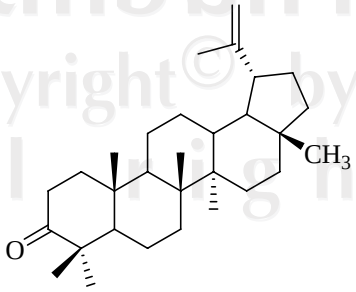
^{13}C NMR ($CDCl_3$, 125 MHz):

δ 20.5(q, 3′-Me), 20.8(q, 2-Me), 31.5(t, C-6′), 34.7(t, C-7′), 67.8(d, C-5′), 113.5(s, C-8′a), 114.2(s, C-4a), 119.4(d, C-4′), 125.5(d, C-3), 127.4(s, C-2′), 128.8(s, C-8a), 129.8(s, C-1), 137.4(d, C-6), 140.3(d, C-7), 144.3(s, C-4′a), 145.8(s, C-3′), 149.2(s, C-2), 159.4(s, C-1′), 161.7(s, C-4), 185.1(s, C-8), 190.5(s, C-5) and 203.8(s, C-8′).

2. Spectral data of isolated compounds from *Diospyros rhodocalyx* woods

The isolated compounds were characterized by their spectroscopic data including NMR and MS spectra.

Compound 5

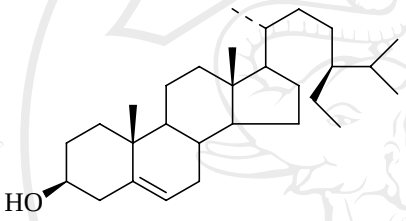


Common name Lupeol

Physical appearance Colorless solid

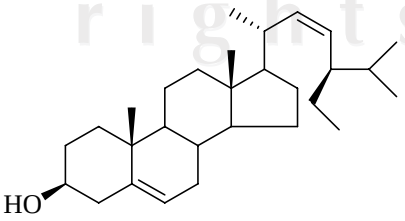
mp	212-214 °C (Lit.[28])
$[\alpha]_D^{24}$	+27.0 ° (*c* 0.1, $CHCl_3$) (Lit.[28])
Molecular formula	$C_{30}H_{50}O$
HRMS (ESI-TOF) *m/z*	449.3861 $[M+Na]^+$ (calcd. For $C_{30}H_{50}ONa$, 449.3861)

Compound 11



Common name	β-sitosterol
Physical appearance	Clear white solid
mp	141 °C (Lit.[30])
$[\alpha]_D^{24}$	+206.52 ° (*c* 0.1, MeOH) (Lit.[30])
Molecular formula	$C_{29}H_{50}O$
HRMS (ESI-TOF) *m/z*	437.7239 $[M+Na]^+$ (calcd. For $C_{29}H_{50}ONa$, 437.7239)

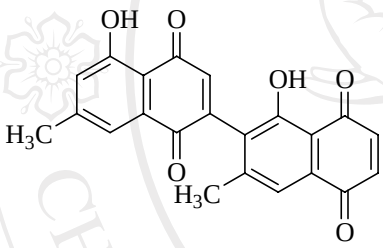
Compound 12



Common name	Stigmasterol

Physical appearance	White solid
mp	166 °C (Lit.[30])
$[\alpha]_D^{22}$	- 57° (*c* 0.1,$CHCl_3$) (Lit.[30])
Molecular formula	$C_{29}H_{48}O$
HRMS (ESI-TOF) *m/z*	435.7050 $[M+Na]^+$ (calcd. For $C_{29}H_{50}ONa$, 435.7050)

Compound 21



Common name	Diospyrin
Physical appearance	Orange-red solid
mp	258 °C
$[\alpha]_D^{24}$	+288 ° (*c* 0.5, H_2O) (Lit.[29])
Molecular formula	$C_{22}H_{14}O_6$
HRMS (ESI-TOF) *m/z*	397.1216 $[M+Na]^+$ (calcd. For $C_{22}H_{14}ONa$, 397.1216)

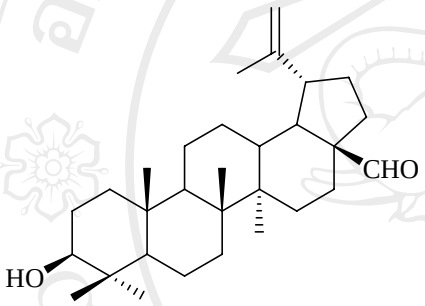
^{1}H NMR ($CDCl_3$, 400 MHz):

δ 2.24 (3H, s, 7-CH_3), and 2.39 (3H, s, 7′-CH_3), 6.83(1H, s, H-3′), 6.89(1H, s, H-2), 6.89(1H, s, H-3), 7.06(1H, br, H-6′), 7.44(1H, brs, H-8′), 7.49(1H, s, H-8), 11.81 (1H, s, H-5′) and 12.07(1H, s, H-5).

^{13}C NMR ($CDCl_3$, 100 MHz):

δ 21.O(7-Me), 22.2(7′-Me), 112.9(C-4a), 113.1(C-4′a), 120.7(C-8), 121.2(C-8′), 124.2(C-6′), 128.8(C-6), 131.3(C-8a), 131.6(C-8′a), 138.7(C-3′), 138.8(C-3), 139.4(C-2), 145.7(C-2′), 159.1(C-5), 161.7(C-5′), 182.5(C-1′), 184.1(C-1), 188.9(C-4′) and 189.7(C-4).

Compound 67



Common name	Betulinaldehyde
Physical appearance	Opaque white solid
mp	192-193 °C (Lit.[28])
$[\alpha]_D^{27}$	+19.0° (*c* 1.10, $CHCl_3$) (Lit.[28])
Molecular formula	$C_{30}H_{48}O_2$
HRMS (ESI-TOF) *m/z*	463.3654 $[M+Na]^+$ (calcd. For $C_{30}H_{48}O_2Na$, 463.3654)

^{1}H NMR ($CDCl_3$, 400 MHz):

δ 0.68(3H, s, 24-CH_3), 0.75(3H, s, 25-CH_3), 0.84(3H, s, 23-CH_3), 0.90(3H, s, 26-CH_3), 1.19(3H, s, 27-CH_3), 1.62(3H, s, 30- CH_3), 2.80(1H, m, H-19), 3.2(1H, q, H-3), 4.56(1H, d, *J*= 2.0 Hz, H-29), 4.69(1H, d, *J*= 2.0 Hz, H-29) and 9.6(1H, s, H-28).

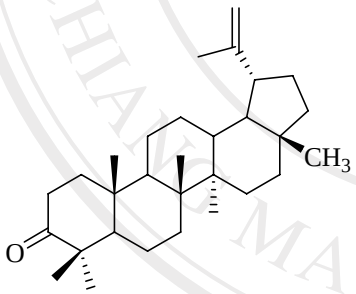
^{13}C NMR ($CDCl_3$, 100 MHz):

δ 14.2(C-27), 15.3(C-24), 15.8(C-25), 16.1(C-26), 18.2(C-6), 19.0(C-30), 20.7(C-11), 25.5(C-12), 27.3(C-2), 27.9(C-23), 28.8(C-16), 29.2(C-15), 29.8(C-21), 33.2(C-22), 34.3(C-7), 37.1(C-10), 38.6(C-1), 38.7(C-13), 38.8(C-4), 40.8(C-8), 42.5(C-14), 47.5(C-19), 48.0(C-18), 50.4(C-9), 55.2(C-5), 59.3(C-17), 78.9(C-3), 110.1(C-29), 149.7(C-20) and 206.7(C-28).

3. Spectral data of isolated compounds from *Diospyros glandulosa* woods

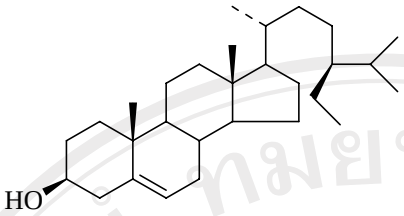
The isolated compounds were characterized by their spectroscopic data including NMR and MS spectra.

Compound 5



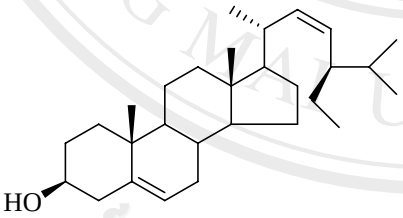
Common name	Lupeol
Physical appearance	Colorless solid
mp	212-214°C (Lit.[28])
$[\alpha]_D^{24}$	+27.0 ° (*c* 0.1, $CHCl_3$) (Lit.[28])
Molecular formula	$C_{30}H_{50}O$
HRMS (ESI-TOF) *m/z*	449.3861 $[M+Na]^+$ (calcd. For $C_{30}H_{50}ONa$, 449.3861)

Compound 11



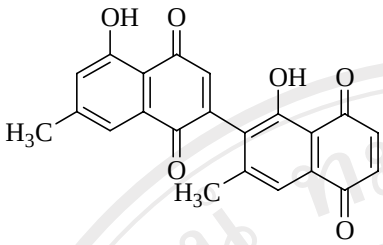
Common name	β-sitosterol
Physical appearance	Clear white solid
mp	141 °C (Lit.[30])
$[\alpha]_D^{24}$	+206.52 ° (*c* 0.1, MeOH) (Lit.[30])
Molecular formula	$C_{29}H_{50}O$
HRMS (ESI-TOF) *m/z*	437.7239 $[M+Na]^+$ (calcd. For $C_{29}H_{50}ONa$, 437.7239)

Compound 12



Common name	Stigmasterol
Physical appearance	White solid
mp	166 °C (Lit.[30])
$[\alpha]_D^{22}$	- 57° (*c* 0.1,$CHCl_3$) (Lit.[30])
Molecular formula	$C_{29}H_{48}O$
HRMS (ESI-TOF) *m/z*	435.7050 $[M+Na]^+$ (calcd. For $C_{29}H_{50}ONa$, 435.7050)

Compound 21



Common name	Diospyrin
Physical appearance	Orange-red solid
mp	258 °C
$[\alpha]_D^{24}$	+288 ° (*c* 0.5, H_2O) (Lit.[29])
Molecular formula	$C_{22}H_{14}O_6$
HRMS (ESI-TOF) *m/z*	397.1216 $[M+Na]^+$ (calcd. For $C_{22}H_{14}ONa$, 397.1216)

APPENDIX C

Spectrum of isolated compounds

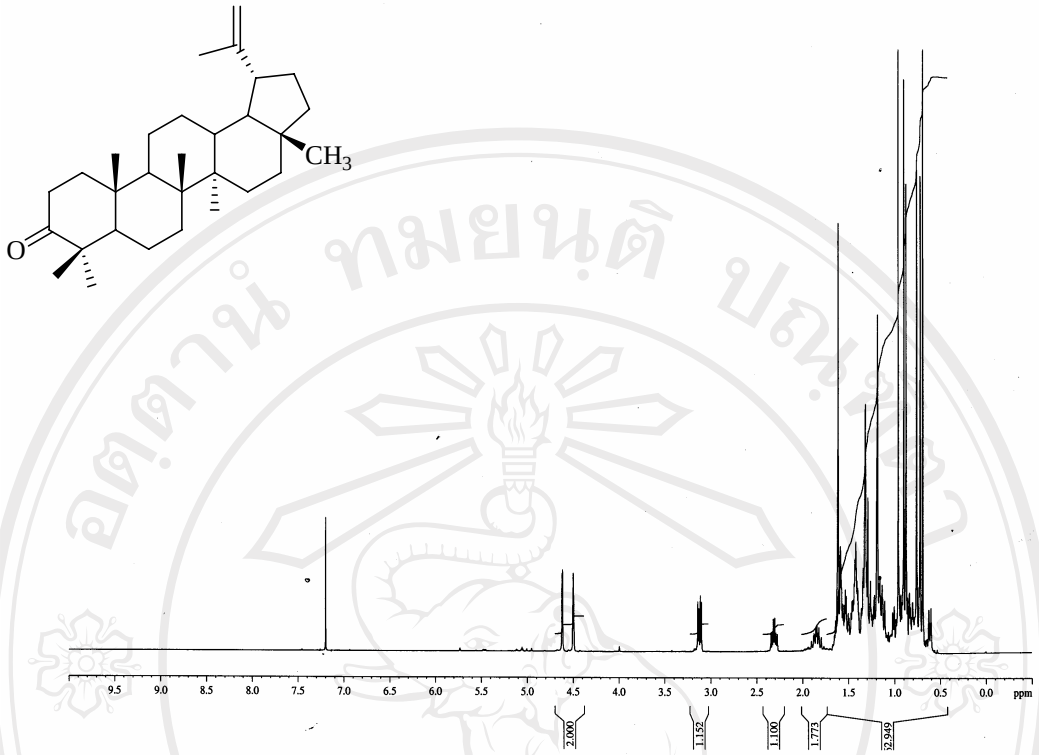


Figure 4. 500 MHz ^{1}H NMR ($CDCl_3$) spectrum of compound **5**

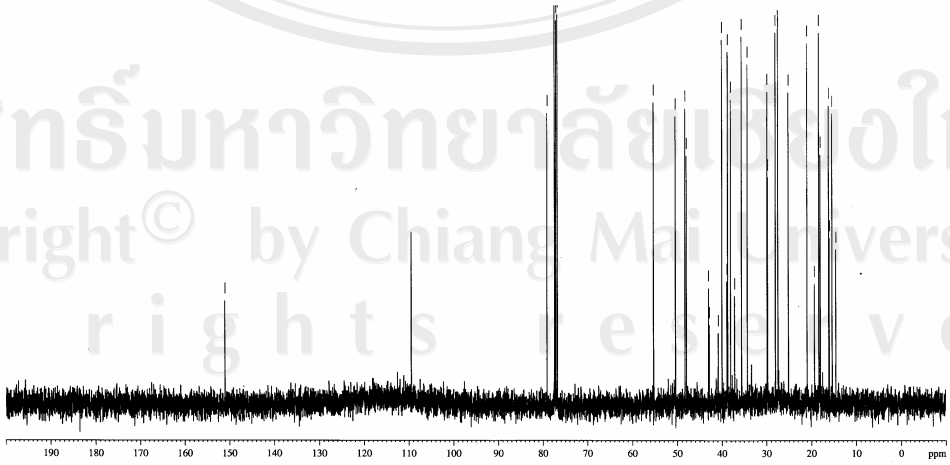


Figure 5. ^{13}C NMR ($CDCl_3$) and DEPT 135 spectra of compound

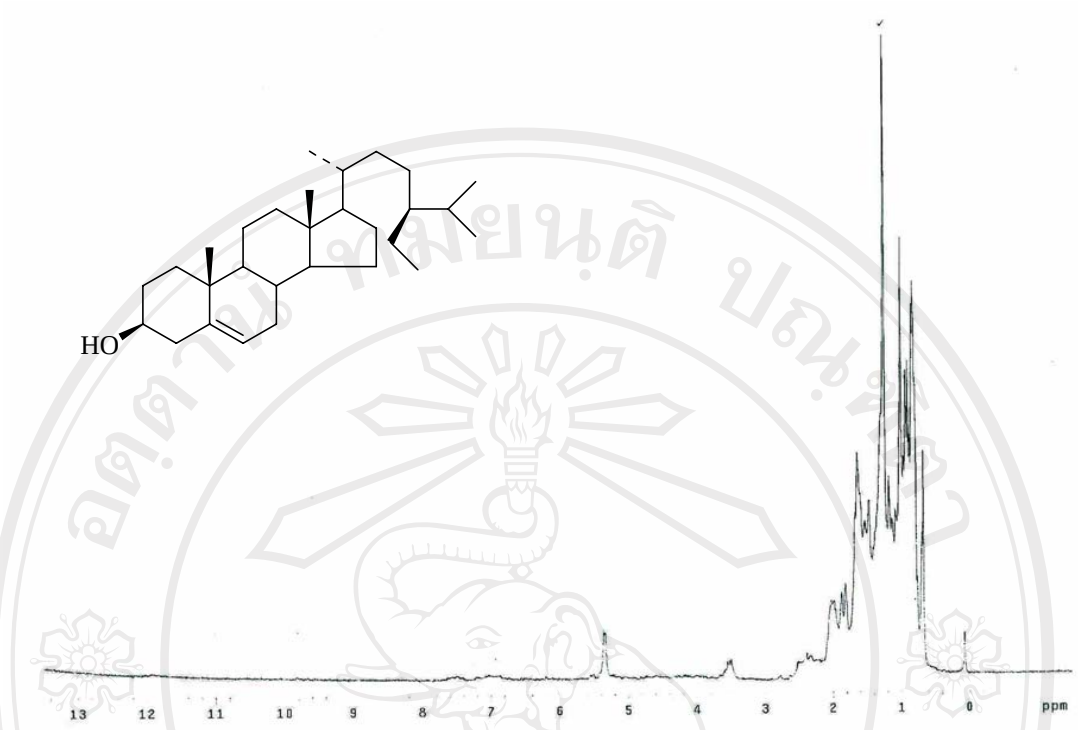


Figure 6. 500 MHz ^{1}H NMR ($CDCl_3$) spectrum of compound **11**

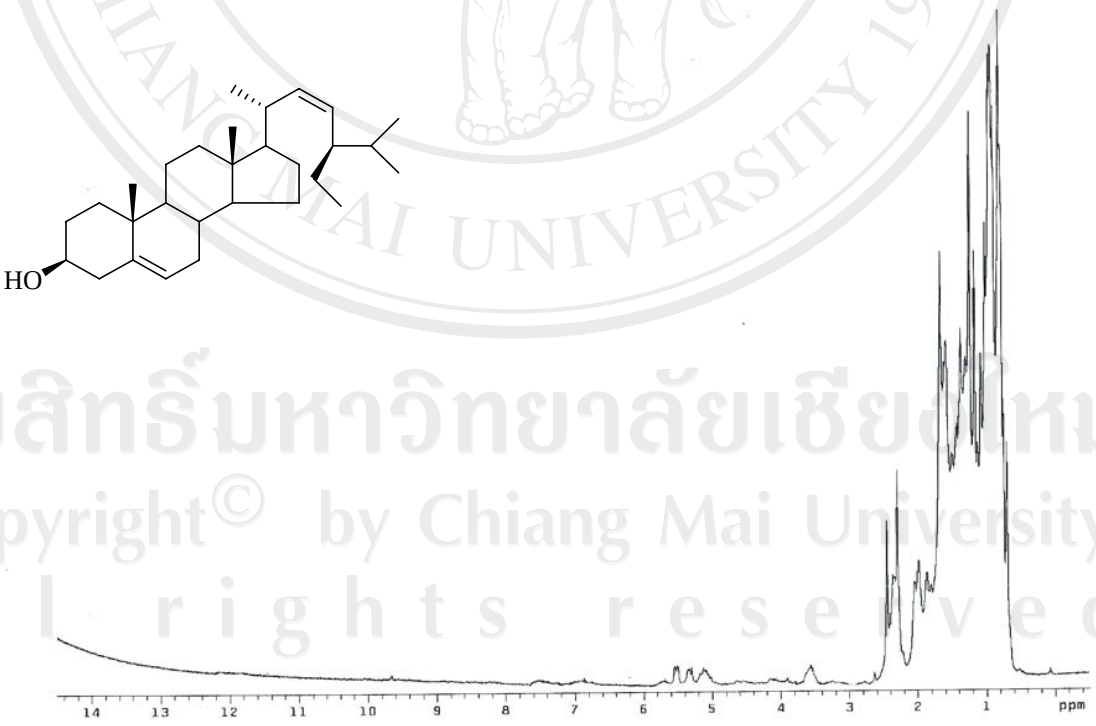


Figure 7. 500 MHz ^{1}H NMR ($CDCl_3$) spectrum of compound **12**

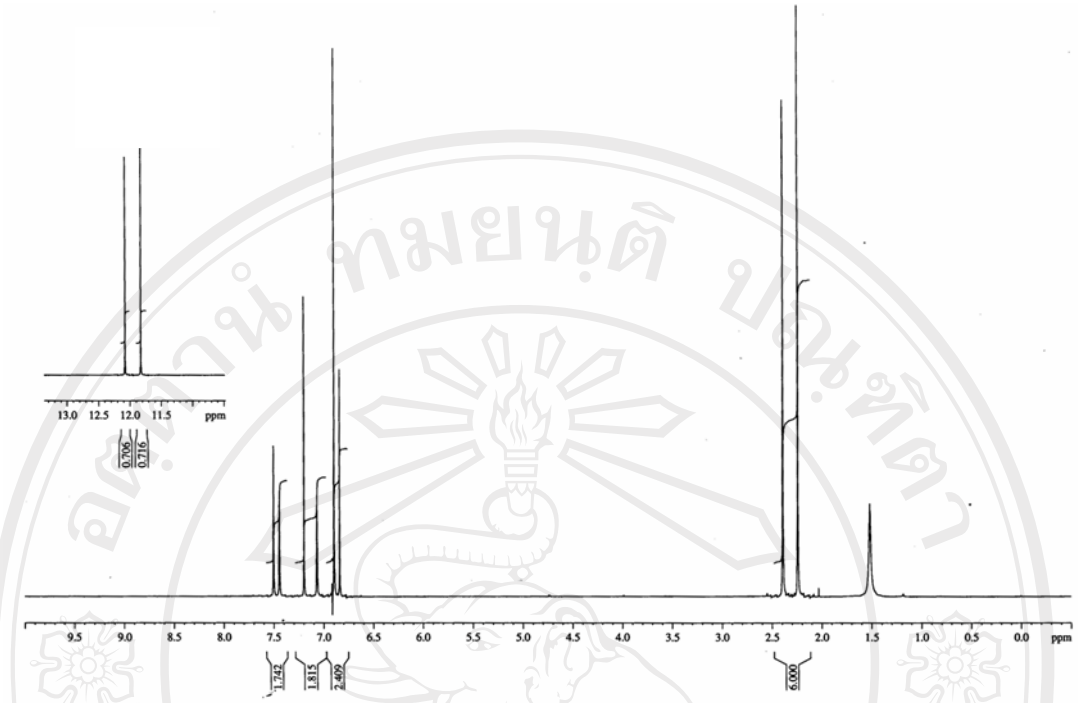


Figure 8. 500 MHz ^{1}H NMR ($CDCl_3$) spectrum of compound **21**

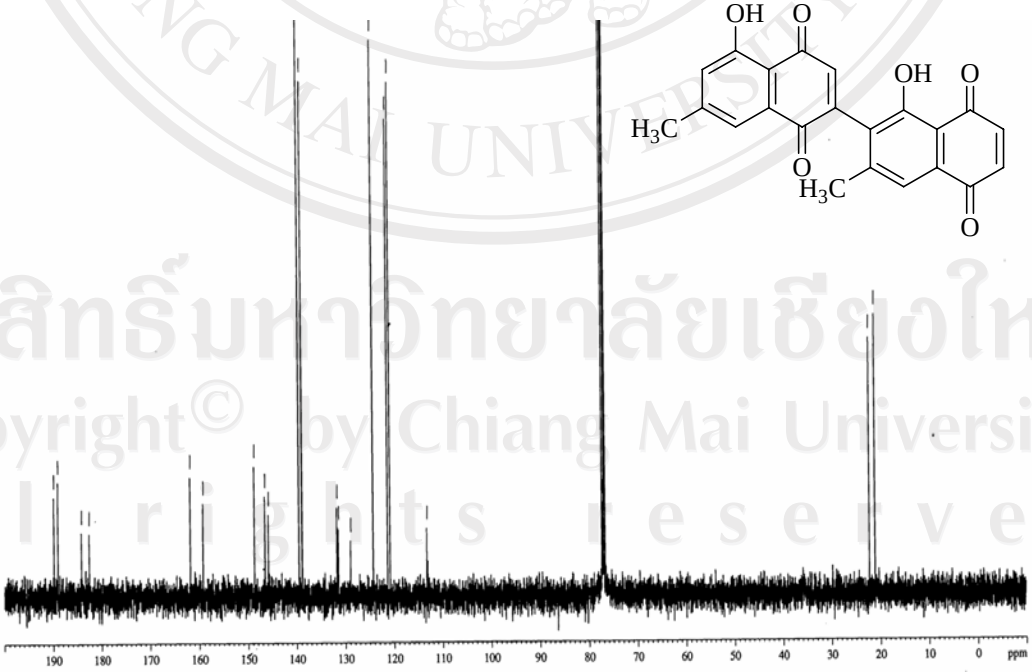


Figure 9. ^{13}C NMR ($CDCl_3$) spectra of compound **21**

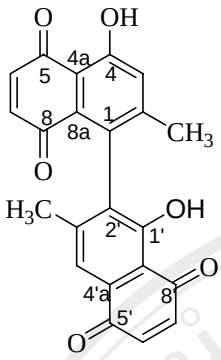


Figure 10. 500 MHz ^{1}H NMR ($CDCl_3$) spectrum of compound **23**

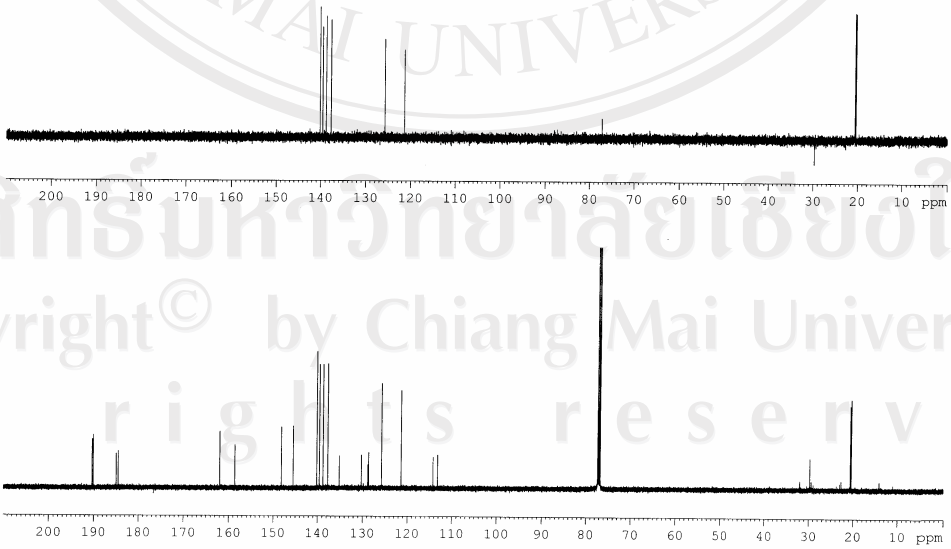


Figure 11. ^{13}C NMR ($CDCl_3$) and DEPT 135 spectra of compound **23**

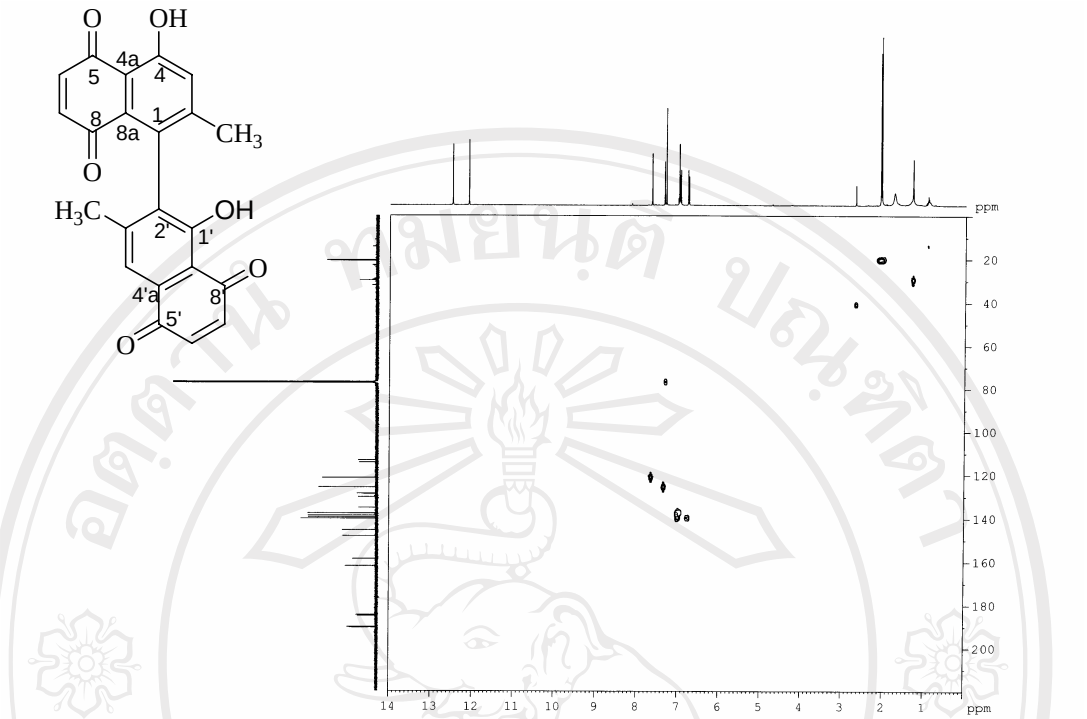


Figure 12. HMQC of compound **23**

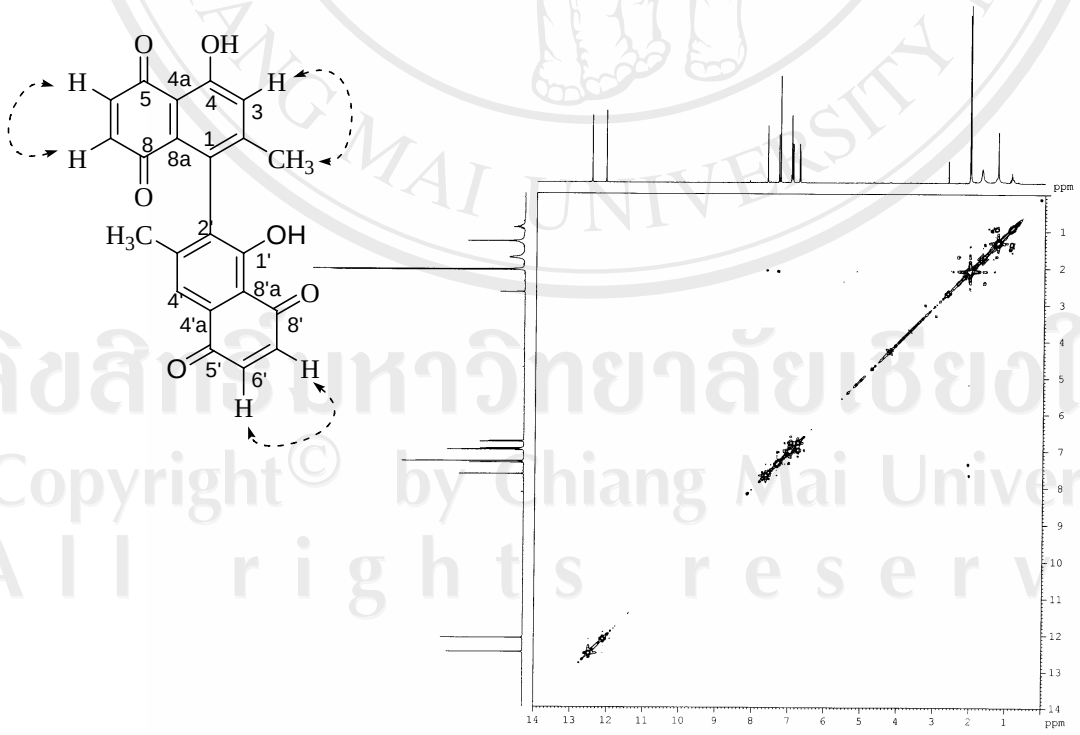


Figure 13. ^{1}H-^{1}H COSY spectrum of compound **23**

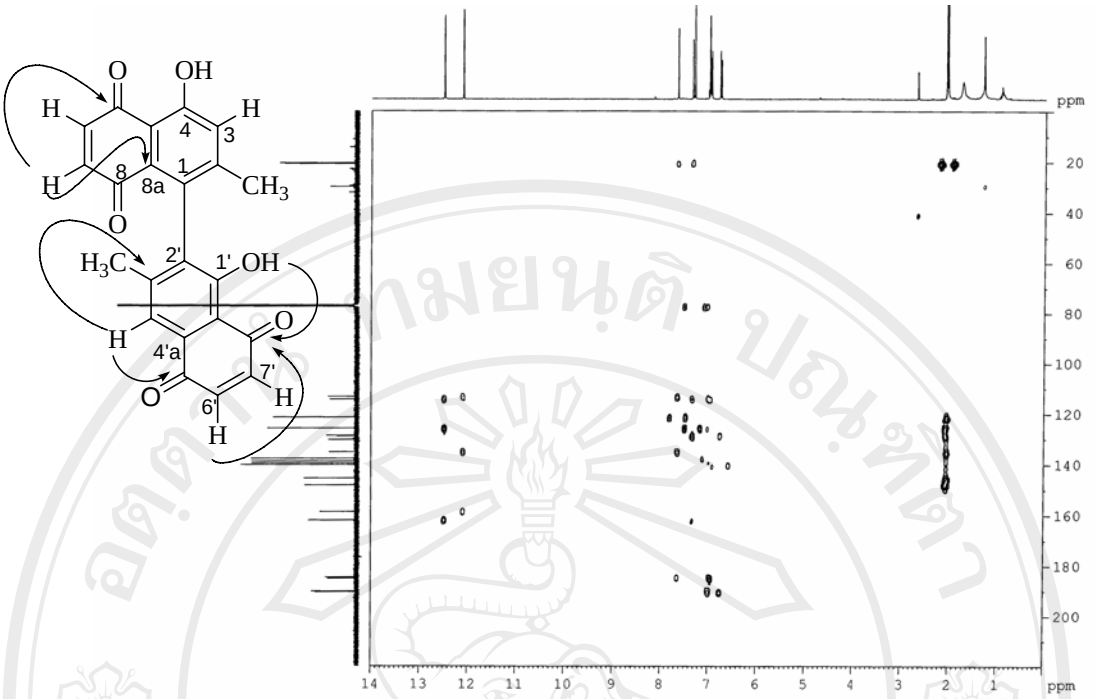


Figure 14. Long range ^{1}H-^{13}C correlations (HMBC) of compound **23**

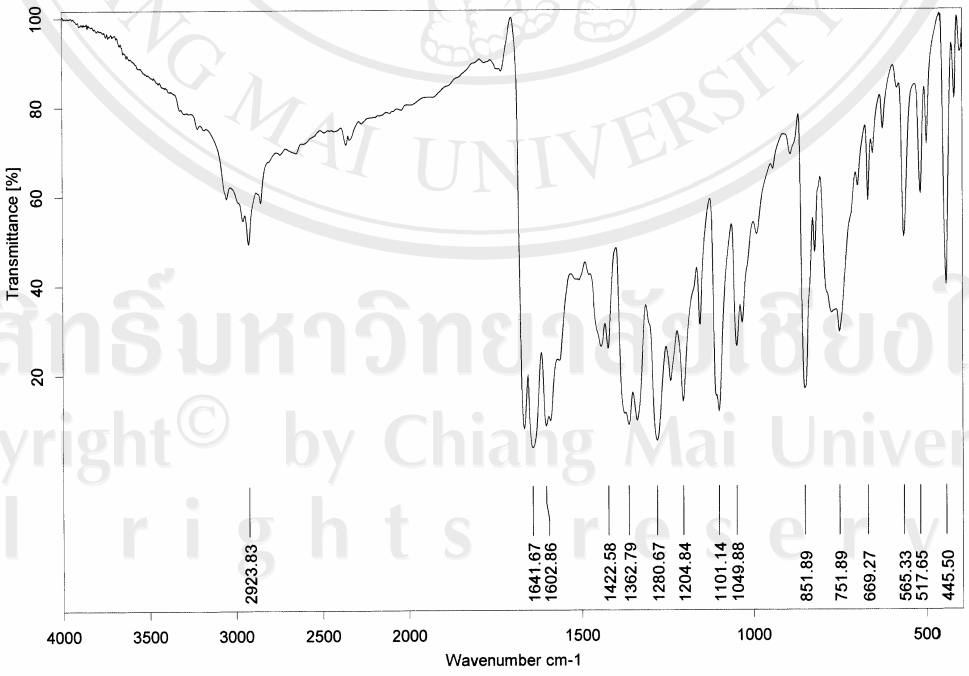


Figure 15. IR spectrum of compound **23**

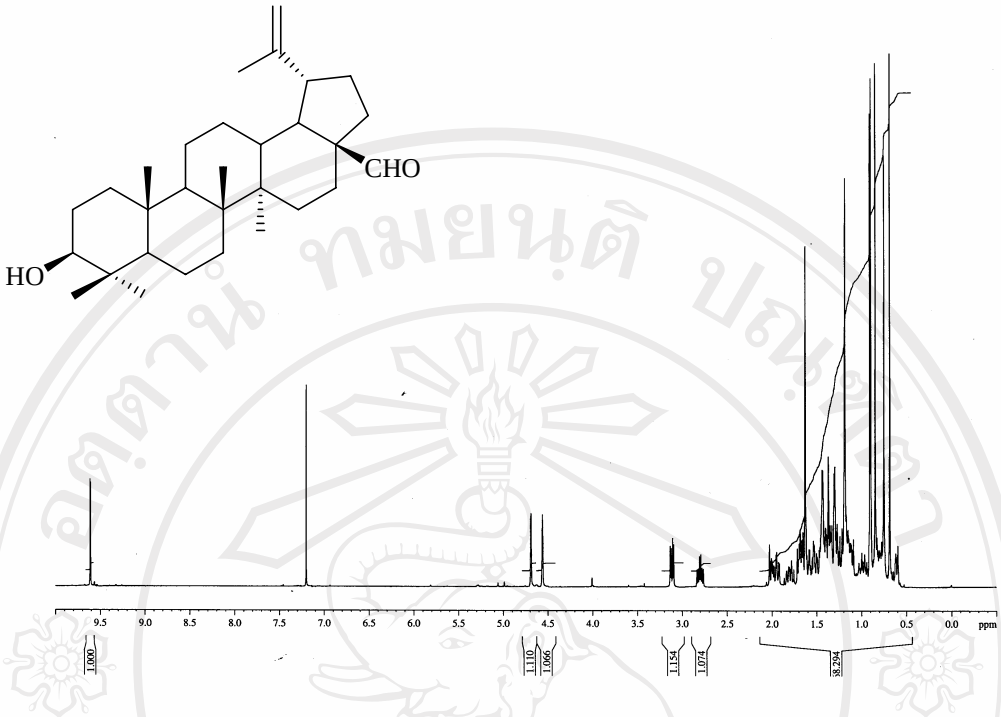


Figure 16. 500 MHz ^{1}H NMR ($CDCl_3$) spectrum of compound **67**

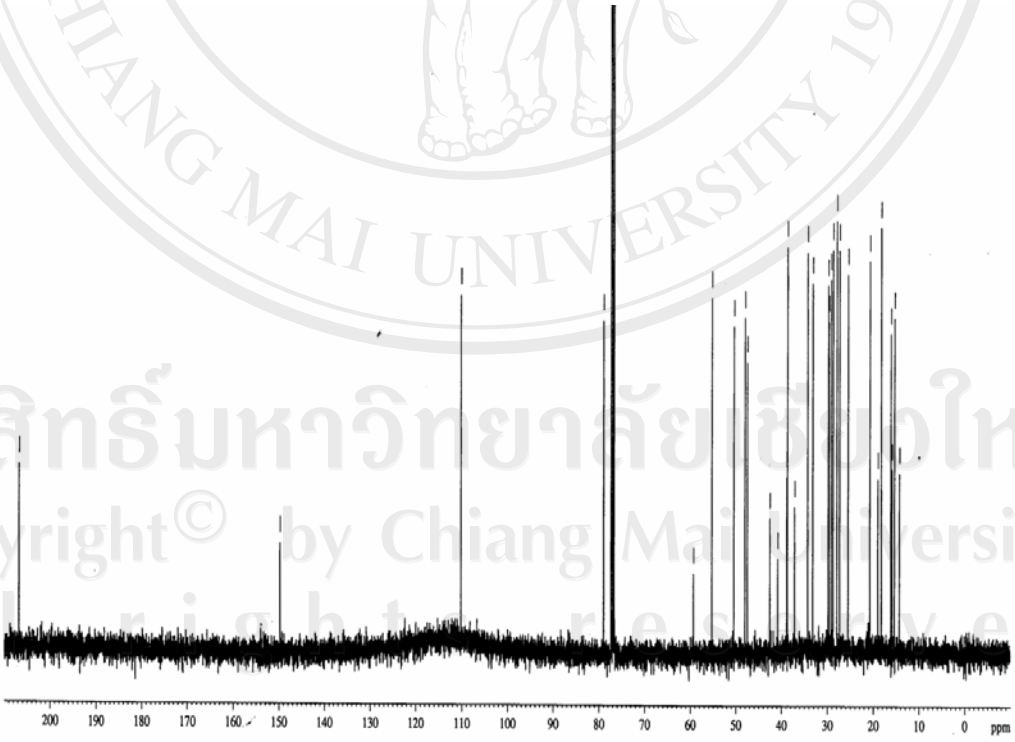


Figure 17. ^{13}C NMR ($CDCl_3$) spectra of compound **67**

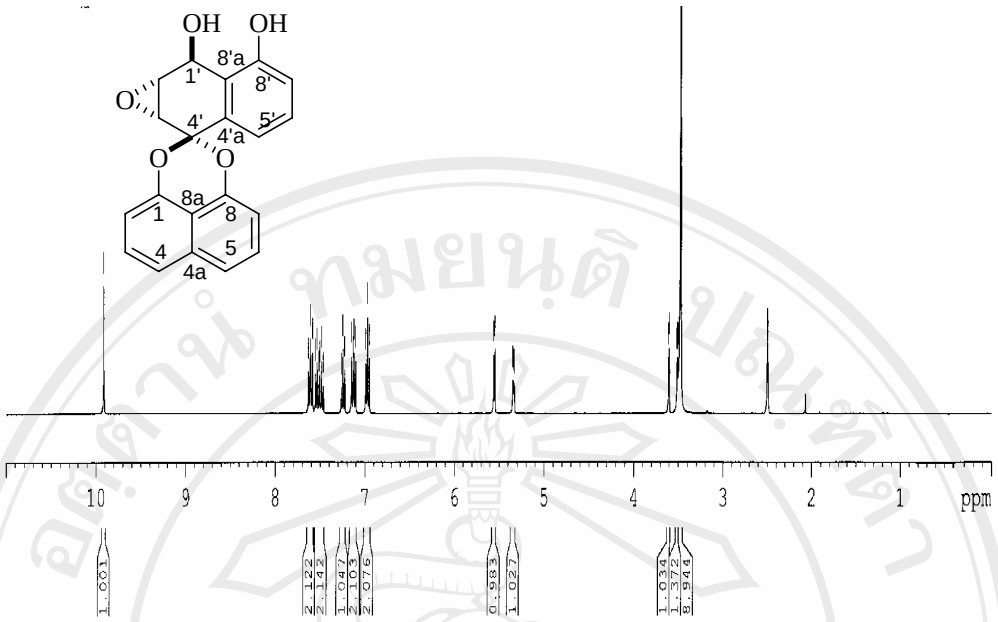


Figure 18. 500 MHz ^{1}H NMR (DMSO-d_6) spectrum of compound **70**

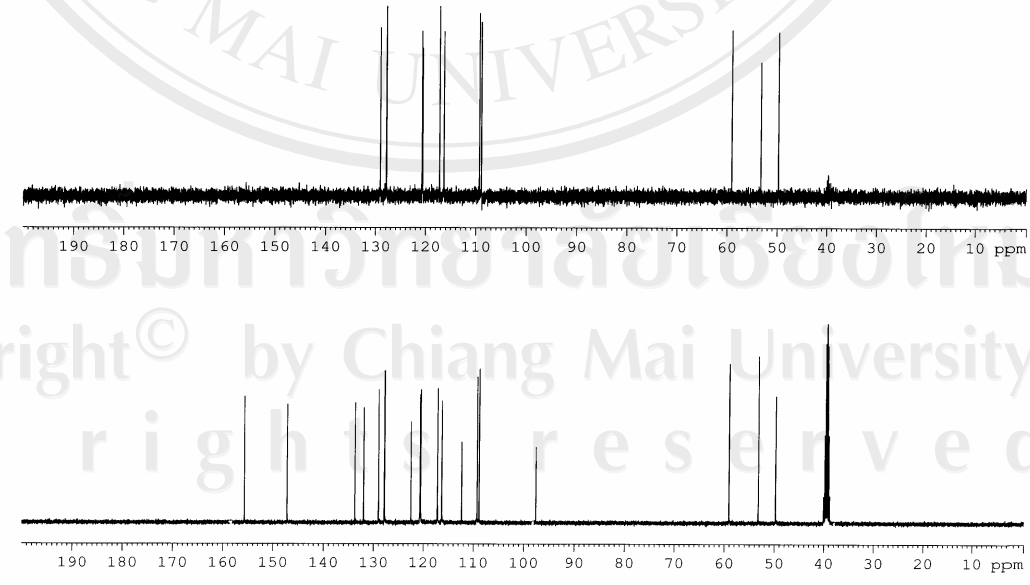


Figure 19. ^{13}C NMR (DMSO-d_6) and DEPT 135 spectra of compound **70**

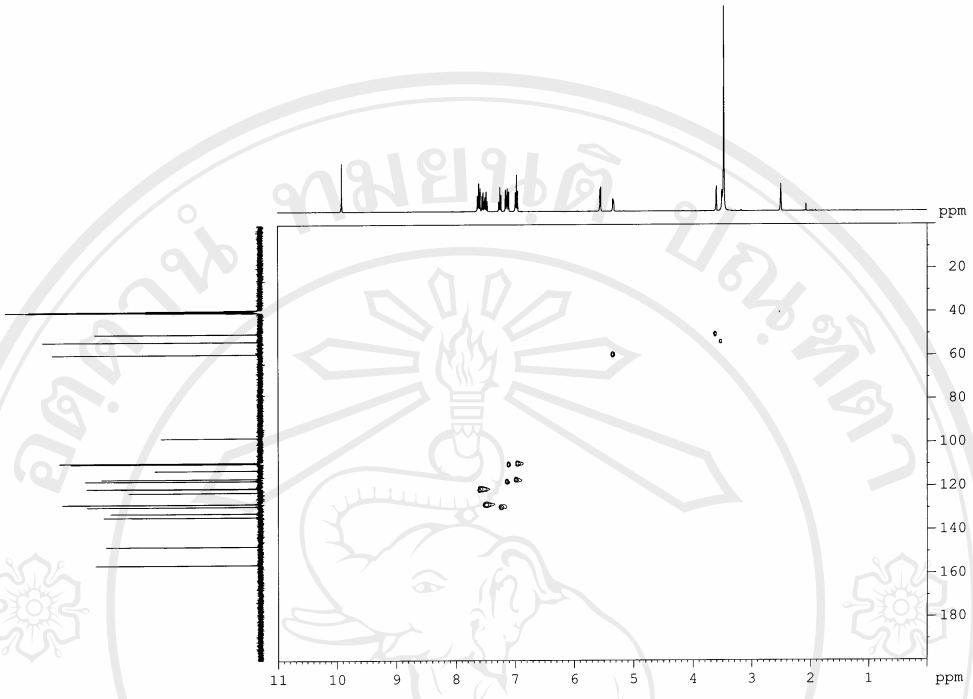


Figure 20. HMQC of compound **70**

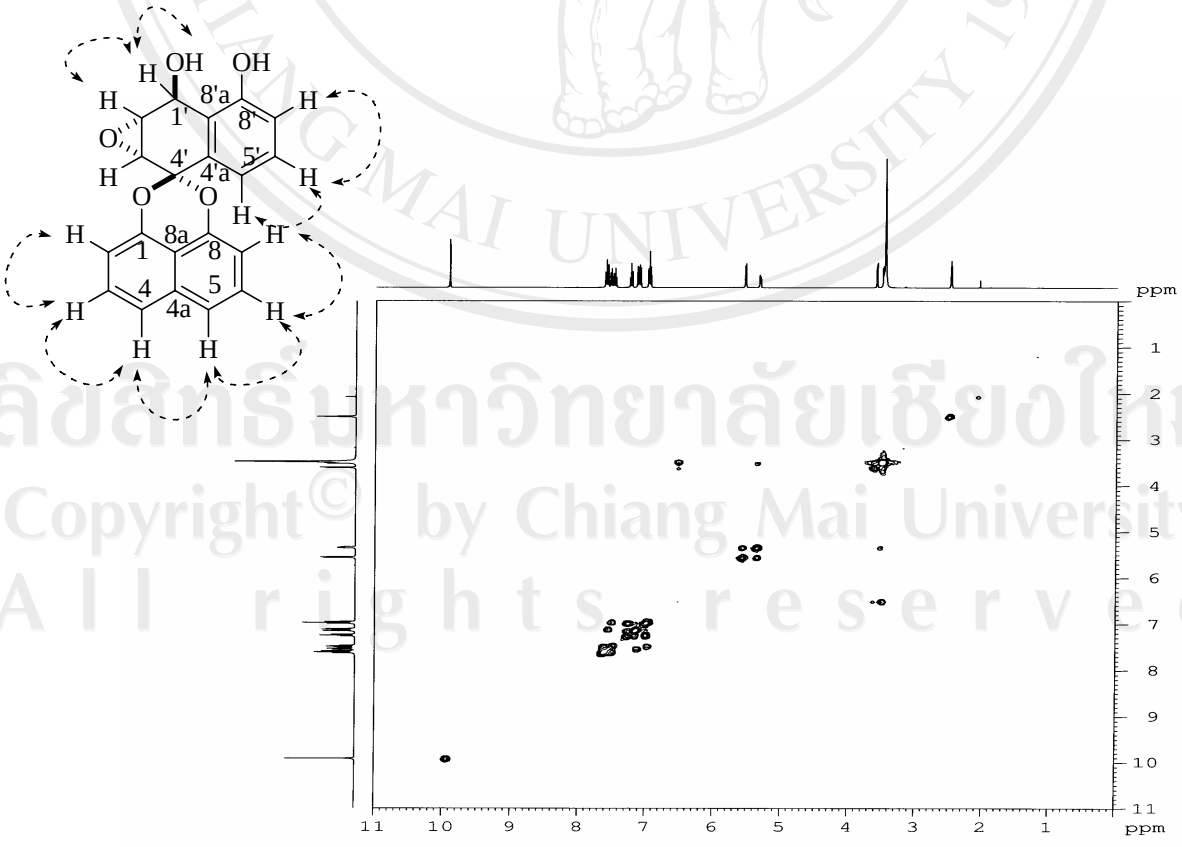


Figure 21. ^{1}H-^{1}H COSY spectrum of compound **70**

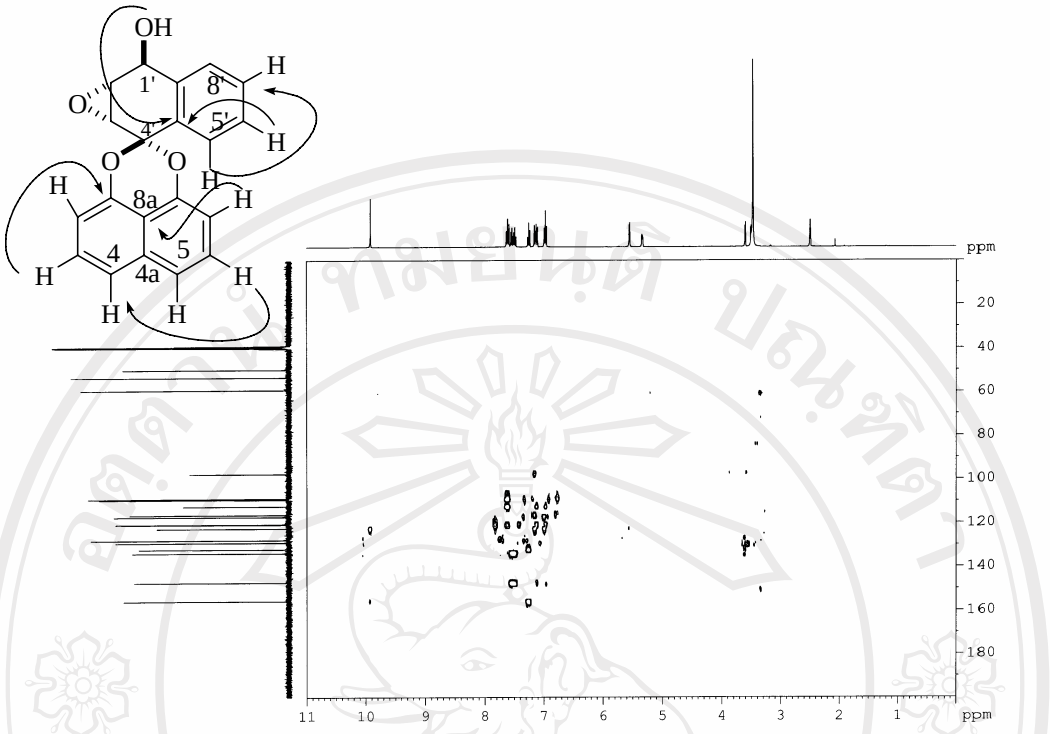


Figure 22. Long range ^{1}H-^{13}C correlations (HMBC) of compound **70**

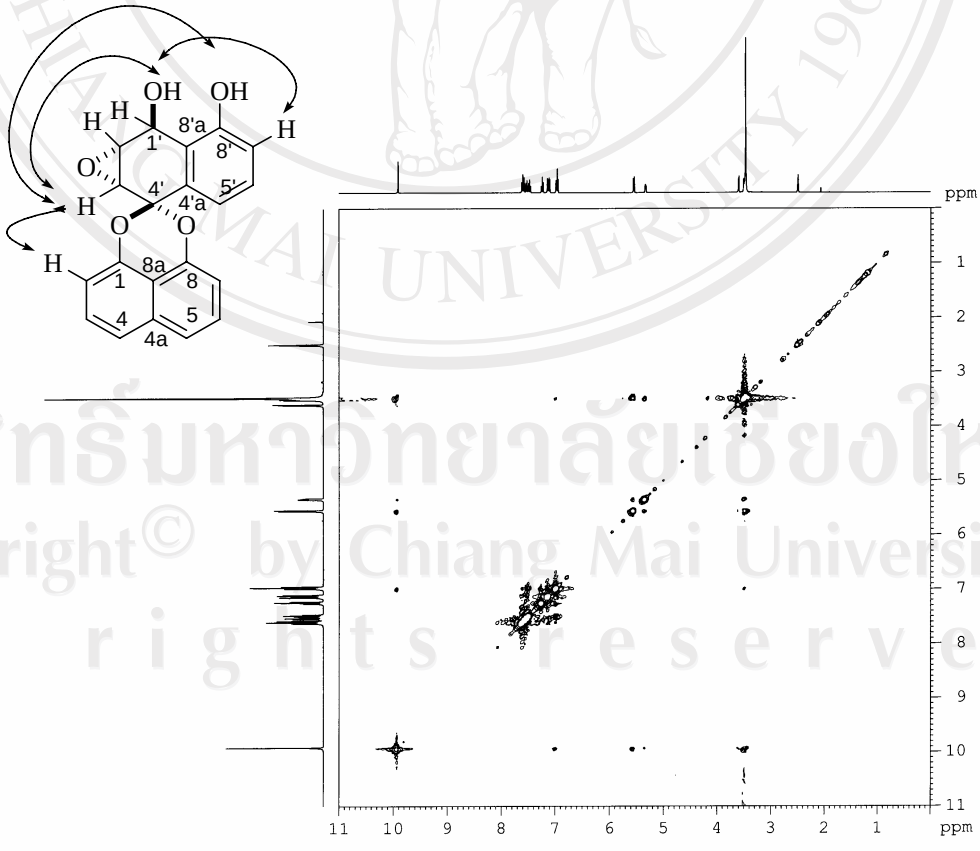


Figure 23. NOESY spectrum of compound **70**

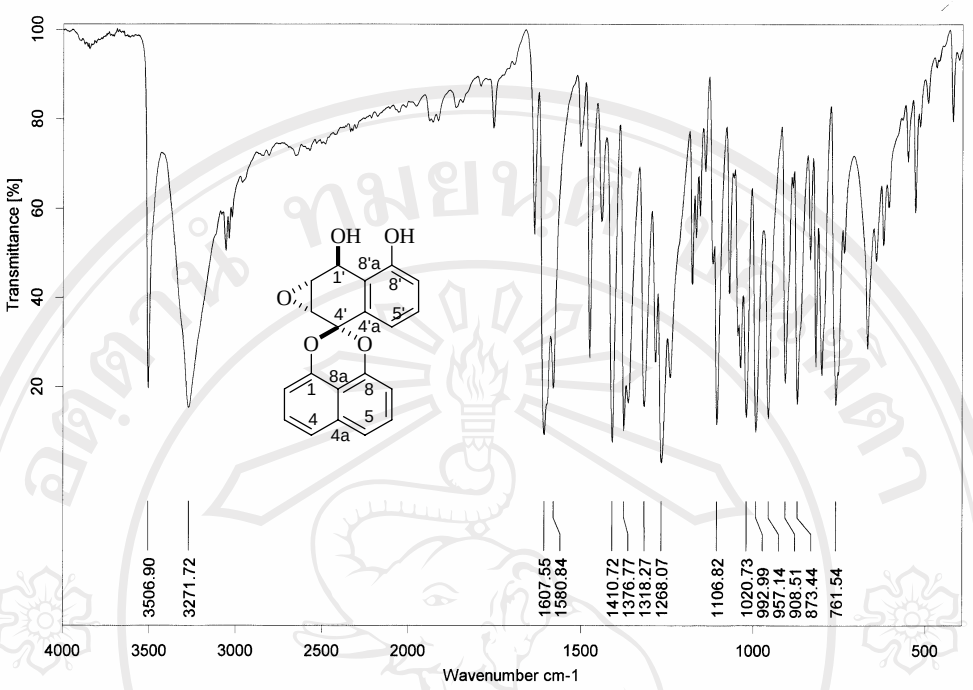


Figure 24. IR spectrum of compound **70**

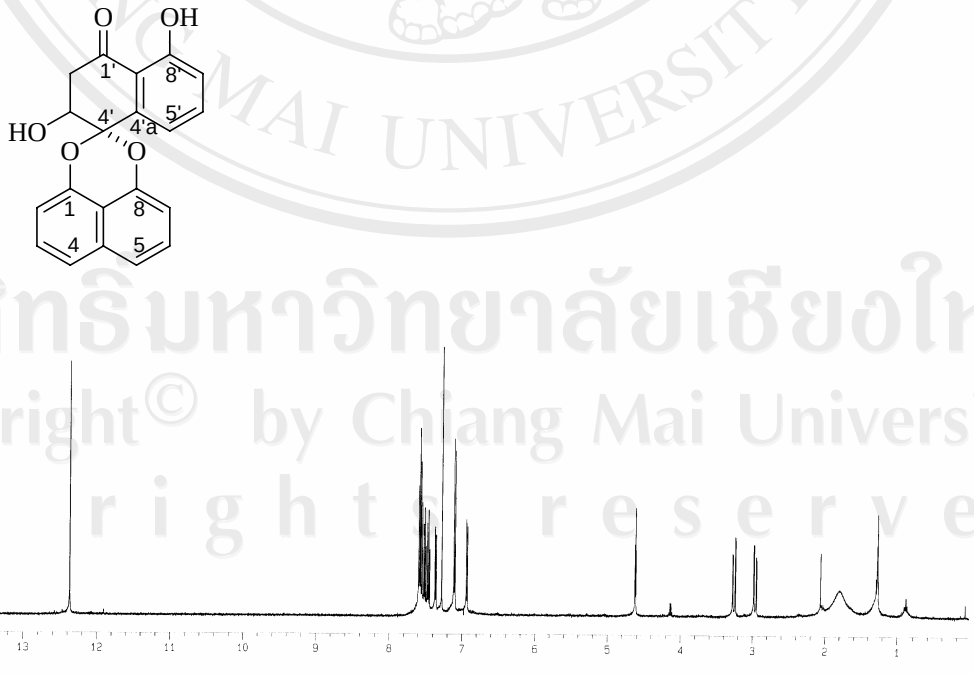


Figure 25. 500 MHz ^{1}H NMR ($CDCl_3$) spectrum of compound **71**

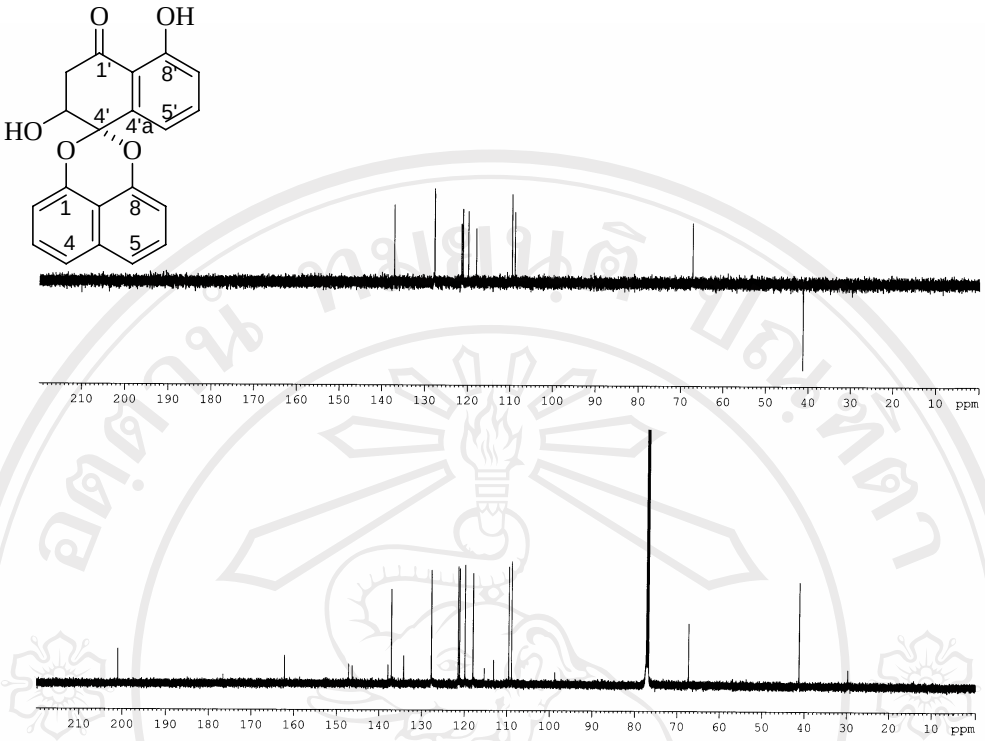


Figure 26. ^{13}C NMR($CDCl_3$) and DEPT 135 spectra of compound **71**

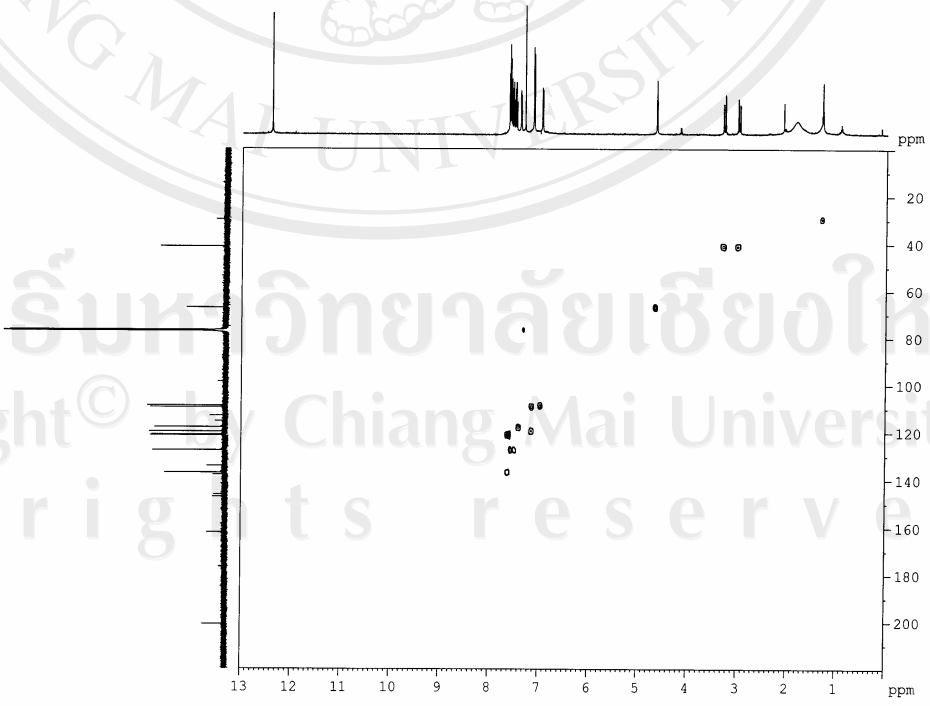


Figure 27. HMQC of compound **71**

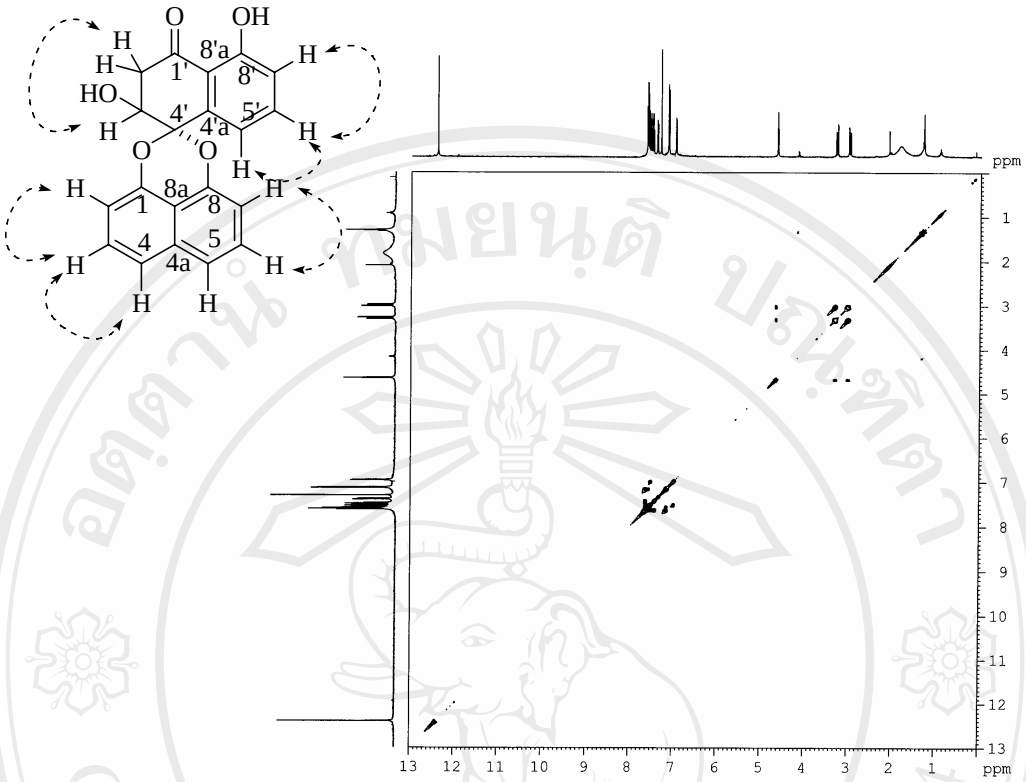


Figure 28. ^{1}H-^{1}H COSY spectrum of compound **71**

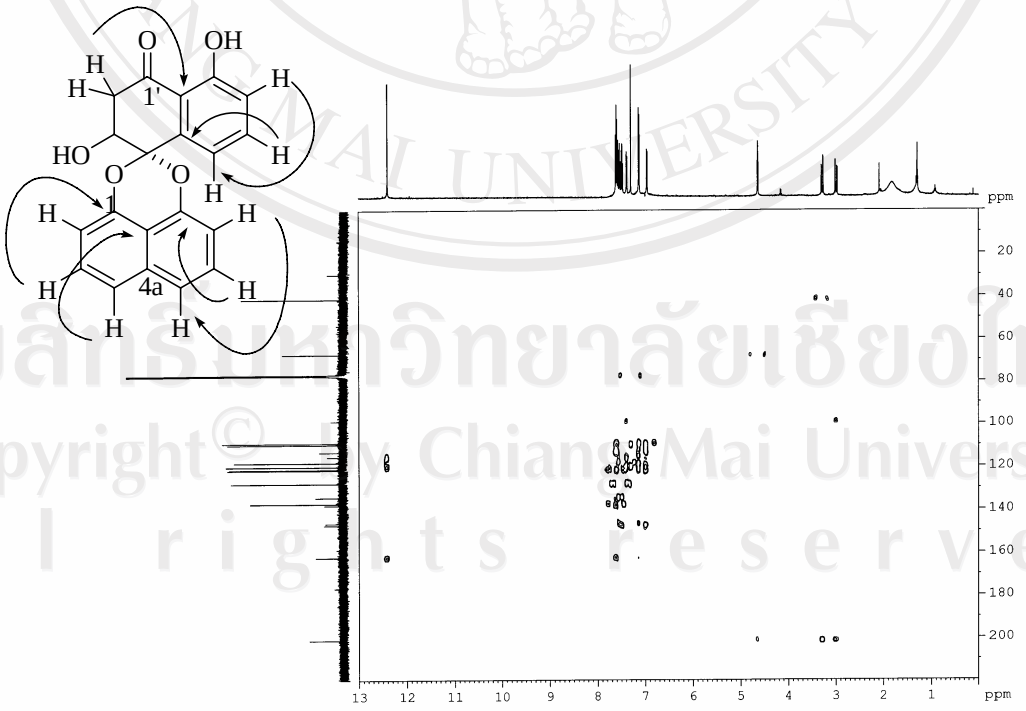


Figure 29. Long range ^{1}H-^{13}C correlations (HMBC) of compound **71**

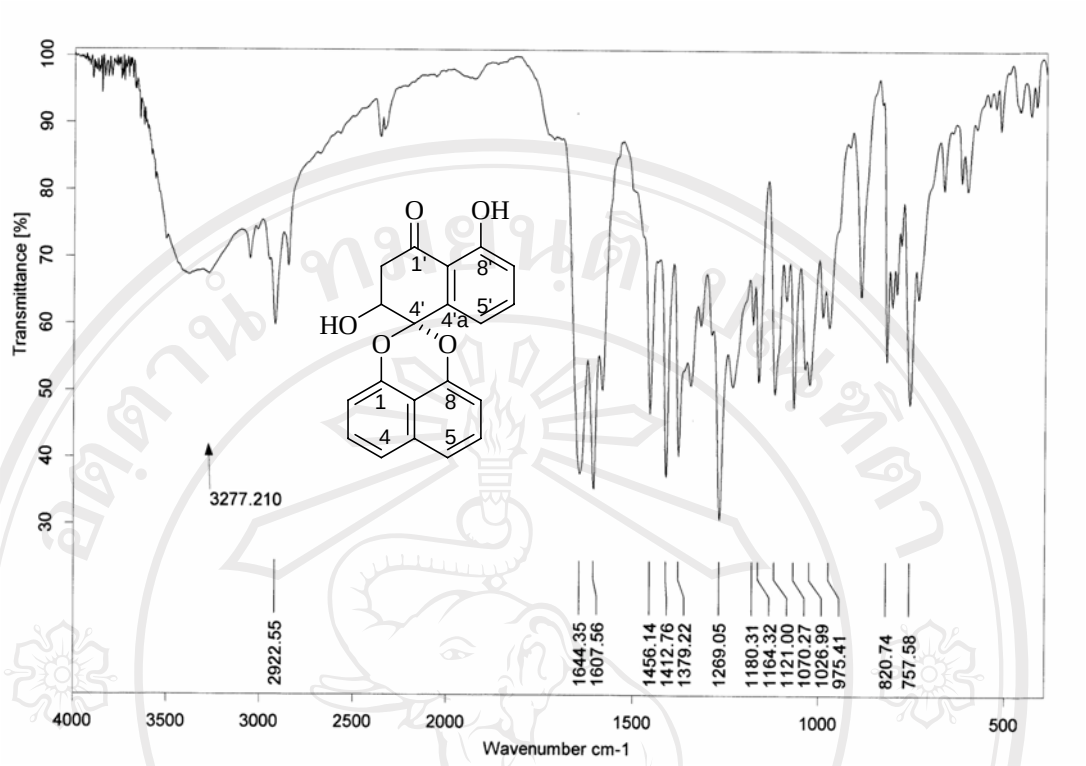


Figure 30. IR spectrum of compound **71**

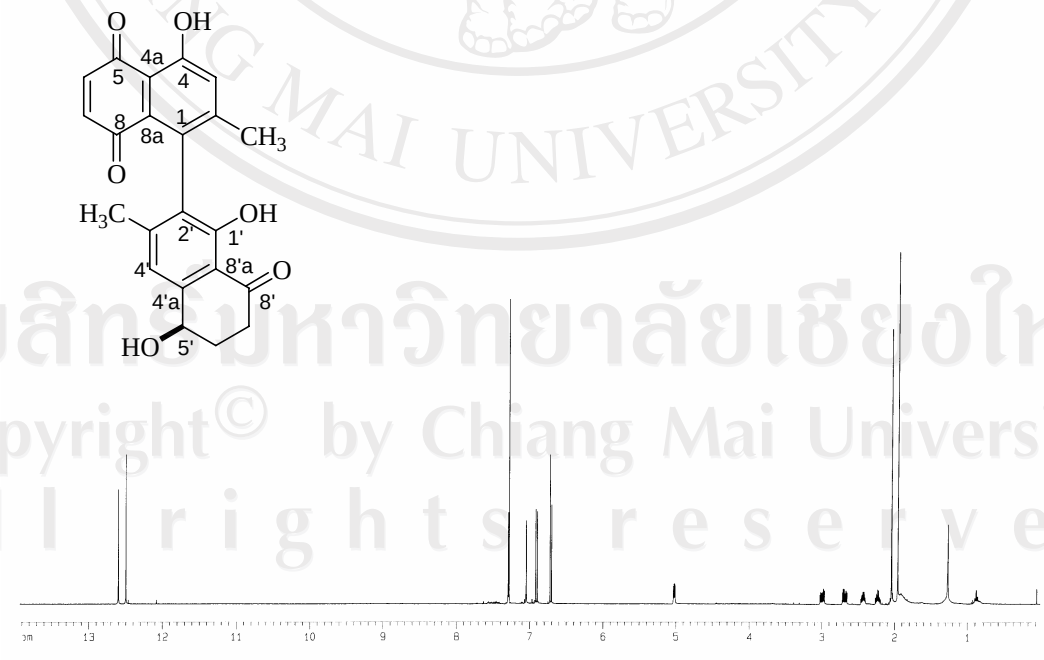


Figure 31. 500 MHz ^{1}H NMR ($CDCl_3$) spectrum of compound **72**

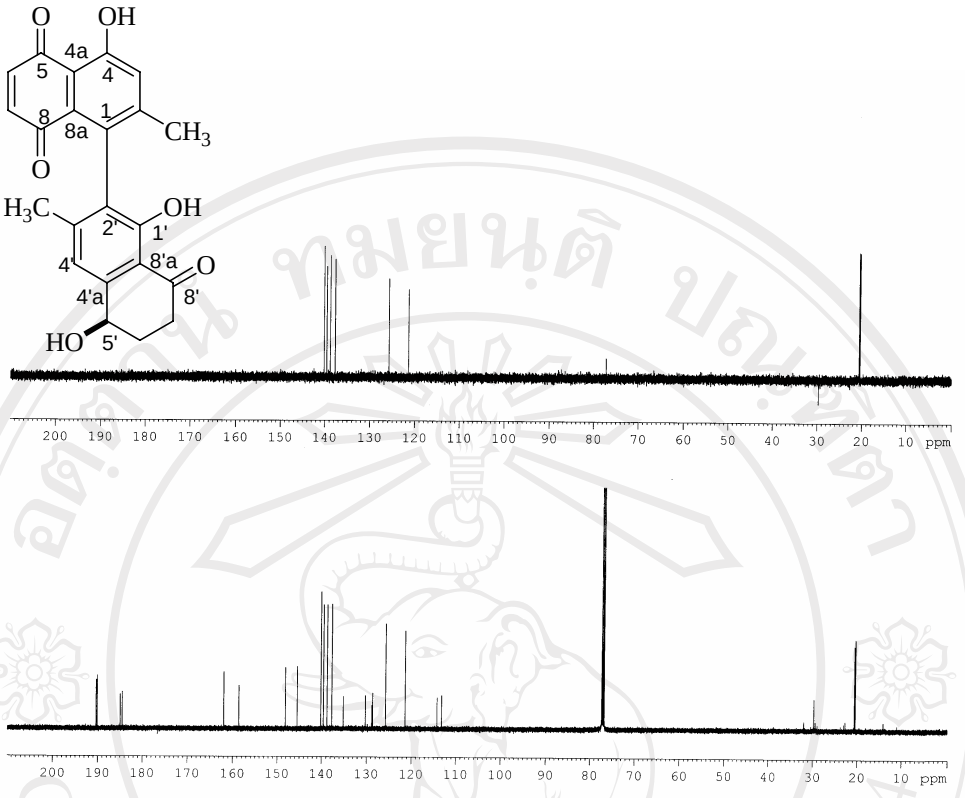


Figure 32. ^{13}C NMR ($CDCl_3$) and DEPT 135 spectra of compound **72**

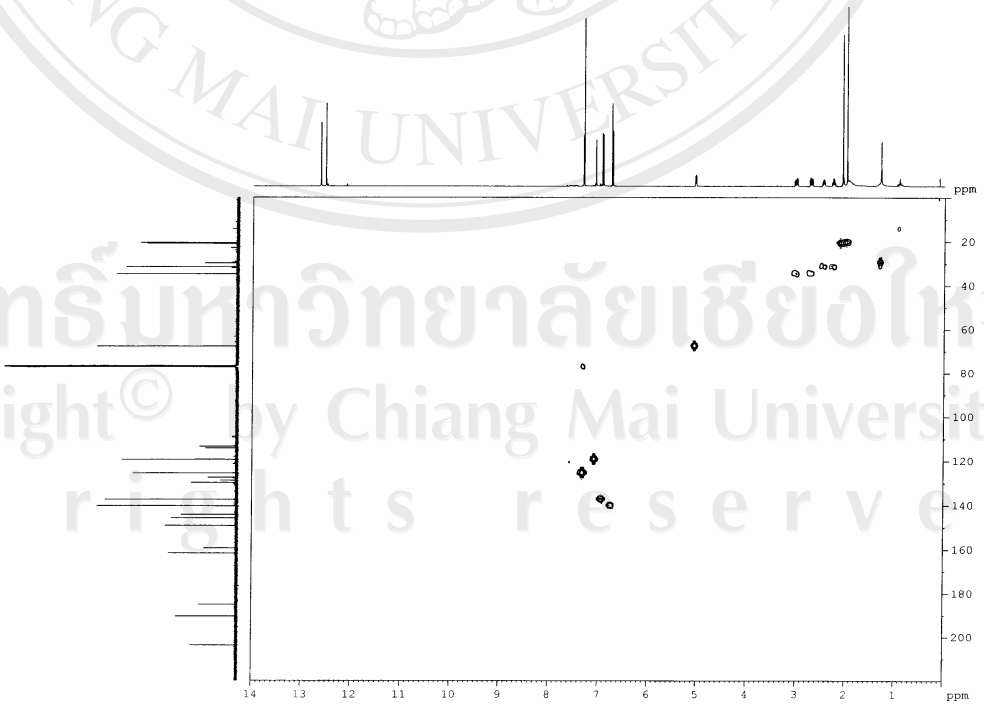


Figure 33. HMQC of compound **72**

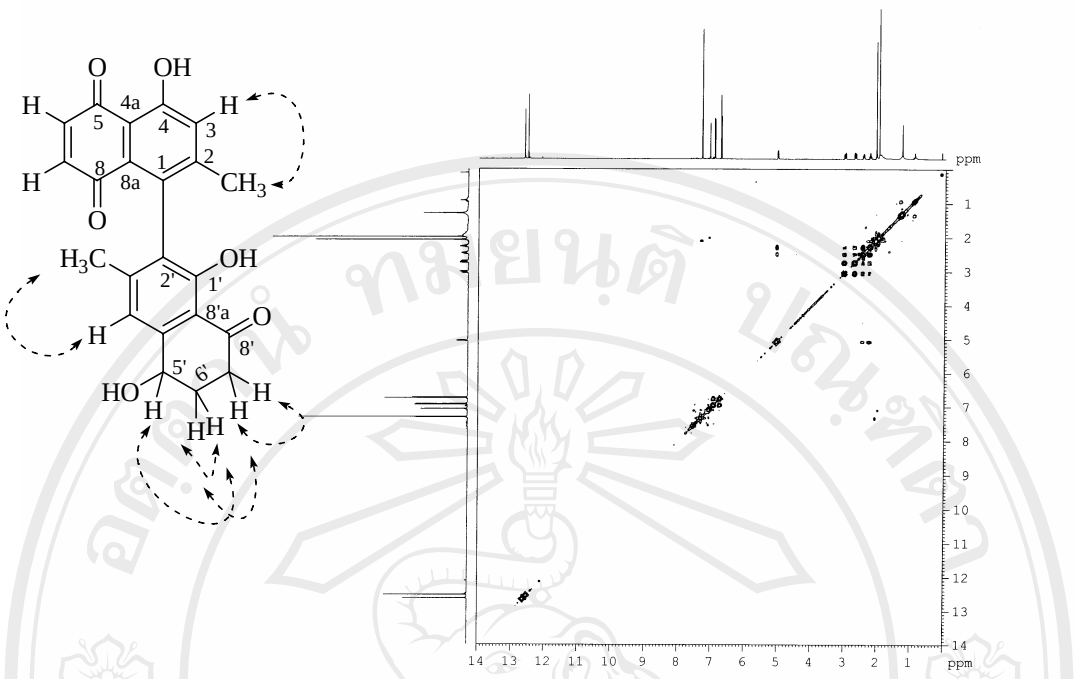


Figure 34. ^{1}H-^{1}H COSY spectrum of compound **72**

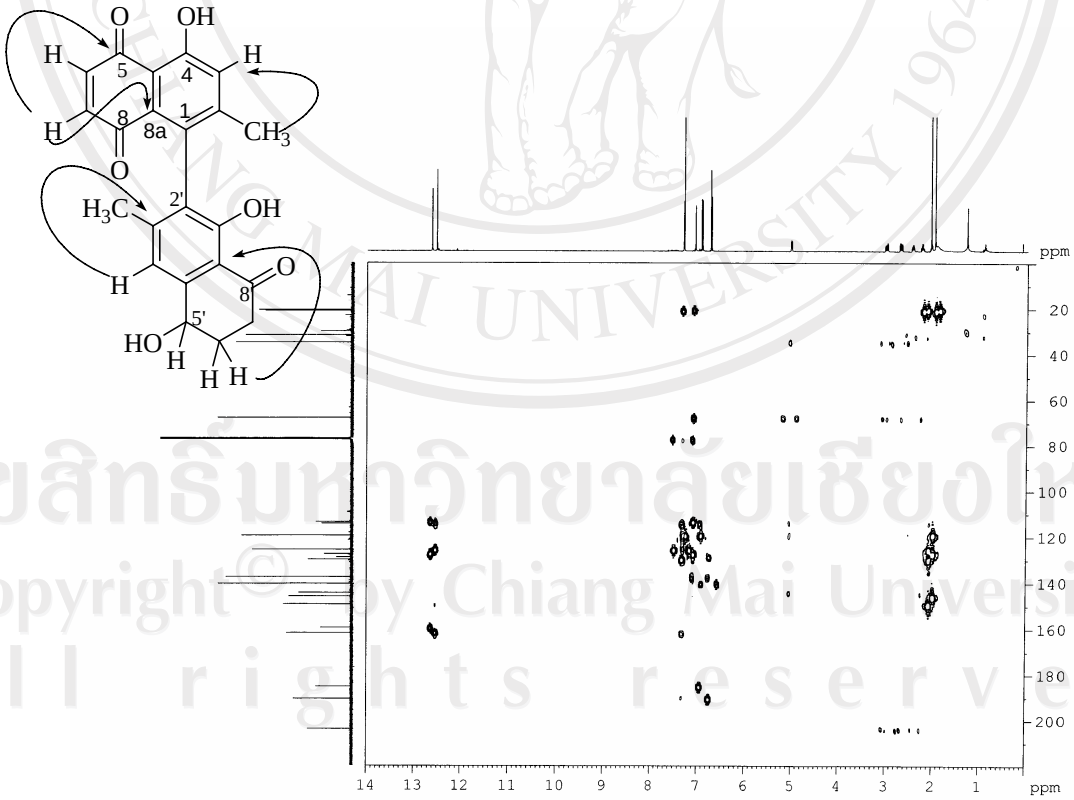


Figure 35. Long range ^{1}H-^{13}C correlations (HMBC) of compound **72**

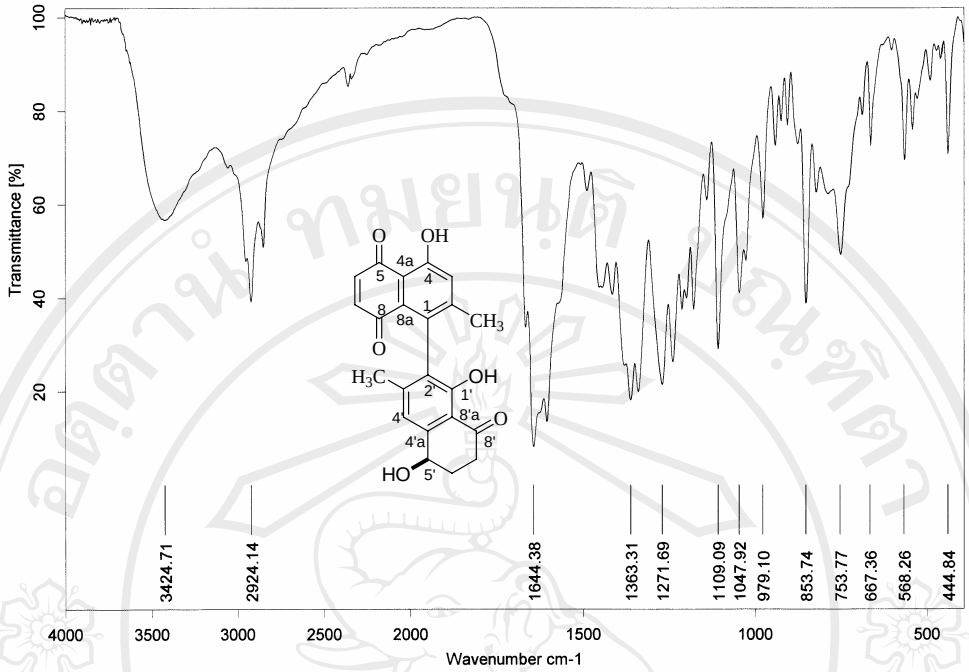


Figure 36. IR spectrum of compound **72**

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Previous Employment

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Master's degree thesis title: Esterification of Andrographolide

Publication

1. **Prajoubklang, A**., Sirithunyalug, B., Charoenchai, P., Suvannakad, R., Sriubolmas, N., Piyamongkol, S., Kongsaeree, P. and Kittakoop, P. 2005. Bioactive Deoxypreussomerins and Dimeric Naphthoquinones from *Diospyros ehretioides* Fruits: Deoxypreussomerins May Not Be Plant Metabolites But May Be from Fungal Epiphytes or Endophytes. *Chemistry & Biodiversity*, **2**: 1358-1367.
2. **Prajoubklang, A.,** Sirithunyalug, B., Charoenchai, P., Suvannakad, R., Sriubolmas, N., Piyamongkol, S., Kongsaeree, P. and Kittakoop, P. Bioactive Deoxypreussomerins and Dimeric Naphthoquinones from *Diospyros ehretioides* Fruits: Deoxypreussomerins May Not Be Plant Metabolites But May Be from Fungal Epiphytes or Endophytes. *Proceeding of Biothailand 2005*, 2-5 November 2005, The Queen Sirikit National Convention Center, Bangkok, Thailand.
3. Sirithunyalug, B., **Theerachayanan, T.,** Piyamongkol, S. and Kittakoop, P. Biological activities of compounds isolated from *Diospyros ehretioides* Fruits. *Proceeding of The Seventh Joint Seminar Recent Advances in Natural Product Research and Its Application.* 1-4 December 2006, Toyama, Japan.
4. **Theerachayanan, T.,** Sirithunyalug, B. and Piyamongkol, S. Antimalarial and Antimycobacterial Activities of Dimeric naphthoquinones from *Diospyos glandulosa* and *Diospyros rhodocalyx*. *Chiang Mai University Journal, in press.*

Special Training:

1. Short course on Intellectual Property: How to & Management, 18 February 2005, Sheraton Chiang Mai Hotel, sponsored by Intellectual Property Alumni Association (IPAA) and Japan External Trade Organization (JETRO)