

Chapter 3 Experimental

1. Source of plant material

The plant material of *Glyptopetalum sclerocarpum* Laws. was collected from Chaiyaphum province, in the north-eastern part of Thailand, during May 1993. It was identified by Dr. Thawatchai Santisuk, Royal Forest Department, Bangkok, Thailand. A voucher specimen was deposited in the herbarium of the Department of Pharmaceutical Botany, Faculty of Pharmaceutical Science, Bangkok, Thailand.

2. Phytochemical techniques

2.1) Chromatographic techniques

2.1.1) Thin-layer chromatography (TLC)

Technique	: One way ascending
Adsorbent	: Silica gel 60G (No. 7731, Merck) and silica gel 60 HF ₂₅₄ (No. 7739, Merck) in ratio 2:1
Plate sizes	: 5 x 20 cm, 10 x 20 cm or 20 x 20 cm
Layer thickness	: 0.25 mm
Activation	: Air-dried for 15 minutes and then warm in hot-air oven at 110 °C for 1 hour
Solvent system	: Various solvent systems depending on materials
Distance	: 15 cm
Detection	: 1) Visual detection under daylight 2) Visual detection under UV light (254 and 366 nm) 3) Spraying with 1% H ₂ SO ₄ in EtOH and heating the plates at 110 °C for 15 minutes

2.1.2) Column chromatography

2.1.2.1) Conventional column chromatography (cc)

Column sizes	: The glass column 3/4 - 4 inches in diameter were used depending on the quantity of sample to be separated.
Adsorbent	: Silica gel 60 (No. 9385, Merck, particle size 0.040-0.063 mm)
Packing method	: Wet packing
Solvent system	: Various solvent systems depending on materials

2.1.2.2) Flash chromatography (fc)

Column sizes	: The glass column 3/4 - 2 inches in diameter were used depending on the quantity of sample to be separated.
Adsorbent	: 1) Silica gel 60 (Merck no 15111, particle size 0.015-0.040 mm) 2) Silica gel 60 (Merck no. 9385, particle size 0.040-0.063 mm)
Packing method	: Wet packing
Solvent system	: Various solvent systems depending on materials

2.1.2.3) Gel filtration chromatography

Column sizes	: The glass column 1 inches in diameter
Adsorbent	: Sephadex [®] LH20 (Pharmacia Biotech)
Packing method	: Wet packing
Solvent system	: Various solvent systems depending on materials

2.2) Melting point

Melting points were determined on a Fisher-Johns Melting Point Apparatus (Faculty of Pharmaceutical Science, Chulalongkorn University). The melting point were uncorrected.

2.3) Optical rotation

Optical rotations were measured on a Perkin Elmer 341 polarimeter (Faculty of Pharmaceutical Science, Chulalongkorn University).

2.4) Spectroscopy

2.4.1) Ultraviolet (UV) absorption spectra

The UV absorption spectra were obtained on a Hitachi U-2000 Spectrophotometer (Faculty of Pharmacy, Silpakorn University). Methanol was employed as the solvent for all compounds.

2.4.2) Infrared (IR) spectra

The IR spectra were obtained on a Nicolet Magna-IR Spectrometer 750 (Faculty of Pharmacy, Silpakorn University). The material were examined as dry film on KBr cell.

2.4.3) Mass spectra (MS)

The EIMS were determined by direct inlet system operating at 70 eV. Two different instruments were used to obtain low resolution MS.

1. JEOL Mass Spectrometer Model DX300 (The Scientific and Technological Research Equipment Center, Chulalongkorn University) for compounds GS-T-1, GS-T-2, GS-Y1-1 and GS-Y1-2.

2. Fisons AG Trio 2000 quadrupole Mass Spectrometer (Department of Chemistry, Faculty of Science, Chulalongkorn University) for the rest of the compounds.

2.4.4) Proton and carbon nuclear magnetic resonance (^1H NMR and ^{13}C NMR) spectra

The ^1H and ^{13}C NMR spectra were obtained on a JEOL JNM-A500 (Alpha series) 500 MHz NMR Spectrometer (The Scientific and Technological Research Equipment Center, Chulalongkorn university). Deuteriochloroform (CDCl_3) containing 0.03% v/v tetramethylsilane (TMS) was used as the operating solvent. The chemical shifts were reported in ppm scale using the chemical shift of TMS at 0.00 ppm as the reference signal. The operating parameters were adjusted to required experiments of ^1H NMR, ^{13}C NMR, DEPT, ^1H - ^1H cosy, ^1H - ^{13}C cosy, HMQC, HMBC ($^nJ_{\text{C-H}} = 8$ Hz) and NOESY.

2.5) Solvents

The solvents of commercial grade were redistilled before used.

3. Extraction and separation

The extraction and separation of chemical constituents from the stem bark of *Glyptopetalum sclerocarpum* Laws. are summarized in Scheme 10. The process was as follows :

3.1) Extraction

The air-dried and ground stem bark (700 g) was extracted successively by maceration with *n*-hexane (7 days for 4 times), chloroform (3 days for 4 times) and methanol (3 days for 4 times), to yield 49.4, 26.0 and 23.3 g of the extracts, respectively. Each extract was examined for its brine-shrimp lethality (BSL) activity. The *n*-hexane and chloroform extracts which showed positive result ($BSLD_{50} < 10 \mu\text{g/ml}$), were dissolved in a small amount of chloroform and added with acetone. The elastic gum formed in this step (4.7 and 2.0 g) were discarded. The solution of both extracts were evaporated to dryness and crystallized in acetone. The chloroform extract gave 2.7 g of red crystal (C-CRY), leaving 18.6 g of mother liquor (C-ML), while *n*-hexane extract gave 6.0 g of red crystal (H-CRY-1) and 36.0 g of mother liquor (H-ML-1). The crystal H-CRY-1 was further recrystallized in acetone to yield 2.4 g of more purified red crystalline material (H-CRY-2) and 3.6 g of another portion of mother liquor (H-ML-2). $BSLD_{50}$ of all extractive fractions were less than $10 \mu\text{g/ml}$.

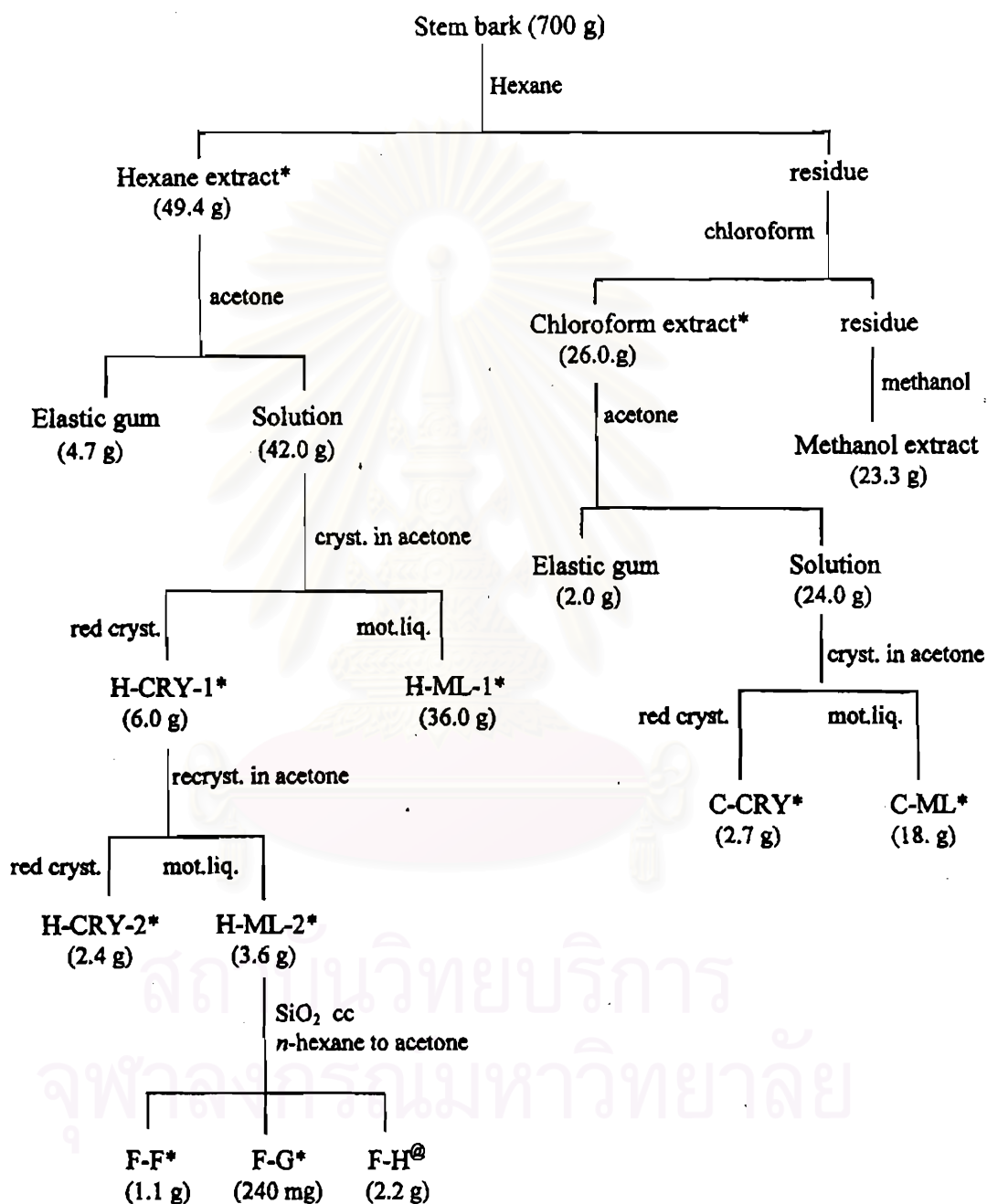
3.2) Separation

The H-ML-1 fraction was subjected to a gel filtration cc using a solvent system of $\text{CHCl}_3/\text{MeOH}$ (2:1). Based on color and the TLC monitoring, four fractions were obtained : fraction H-ML-1/1 (16.0 g) with dark color, fractions H-ML-1/2 (7.4 g) and H-ML-1/3 (12.2 g) with red color, and finally, a colorless fraction H-ML-1/4 (191 mg). BSL activity was not detected in fraction H-ML-1/1 and was quite weak in fraction H-ML-1/4 ($BSLD_{50} 80 \mu\text{g/ml}$). Only the two red fractions exhibited very strong activity ($BSLD_{50} < 10 \mu\text{g/ml}$) and were selected for further study.

TLC examination revealed the chemical constituents of fractions H-ML-1/2 and H-ML-1/3 to be similar, except for the non-polar colorless compounds presented in the latter. Therefore, fraction H-ML-1/3 was subjected to partition between *n*-hexane and methanol. The non-polar and non-bioactive constituents were easily separated in *n*-hexane layer (6.6 g). The red methanol layer (2.3 g) was combined with fraction H-ML-1/2 and crystallized in acetone to obtain 3.1 g of red crystal and 6.6 g of mother liquor. The crystal still consisting of several red substances, was further fractionated by cc using CHCl₃/MeOH gradient (99:1 to 1:2) into fractions F-A (1.7 g) and F-B (1.1 g). The mother liquor was subjected to another cc eluted with gradient of *n*-hexane with increasing amount of acetone, to give fractions F-C (0.8 g), F-D (1.5 g) and F-E (4.1 g).

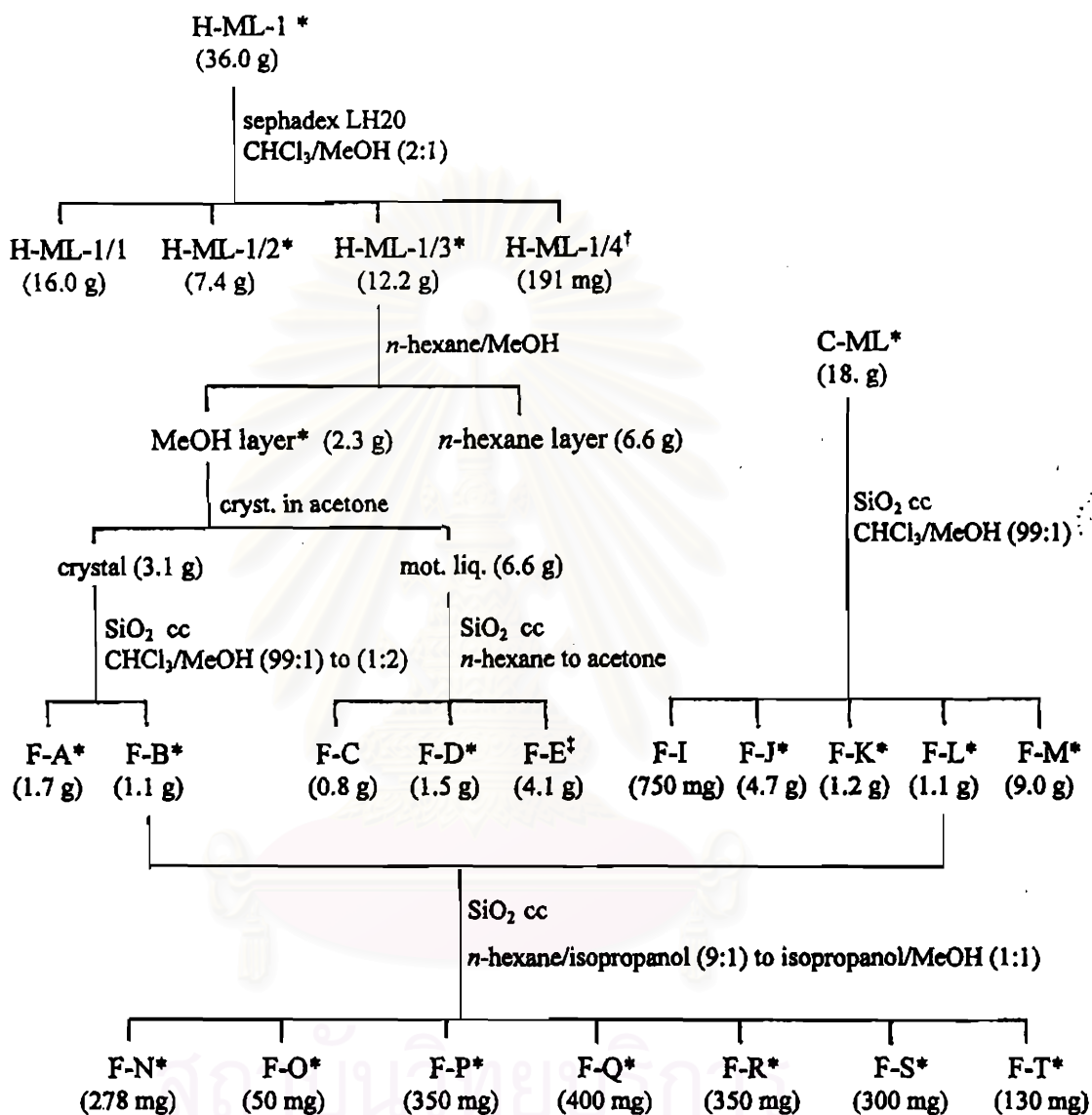
The second mother liquor portion from hexane extract, H-ML-2, was chromatographed over SiO₂ eluted with *n*-hexane/acetone gradient. It was separated into fractions F-F (1.1 g), F-G (240 mg) and F-H (2.2 g) based on their TLC pattern. Cc of the mother liquor from chloroform extract, C-ML, using CHCl₃/MeOH (99:1) solvent system also gave 5 eluted fractions : F-I (750 mg), F-J (4.7 g), F-K (1.2 g), F-L (1.1 g) and F-M (9.0 g). All fractions, except F-C and F-I, gave positive results to BSL test.

Fractions F-B (1.1 g) and F-L (1.1 g) possessed several similar chemical constituents, they were thus combined and further fractionated by cc using various solvent system : *n*-hexane/isopropanol (9:1), *n*-hexane with increasing amount of isopropanol, isopropanol and isopropanol with increasing amount of MeOH to the final ratio of (1:1). Seven fractions, F-N (278 mg), F-O (50 mg), F-P (350 mg), F-Q (400 mg), F-R (350 mg), F-S (300 mg) and F-T (130 mg), were divided based on TLC examination. All of them gave positive results to BSL test.



Scheme 10. Extraction and separation of chemical constituents from the stem bark of *Glyptopetalum sclerocarpum*.

(* BSLD₅₀ < 10 µg/ml, @ BSLD₅₀ = 30.0 µg/ml)



Scheme 10. (cont.) (* BSLD₅₀ < 10 µg/ml,

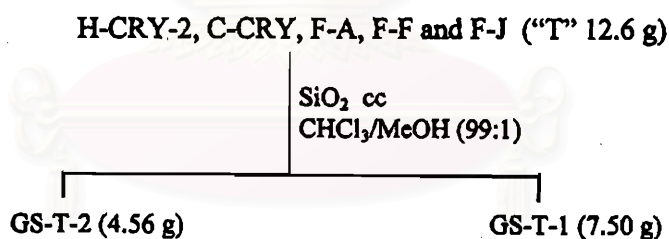
[†] BSLD₅₀ = 80.0 µg/ml, [‡] BSLD₅₀ = 26.6 µg/ml)

4. Isolation of pure compounds

Only the separated fractions that showed positive results to BSL test, except for F-D, F-N and F-S were further isolated. F-D consisted of the main red compounds similarly found in F-A and F-B, but it contaminated with several non-interesting compounds, while fractions F-N and F-S possessed rather small quantities of several chemical constituents. Therefore, these fractions were not pursued.

4.1) Isolation of compounds in "T" series

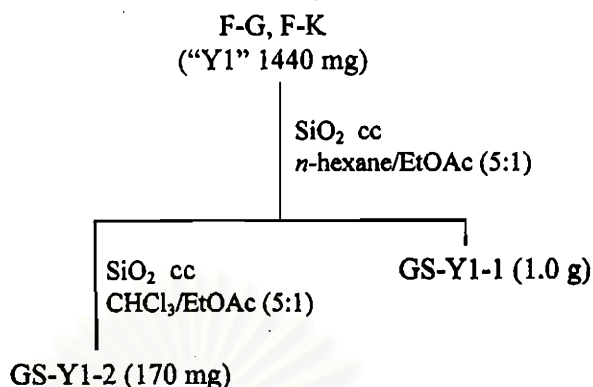
Fractions H-CRY-2, C-CRY, F-A, F-F and F-J were all characterized as being red crystalline matter. They possessed the same two red compounds. The more polar one showed TLC *R_f* values as 22 β -hydroxy-tingenone (1) previously isolated from this plant, and was coded as GS-T-1 for this investigation. All fractions were combined to give crude "T" (12.6 g) and subjected to cc using CHCl₃/MeOH (99:1) solvent system to yield 7.50 g of GS-T-1 and a less polar compound coded as GS-T-2 (4.56 g) (Scheme 11).



Scheme 11. Isolation of "T" compound series.

4.2) Isolation of compounds in "Y1" series

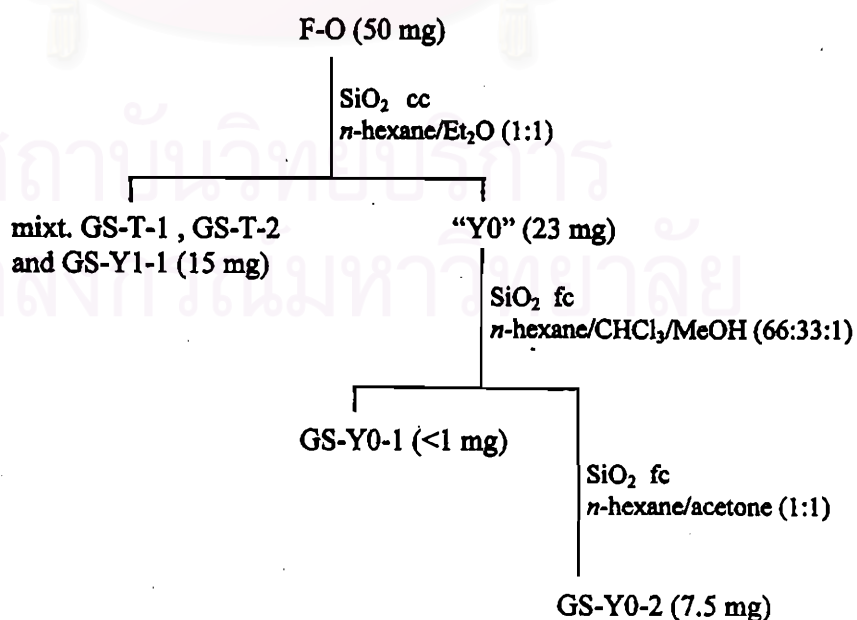
Based on their similar TLC pattern, fractions F-G and F-K were combined to give crude "Y1" (1440 mg). It was chromatographed over SiO₂ eluted with *n*-hexane/EtOAc (5:1) to afford a pure red compound GS-Y1-1 (1.0 g) in the second fraction. This compound showed the same TLC *R_f* values as 20-hydroxy-20-epi-tingenone (2) previously reported from this plant. The first eluted fractions (290 mg) consisted of GS-T-1, GS-T-2 and another red compound coded as GS-Y1-2. It was purified by cc using solvent system of CHCl₃/EtOAc gradient (10:1 to 5:1). The yield of this compound was 170 mg (Scheme 12).



Scheme 12. Isolation of "Y1" compound series.

4.3) Isolation of compounds in "Y0" series

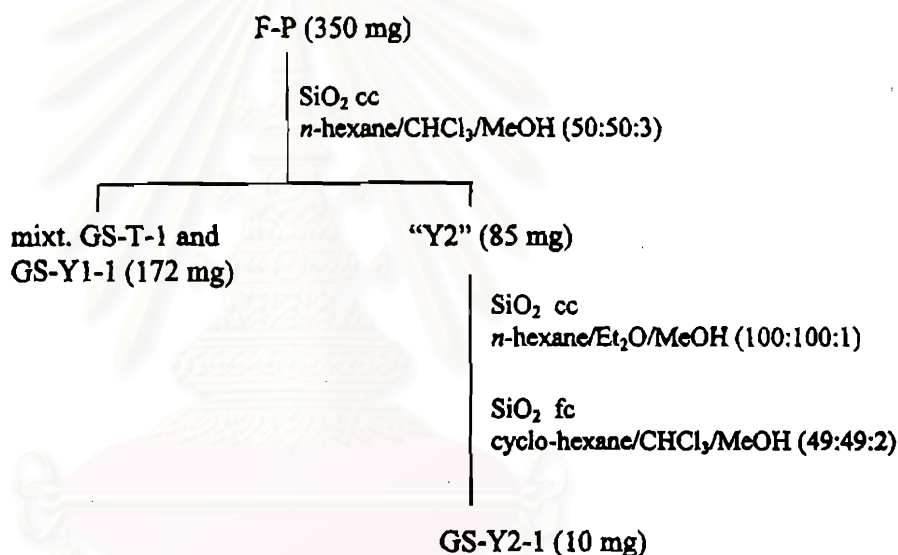
Fraction F-O (50 mg) consisted of GS-T-1, GS-T-2, GS-Y1-1 and a few other red compounds. A mixture of GS-T-1, GS-T-2, GS-Y1-1 was separated into less polar fraction by cc eluted with *n*-hexane/EtO₂ (1:1) solvent system. The more polar fraction, named "YO" (23 mg), was subjected to fc (Merck no.15111) using *n*-hexane/CHCl₃/MeOH (66:33:1) solvent system. The first eluted fraction yielded a very small amount of pure compound GS-Y0-1 (< 1 mg) which was not further investigated. The second fraction (13.5 mg) was purified by another fc (Merck no.15111) eluted with *n*-hexane/acetone (1:1) to afford compound GS-Y0-2 (7.5 g) (Scheme 13).



Scheme 13. Isolation of "Y0" compound series.

4.4) Isolation of compounds in "Y2" series

Fraction F-P (350 mg) consisted of GS-T-1, GS-Y1-1 and another major red compound. It was chromatographed over cc using solvent system of *n*-hexane/CHCl₃/MeOH (50:50:3) to separate the mixture of GS-T-1 and GS-Y1-1 into a less polar fraction (172 mg). The more polar fraction (85 mg), named "Y2" was purified by cc and fc (Merck no.15111) using *n*-hexane/Et₂O/MeOH (100:100:1) and cyclo-hexane/CHCl₃/MeOH (49:49:2) solvent systems, respectively, to yield pure compound GS-Y2-1 (10 mg) (Scheme 14).

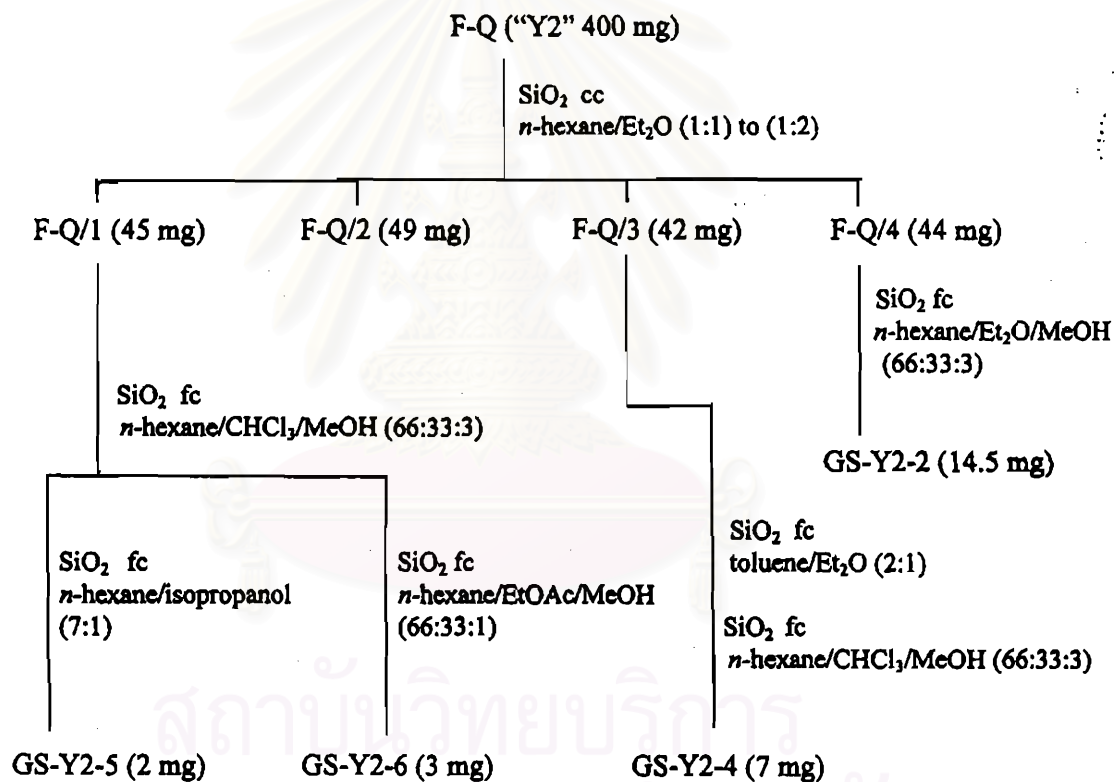


Scheme 14. Isolation of GS-Y2-1.

Fraction F-Q (400 mg) possessed a number of red compounds which showed nearly equal TLC *R_f* values to compound GS-Y2-1. They were thus coded as compounds in "Y2" series. F-Q was chromatographed over SiO₂ cc using the solvent system of *n*-hexane/Et₂O gradient (1:1 to 1:2). Based on the separated red chromatographic bands, four subfractions were collected : F-Q/1 (45 mg), F-Q/2 (49 mg), F-Q/3 (42 mg) and F-Q/4 (44 mg).

Subfraction F-Q/4 contained a main red compound, coded GS-Y2-2. It was isolated by fc (Merck no.15111) eluted with *n*-hexane/Et₂O/MeOH (66:33:3) to yield 14.5 mg of pure compound.

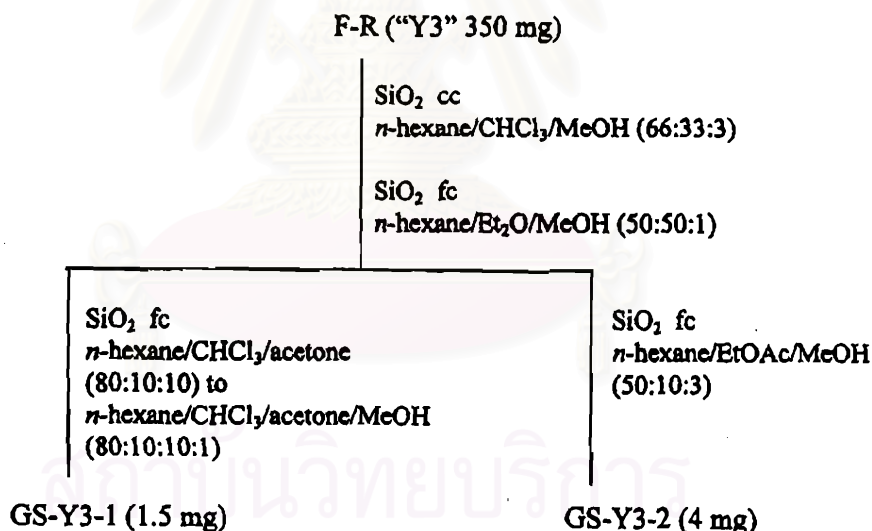
Another red compound, GS-Y2-4 (7 mg), was successively purified from subfraction F-Q/3 by two fc (Merck no.15111) using the solvent system of toluene/Et₂O (2:1) and *n*-hexane/CHCl₃/MeOH (66:33:3), respectively. A fc (Merck no.15111) eluted with *n*-hexane/CHCl₃/MeOH (66:33:3) solvent system was used to separate the two main red compounds from subfraction F-Q/1. The less polar compound, GS-Y2-5 (2 mg), was purified by fc (Merck no.15111) using *n*-hexane/isopropanol (7:1) solvent system, while the more polar one, GS-Y2-6 (3 mg), was purified by fc (Merck no.15111) eluted with *n*-hexane/EtOAc/MeOH (66:33:1) solvent system (Scheme 15).



Scheme 15. Isolation of GS-Y2-2, GS-Y2-4, GS-Y2-5 and GS-Y2-6.

4.5) Isolation of compounds in "Y3" series

Fraction F-R (350 mg) was named "Y3" and divided into three subfractions by cc using the solvent system of *n*-hexane/CHCl₃/MeOH (66:33:3). The first and the last subfractions consisted of small amount of several compounds. Therefore, only the second subfraction (30 mg) was further studied. Two main red compounds were separated by fc (Merck no.15111) eluted with *n*-hexane/Et₂O/MeOH (50:50:1) solvent system. The less polar compound, GS-Y3-1 (1.5 mg), was further purified with fc (Merck no.15111) using solvent system gradiented from *n*-hexane/CHCl₃/acetone (80:10:10) to *n*-hexane/CHCl₃/acetone/MeOH (80:10:10:1). The more polar compound, GS-Y3-2 (4 mg), was purified by fc (Merck no.15111) eluted with *n*-hexane /EtOAc/MeOH (50:10:3) solvent system (Scheme 16). Later, GS-Y3-1 was proved to be identical with GS-Y2-2.



Scheme 16. Isolation of "Y3" compound series.

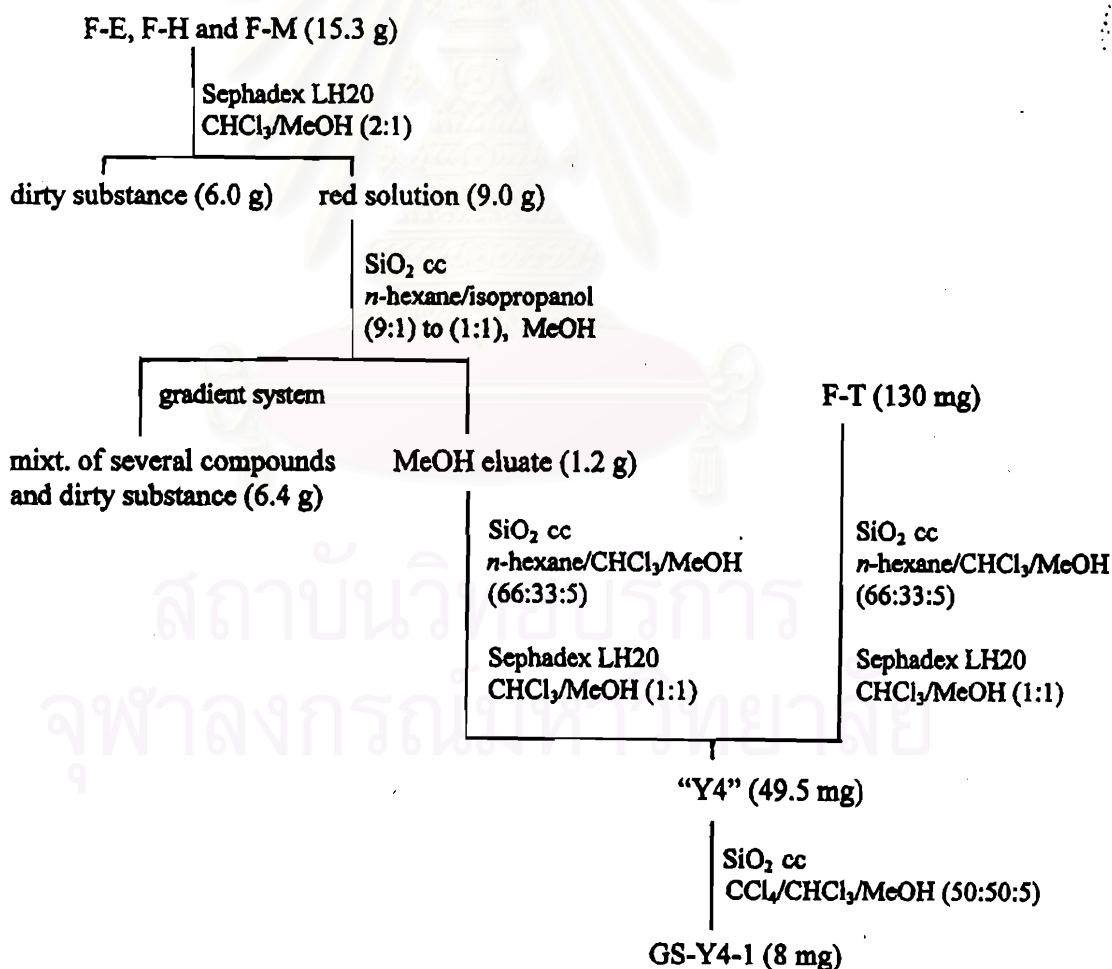
4.6) Isolation of compounds in "Y4" series

Fractions F-E (4.1 g), F-H (2.2 g) and F-M (9.0 g), all of which possessed several polar compounds together with a number of dirty substance, were combined and partly purified by a gel filtration cc eluted with CHCl₃/MeOH (2:1). The remaining reddish material (9.0 g) was then subjected to cc using *n*-hexane/isopropanol gradient system (9:1 to 1:1) and finally eluted with MeOH. The MeOH eluted fraction (1.2 g) was further separated by cc using *n*-hexane/CHCl₃/MeOH (66:33:5) solvent system into two

fractions. The less polar fraction (77 mg) was further clarified by a gel filtration cc eluted with $\text{CHCl}_3/\text{MeOH}$ (1:1) to obtain 18.5 mg of crude "Y4".

Fraction F-T (130 mg) also possessed the main constituents and nature as "Y4". It was subjected to cc using *n*-hexane/ $\text{CHCl}_3/\text{MeOH}$ (66:33:5) solvent system and subsequently to a gel filtration cc eluted with $\text{CHCl}_3/\text{MeOH}$ (1:1) to obtain a clear red material (31 mg), prior to combine with previous "Y4" fraction.

The collective "Y4" possessed only a main red compound coded GS-Y4-1. It was purified by fc (Merck no.15111) using the solvent system of $\text{CCl}_4/\text{CHCl}_3/\text{MeOH}$ (50:50:5) to yield 8 mg of pure compound (Scheme 17).



Scheme 17. Isolation of "Y4" compound series.

5. Acid rearrangement of GS-T-1, GS-T-2 and GS-Y1-1

5.1) Qualitative analysis

5.1.1) Reaction procedure

Sample compounds (100 mg) were dissolved in 100 ml of methanol and concentrated HCl was added dropwise to the final concentration of 1% v/v (pH 2, detected by universal indicator, Merck, Art 9535). The reaction was allowed to go on at room temperature (28-30°C) for 1 hour. Then the reactant was diluted with distilled water and extracted with ethyl acetate. The ethyl acetate extract was washed with distilled water to remove its acidity, and further dried over anhydrous sodium sulfate. Nearly all of the amount of the starting mass was recovered. The extracts obtained from compound GS-T-1, GS-T-2 and GS-Y1-1 were coded as crude GS-T-1-ACID, GS-T-2-ACID and GS-Y1-1-ACID, respectively. Based on TLC monitoring, only a main product of the reaction per each sample compound was detected together with a very small amount of remaining substrate.

5.1.2) Isolation of acid-rearranged products

Crude GS-T-1-ACID was subjected to a fc (Merck no.9382) eluted with *n*-hexane/Et₂O (4:1) to obtain 60 mg of pure compound GS-T-1-ACID.

Similarly, crude GS-T-2-ACID and crude GS-Y1-1-ACID were purified to give pure GS-T-2-ACID (20 mg) and GS-Y1-1-ACID (40 mg). The ratio of ingredient in mobile phases were 4:1 and 2:1, respectively.

5.2) Quantitative analysis

Sample compounds were dissolved in 1% v/v HCl in methanol to a concentration of about 0.03-0.06 mg/ml. Their UV absorption were measured at 420 and 306 nm, immediately and after 5, 10, 15, 20, 30 and 60 minutes. Progress of the reactions were shown as plotting between time and concentration, calculated from their molar absorptivity (ϵ), of starting compounds and their acid-rearranged products.

6. Characterization of the isolated and acid-rearranged compounds

GS-T-1

Red prism crystals (acetone). Melting point : 202-204°C Soluble in chloroform, ethyl acetate and acetone. $[\alpha]_D -244^\circ$ ($c = 0.18$, CHCl_3).

UV λ_{max} , (MeOH), nm (log ϵ) : 252 (3.94) sh, 421 (4.09) (Figure 40, page 153).

IR ν_{max} , (dry film), cm^{-1} : 3600-3100, 2959, 2931, 1707, 1593, 1439 (Figure 41, page 153).

EIMS 70 eV, m/z (rel. int.) : 436 $[\text{M}]^+$ (43), 422 (13), 267 (6), 253 (18), 241 (32), 227 (18), 202 (100), 201 (59), 200 (26), 43 (17) (Figure 42, page 154).

^1H NMR (500 MHz, CDCl_3) : 7.05 (1H, *dd*, $J = 7.1, 1.5$ Hz), 6.97 (1H, *br s*), 6.54 (1H, *d*, $J = 1.5$ Hz), 6.39 (1H, *d*, $J = 7.1$ Hz), 4.55 (1H, *d*, $J = 3.7$ Hz), 3.66 (1H, *d*, 3.7 Hz), 2.66 (1H, *ddq*, $J = 12.9, 6.5, 6.5$ Hz), 2.27 (1H, *m*), 2.27 (1H, *m*), 2.23 (3H, *s*), 2.22 (1H, *dd*, $J = 14.3, 6.5$ Hz), 2.03 (1H, *td*, $J = 13.4, 7.0$ Hz), 1.88 (1H, *td*, $J = 13.5, 4.7$ Hz), 1.85 (1H, *m*), 1.84 (1H, *m*), 1.82 (1H, *d*, $J = 7.4$ Hz), 1.78 (1H, *ddd*, $J = 14.3, 12.9, 7.4$ Hz), 1.66 (1H, *ddd*, $J = 13.5, 5.3, 1.9$ Hz), 1.61 (1H, *m*), 1.52 (3H, *s*), 1.37 (3H, *s*), 1.07 (3H, *d*, 6.49 Hz), 0.98 (3H, *s*) 0.87 (3H, *s*) (Figure 43, page 155).

^{13}C NMR (125 MHz, CDCl_3) : 213.5, 178.4, 168.4, 164.7, 146.0, 133.7, 127.7, 119.8, 118.1, 117.2, 76.3, 45.0, 44.8, 44.3, 42.6, 40.8, 40.5, 39.1, 34.0, 32.0, 29.9, 29.5, 28.2, 25.0, 21.6, 20.5, 14.7, 10.2 (Figure 44, page 157).

GS-T-2

Red prism crystals (acetone). Melting point 140°C. Soluble in chloroform, ethyl acetate and acetone. $[\alpha]_D -237^\circ$ ($c = 0.20$, CHCl_3).

UV λ_{max} , (MeOH), nm (log ϵ) : 252 (4.14) sh, 421 (4.27) (Figure 45, page 158).

IR ν_{max} , (dry film), cm^{-1} : 3500-3100, 2954, 1707, 1593, 1438 (Figure 46, page 158).

EIMS 70 eV, m/z (rel. int.) : 420 $[\text{M}]^+$ (50), 406 (13), 267 (5), 253 (16), 241 (36), 227 (19), 202 (100), 201 (60), 200 (34) (Figure 47, page 159).

^1H NMR (500 MHz, CDCl_3) : 7.04 (1H, *dd*, $J = 7.0, 1.5$ Hz), 6.98 (1H, *br s*), 6.55 (1H, *d*, $J = 1.5$ Hz), 6.38 (1H, *d*, $J = 7.0$ Hz), 2.92 (1H, *d*, $J = 15.1$ Hz), 2.50 (1H, *ddq*, $J = 13.1, 6.5, 6.5$ Hz), 2.26 (1H, *ddd*, $J = 13.7, 4.9, 2.1$ Hz), 2.23 (3H, *s*), 2.20

(1H, *dd*, $J = 15.0, 6.4$ Hz), 2.02 (1H, *td*, $J = 13.7, 5.8$ Hz), 1.91 (1H, *td*, $J = 14.0, 4.9$ Hz), 1.87 (1H, *m*), 1.86 (1H, *d*, $J = 15.1$ Hz), 1.84 (1H, *m*), 1.81 (1H, *td*, $J = 14.0, 4.1$ Hz), 1.76 (1H, *ddd*, $J = 15.0, 13.1, 7.2$ Hz), 1.67 (1H, *d*, $J = 7.2$ Hz), 1.65 (1H, *m*), 1.51 (3H, *s*), 1.46 (1H, *ddd*, $J = 14.0, 4.1, 2.3$ Hz), 1.35 (3H, *s*), 1.02 (3H, *s*), 1.00 (3H, *s*), 0.98 (3H, *s*) (Figure 48, page 160).

^{13}C NMR (125 MHz, CDCl_3) : 213.6, 178.4, 168.7, 164.7, 146.0, 133.6, 127.7, 119.8, 118.1, 117.1, 52.5, 44.6, 43.5, 42.7, 41.8, 40.6, 39.0, 38.2, 35.5, 33.8, 32.5, 32.0, 29.9, 28.5, 21.5, 19.7, 15.1, 10.2 (Figure 49, page 162).

GS-Y0-2

Orange-red powder (acetone). Melting point 203-205°C. Soluble in chloroform, ethyl acetate and acetone. $[\alpha]_{\text{D}}^{-7^\circ} - +7^\circ$ ($c = 0.10, \text{CHCl}_3$).

UV λ_{max} , (MeOH), nm (log ϵ) : 217 (3.97), 263 (3.97), 272 (3.96), 404 (3.41) (Figure 50, page 163).

IR ν_{max} , (dry film), cm^{-1} : 3600-3200, 2927, 1713, 1594, 1443 (Figure 51, page 163).

EIMS 70 eV, m/z (rel. int.) : 422 $[\text{M}]^+$ (2), 267 (1), 253 (2), 241 (2), 227 (3), 202 (6), 201 (9), 200 (3), 43 (100) (Figure 52, page 164).

^1H NMR (500 MHz, CDCl_3) : 9.71 (1H, *s*), 7.01 (1H, *dd*, $J = 7.0, 1.5$ Hz), 6.95 (1H, *br s*), 6.48 (1H, *d*, $J = 1.5$ Hz), 6.28 (1H, *d*, $J = 7.0$ Hz), 2.81 (1H, *dd*, $J = 7.6, 2.2$ Hz), 2.81 (1H, *dd*, $J = 19.3, 2.2$ Hz), 2.67 (1H, *dd*, $J = 19.3, 7.6$ Hz), 2.31 (1H, *dd*, $J = 10.1, 2.1$ Hz), 2.27 (3H, *s*), 2.20 (3H, *s*), 2.15 (1H, *dd*, $J = 8.5, 2.1$ Hz), 1.84 (2H, *m*), 1.71 (2H, *m*), 1.60 (1H, *m*), 1.44 (6H, *s*), 1.00 (3H, *s*), 0.96 (1H, *dd*, $J = 8.5, 2.1$ Hz), 0.48 (3H, *s*) (Figure 53, page 165).

^{13}C NMR (125 MHz, CDCl_3) : 206.5 (*s*), 205.5 (*d*), 178.4 (*s*), 167.7 (*s*), 164.2 (*s*), 146.0 (*s*), 133.6 (*d*), 127.7 (*s*), 119.7 (*d*), 118.0 (*d*), 117.1 (*s*), 47.3 (*s*), 43.7 (*s*), 42.8 (*s*), 42.6 (*d*), 40.6 (*s*), 39.3 (*t*), 38.2, 33.8 (*t*), 30.1 (*q*), 29.1 (*t*), 28.7 (*t*), 28.4 (*t*), 24.7 (*q*), 22.0 (*q*), 16.5 (*q*), 10.2 (*q*) (Figure 54, page 167).

GS-Y1-1

Orange-red powder (acetone). Melting point 198-200°C. Soluble in chloroform, ethyl acetate and acetone. $[\alpha]_D^{+35}$ ($c = 0.16$, CHCl_3).

UV λ_{max} , (MeOH), nm (log ϵ) : 252 (4.07) sh, 421 (4.13) (Figure 59, page 175).

IR ν_{max} , (dry film), cm^{-1} : 3600-3100, 2952, 1712, 1591, 1440 (Figure 60, page 175).

EIMS 70 eV, m/z (rel. int.) : 436 $[\text{M}]^+$ (44), 422 (7), 421 (7), 418 (3), 403 (4), 267 (6), 253 (13), 241 (40), 227 (13), 202 (60), 201 (20), 200 (20), 43 (48) (Figure 61, page 176).

^1H NMR (500 MHz, CDCl_3) : 7.02 (1H, *dd*, $J = 1.4, 7.0$ Hz), 6.97 (1H, *br s*), 6.54 (1H, *d*, $J = 1.4$ Hz), 6.37 (1H, *d*, $J = 7.02$ Hz), 3.24 (1H, *s*), 3.00 (1H, *d*, $J = 14$ Hz), 2.28 (1H, *dd*, $J = 15.6, 9.0$ Hz), 2.22 (3H, *s*), 2.21 (1H, *m*), 2.20 (1H, *dd*, $J = 15.6, 4.3$ Hz), 1.95 (1H, *td*, $J = 14.0, 6.4$ Hz), 1.95 (1H, *d*, $J = 14$ Hz), 1.94 (1H, *dd*, $J = 9.0, 4.3$ Hz), 1.89 (1H, *m*), 1.84 (1H, *m*), 1.81 (1H, *td*, $J = 14.0, 5.0$ Hz), 1.76 (1H, *ddd*, $J = 14.0, 6.4, 3.0$ Hz), 1.73 (1H, *m*), 1.65 (1H, *m*), 1.48 (3H, *s*), 1.37 (3H, *s*), 1.36 (3H, *s*), 1.13 (3H, *s*), 0.89 (3H, *s*) (Figure 62, page 177).

^{13}C NMR (125 MHz, CDCl_3) : 215.1, 178.4, 169.0, 164.3, 146.1, 133.7, 127.7, 119.7, 118.3, 117.2, 73.7, 50.4, 44.1, 43.3, 43.0, 39.9, 39.8, 38.5, 36.0, 35.6, 33.1, 33.1, 29.8, 29.2, 29.0, 23.1, 19.4, 10.3 (Figure 63, page 179).

GS-Y1-2

Orange-red powder (acetone). Melting point 218-220°C Soluble in chloroform, ethyl acetate and acetone. $[\alpha]_D^{-230}$ ($c = 0.16$, CHCl_3).

UV λ_{max} , (MeOH), nm (log ϵ) : 252 (3.85) sh, 422 (4.00) (Figure 64, page 180)

IR ν_{max} , (dry film), cm^{-1} : 3700-3100, 2928, 1709, 1591, 1443 (Figure 65, page 180)

EIMS 70 eV, m/z (rel. int.) : 436 $[\text{M}]^+$ (24), 422 (3), 421 (3), 403 (5), 267 (6), 253 (18), 241 (19), 227 (11), 202 (90), 201 (100), 200 (41) (Figure 66, page 181).

^1H NMR (500 MHz, CDCl_3) : 7.01 (1H, *dd*, $J = 7.2, 1.5$ Hz), 6.97 (1H, *br s*), 6.51 (1H, *d*, $J = 1.5$ Hz), 6.30 (1H, *d*, $J = 7.2$ Hz), 3.06 (1H, *s*), 2.75 (1H, *d*, $J = 19.2$ Hz), 2.29 (2H, *m*), 2.21 (3H, *s*), 2.20 (1H, *m*), 2.17 (1H, *d*, $J = 19.2$ Hz), 1.97 (1H,

td, $J = 13.6, 7.1$ Hz), 1.88 (1H, *td*, $J = 13.4, 4.6$ Hz), 1.80 (1H, *td*, $J = 13.4, 4.0$ Hz), 1.76 (2H, *m*), 1.75 (1H, *m*), 1.57 (1H, *ddd*, $J = 13.4, 4.6, 2.4$ Hz), 1.50 (1H, *ddd*, $J = 13.4, 4.0, 2.4$ Hz), 1.46 (3H, *s*), 1.38 (3H, *s*), 1.34 (3H, *s*), 1.28 (3H, *s*), 0.62 (3H, *s*) (Figure 67, page 182).

^{13}C NMR (125 MHz, CDCl_3) : 216.3 (*s*), 178.4 (*s*), 168.8 (*s*), 164.6 (*s*), 146.0 (*s*), 133.6 (*d*), 127.6 (*s*), 119.7 (*d*), 117.8 (*d*), 117.0 (*s*), 72.0 (*s*), 47.8, 44.1 (*s*), 43.8 (*d*), 42.7 (*s*), 39.6 (*s*), 38.8, 36.5 (*t*), 34.7 (*q*), 34.7 (*s*), 34.5 (*t*), 33.8 (*t*), 30.8 (*q*), 28.9 (*t*), 28.1 (*t*), 21.7 (*q*), 16.8 (*q*), 10.2 (*q*) (Figure 68, page 184).

GS-Y2-1

Orange-red powder (acetone). Melting point 142-148°C. Soluble in chloroform, ethyl acetate and acetone. $[\alpha]_{\text{D}} -22^\circ$ ($c = 0.07$, CHCl_3).

UV λ_{max} , (MeOH), nm (log ϵ) : 255 (3.79) sh, 421 (3.79) (Figure 73, page 192).

IR ν_{max} , (dry film), cm^{-1} : 3300-3000, 2928, 1716, 1587, 1442 (Figure 74, page 192).

EIMS 70 eV, m/z (rel. int.) : 452 $[\text{M}]^+$ (7), 450 (11), 438 (3), 434 (17), 420 (9), 267 (8), 253 (29), 241 (24), 227 (19), 202 (94), 201 (100), 200 (23), 43 (58) (Figure 75, page 193).

^1H NMR (500 MHz, CDCl_3) : 7.05 (1H, *dd*, $J = 7.2, 1.5$ Hz), 6.96 (1H, *br s*), 6.54 (1H, *d*, $J = 1.5$ Hz), 6.38 (1H, *d*, $J = 7.2$ Hz), 4.98 (1H, *d*, $J = 4.4$ Hz), 3.40 (1H, *d*, $J = 4.4$ Hz), 2.35 (1H, *ddd*, $J = 15.6, 5.5, 2.4$ Hz), 2.28 (1H, *dd*, $J = 16.2, 1.8$ Hz), 2.23 (3H, *s*), 2.22 (1H, *ddd*, $J = 13.8, 5.2, 2.5$ Hz), 2.05 (1H, *dd*, $J = 16.2, 9.2$ Hz), 2.00 (1H, *dt*, $J = 13.8, 6.2$ Hz), 1.92 (1H, *dd*, $J = 13.4, 5.5$ Hz), 1.92 (1H, *dd*, $J = 9.2, 1.8$ Hz), 1.81 (1H, *ddd*, $J = 13.8, 6.2, 2.5$ Hz), 1.76 (1H, *td*, $J = 13.8, 5.2$ Hz), 1.65 (1H, *ddd*, $J = 13.4, 6.3, 2.4$ Hz), 1.58 (1H, *dd*, $J = 15.6, 6.3$ Hz), 1.48 (3H, *s*), 1.40 (3H, *s*), 1.35 (3H, *s*), 1.02 (3H, *s*), 0.86 (3H, *s*) (Figure 76, page 194).

^{13}C NMR (125 MHz, CDCl_3) : 211.5 (*s*), 178.4 (*s*), 169.4 (*s*), 164.7 (*s*), 146.0 (*s*), 134.0 (*d*), 127.5 (*s*), 119.6 (*d*), 118.0 (*d*), 117.2 (*s*), 73.8 (*d*), 72.4 (*s*), 44.8 (*d*), 44.2 (*s*), 42.8 (*s*), 42.4 (*s*), 39.6 (*s*), 38.7 (*q*), 35.1 (*t*), 33.8 (*t*), 29.6 (*t*), 29.4 (*t*), 28.7 (*t*), 26.1 (*q*), 25.4 (*q*), 22.1 (*q*), 19.3 (*q*), 10.3 (*q*) (Figure 77, page 196).

GS-Y2-2

Orange-red powder (acetone). Melting point 210-212°C. Soluble in chloroform, ethyl acetate and acetone. $[\alpha]_D^{+41}$ ($c = 0.10$, CHCl_3).

UV λ_{max} , (MeOH), nm ($\log \epsilon$): 254 (3.70) sh, 419 (3.69) (Figure 82, page 205).

IR ν_{max} , (dry film), cm^{-1} : 3300-3000, 2960, 1719, 1591, 1442 (Figure 83, page 205).

EIMS 70 eV, m/z (rel. int.): 452 $[\text{M}]^+$ (14), 450 (2), 438 (6), 434 (13), 420 (6), 267 (8), 253 (31), 241 (27), 227 (24), 202 (86), 201 (66), 200 (26), 43 (100) (Figure 84, page 206).

^1H NMR (500 MHz, CDCl_3): 7.04 (1H, *dd*, $J = 7.2, 1.3$ Hz), 6.98 (1H, *br s*), 6.54 (1H, *d*, $J = 1.3$ Hz), 6.41 (1H, *d*, $J = 7.2$ Hz), 4.57 (1H, *d*, $J = 4.7$ Hz), 3.40 (1H, *d*, $J = 4.7$ Hz), 2.98 (1H, *br s*), 2.34 (1H, *ddd*, $J = 15.6, 7.7, 3.8$ Hz), 2.23 (1H, *dd*, $J = 15.2, 7.6$ Hz), 2.22 (3H, *s*), 2.21 (1H, *m*), 2.18 (1H, *dd*, $J = 15.2, 6.6$ Hz), 2.09 (1H, *dd*, $J = 7.6, 6.6$ Hz), 1.92 (1H, *td*, $J = 13.7, 5.2$ Hz), 1.88 (1H, *m*), 1.84 (1H, *td*, $J = 13.7, 3.6$ Hz), 1.77 (1H, *ddd*, $J = 13.5, 7.1, 3.8$ Hz), 1.74 (1H, *ddd*, $J = 13.7, 5.2, 2.7$ Hz), 1.66 (1H, *ddd*, $J = 15.6, 7.9, 7.1$ Hz), 1.47 (3H, *s*), 1.40 (3H, *s*), 1.39 (3H, *s*), 0.99 (3H, *s*), 0.92 (3H, *s*) (Figure 85, page 207).

^{13}C NMR (125 MHz, CDCl_3): 215.3 (*s*), 178.4 (*s*), 169.0 (*s*), 164.1 (*s*), 146.1 (*s*), 133.7 (*d*), 127.7 (*s*), 119.6 (*d*), 118.6 (*d*), 117.2 (*s*), 76.7 (*d*), 73.2 (*s*), 43.7 (*s*), 43.1 (*d*), 43.0 (*s*), 40.2 (*s*), 39.8 (*s*), 38.1 (*q*), 35.1 (*t*), 33.0 (*t*), 29.7 (*t*), 29.7 (*t*), 29.5 (*t*), 28.7 (*q*), 26.1 (*q*), 24.1 (*q*), 19.8 (*q*), 10.3 (*q*) (Figure 86, page 209).

GS-Y2-4

Orange-red powder (acetone). Melting point 162-164°C. Soluble in chloroform, ethyl acetate and acetone. $[\alpha]_D^{-87}$ ($c = 0.10$, CHCl_3).

UV λ_{max} , (MeOH), nm ($\log \epsilon$): 254 (3.74) sh, 419 (3.78) (Figure 91, page 222).

IR ν_{max} , (dry film), cm^{-1} : 3300-3000, 2939, 1712, 1590, 1441 (Figure 92, page 222).

EIMS 70 eV, m/z (rel. int.): 450 $[\text{M}]^+$ (14), 436 (5), 267 (2), 253 (11), 241 (7), 227 (6), 202 (17), 201 (24), 200 (10), 43 (100) (Figure 93, page 223).

^1H NMR (500 MHz, CDCl_3) : 6.99 (1H, *dd*, $J = 7.2, 1.4$ Hz), 6.96 (1H, *br s*), 6.51 (1H, *d*, $J = 1.4$ Hz), 6.26 (1H, *d*, $J = 7.2$ Hz), 2.84 (1H, *br s*), 2.65 (1H, *dt*, $J = 13.3, 2.7$ Hz), 2.47 (1H, *dd*, $J = 16.6, 1.7$ Hz), 2.40 (1H, *dd*, $J = 16.6; 6.7$ Hz), 2.22 (1H, *dt*, $J = 13.6, 4.2$ Hz), 2.20 (3H, *s*), 2.12 (1H, *dd*, $J = 6.7, 1.7$ Hz), 1.91 (1H, *td*, $J = 13.6, 5.3$ Hz), 1.87 (1H, *m*), 1.82 (1H, *dt*, $J = 13.6, 4.2$ Hz), 1.75 (1H, *td*, $J = 13.3, 5.2$ Hz), 1.64 (1H, *m*), 1.58 (1H, *ddd*, $J = 12.1, 5.2, 2.7$ Hz), 1.48 (3H, *s*), 1.45 (3H, *s*), 1.34 (3H, *s*), 1.32 (3H, *s*), 0.58 (3H, *s*) (Figure 94, page 224).

^{13}C NMR (125 MHz, CDCl_3) : 199.2 (*s*), 196.8 (*s*), 178.4 (*s*), 167.3 (*s*), 164.0 (*s*), 146.1 (*s*), 133.5 (*d*), 127.7 (*s*), 119.7 (*d*), 118.4 (*d*), 117.1 (*s*), 74.3 (*s*), 48.6 (*s*), 44.6 (*d*), 43.8 (*s*), 42.6 (*s*), 39.9 (*s*), 37.8 (*q*), 33.6 (*t*), 31.9 (*t*), 31.1 (*q*), 28.8 (*t*), 28.6 (*t*), 28.1 (*t*), 28.0 (*q*), 21.9 (*q*), 17.0 (*q*), 10.2 (*q*) (Figure 95, page 226).

GS-Y2-5

Orange-red powder (acetone). Melting point 106-110°C. Soluble in chloroform, ethyl acetate and acetone. $[\alpha]_{\text{D}}^{-72^\circ}$ ($c = 0.10, \text{CHCl}_3$).

UV λ_{max} , (MeOH), nm (log ϵ) : 255 (3.84) sh, 420 (3.81) (Figure 100, page 234).

IR ν_{max} , (dry film), cm^{-1} : 3300-3000, 2923, 1715, 1594, 1445 (Figure 101, page 234).

EIMS 70 eV, m/z (rel. int.) : 452 $[\text{M}]^+$ (9), 450 (20), 436 (9), 434 (21), 420 (6), 267 (6), 253 (30), 241 (26), 227 (12), 202 (31), 201 (100), 200 (12), 43 (45) (Figure 102, page 235).

^1H NMR (500 MHz, CDCl_3) : 7.01 (1H, *dd*, $J = 7.2, 1.4$ Hz), 6.96 (1H, *br s*), 6.51 (1H, *d*, $J = 1.4$ Hz), 6.33 (1H, *d*, $J = 7.2$ Hz), 4.33 (1H, *d*, $J = 2.8$ Hz), 3.89 (1H, *d*, $J = 2.8$ Hz), 2.64 (1H, *ddd*, $J = 14.9, 5.7, 1.6$ Hz), 2.28 (1H, *dd*, $J = 10.1, 1.8$ Hz), 2.26 (1H, *dd*, $J = 12.2, 1.8$ Hz), 2.21 (3H, *s*), 2.20 (1H, *dd*, $J = 12.2, 10.1$ Hz), 2.14 (1H, *ddd*, $J = 13.7, 4.3, 2.5$ Hz), 1.86 (1H, *td*, $J = 13.7, 5.3$ Hz), 1.79 (1H, *dd*, $J = 13.7, 5.3$ Hz), 1.76 (1H, *m*), 1.71 (1H, *td*, $J = 13.7, 4.3$ Hz), 1.62 (1H, *dd*, $J = 13.3, 5.3$ Hz), 1.52 (1H, *m*), 1.44 (3H, *s*), 1.43 (3H, *s*), 1.41 (3H, *s*), 1.28 (3H, *s*), 0.59 (3H, *s*) (Figure 103, page 236).

^{13}C NMR (125 MHz, CDCl_3) : 215.3 (*s*), 178.4 (*s*), 168.7 (*s*), 164.2 (*s*), 146.0 (*s*), 133.8 (*d*), 127.5 (*s*), 119.5 (*d*), 118.2 (*d*), 117.0 (*s*), 75.7 (*d*), 75.5 (*s*), 49.6 (*d*),

45.0 (s), 44.5 (s), 42.8 (s), 39.3 (s), 37.4 (q), 33.2 (t), 31.6 (q), 31.5 (q), 31.0 (t), 29.4 (t), 29.0 (t), 27.9 (t), 21.9 (q), 18.5 (q), 10.2 (q) (Figure 104, page 238).

GS-Y2-6

Orange-red powder (acetone). Melting point 85-88°C. Soluble in chloroform, ethyl acetate and acetone. $[\alpha]_D^{-27^\circ}$ ($c = 0.13$, CHCl_3).

UV λ_{max} , (MeOH), nm (log ϵ) : 255 (3.80) sh, 413 (3.53) (Figure 109, page 248).

IR ν_{max} , film, cm^{-1} : 3300-3000, 2924, 1713, 1591, 1445 (Figure 110, page 248).

EIMS 70 eV, m/z (rel. int.) : 450 $[\text{M}]^+$ (18), 436 (5), 267 (6), 253 (34), 241 (29), 227 (14), 202 (37), 201 (100), 200 (22), 43 (58) (Figure 111, page 249).

^1H NMR (500 MHz, CDCl_3) : 7.00 (1H, *dd*, $J = 7.1, 1.4$ Hz), 6.94 (1H, *br s*), 6.50 (1H, *d*, $J = 1.4$ Hz), 6.28 (1H, *d*, $J = 7.1$ Hz), 2.64 (1H, *dt*, $J = 12.8, 3.0$ Hz), 2.56 (1H, *dd*, $J = 16.1, 9.2$ Hz), 2.35 (1H, *d*, $J = 16.1$ Hz), 2.26 (1H, *m*), 2.23 (1H, *d*, $J = 9.2$ Hz), 2.20 (3H, *s*), 1.87 (2H, *m*), 1.71 (1H, *dt*, $J = 7.9, 2.2$ Hz), 1.67 (1H, *m*), 1.62 (1H, *m*), 1.61 (1H, *m*), 1.56 (3H, *s*), 1.50 (3H, *s*), 1.47 (3H, *s*), 1.35 (3H, *s*), 0.42 (3H, *s*) (Figure 112, page 250).

^{13}C NMR (125 MHz, CDCl_3) : 200.1 (s), 195.6 (s), 178.4 (s), 166.8 (s), 164.0 (s), 146.1 (s), 133.5 (d), 127.8 (s), 119.8 (d), 118.6 (d), 117.1 (s), 74.5 (s), 48.7 (s), 44.3 (d), 43.8 (s), 42.5 (s), 40.7 (s), 38.1 (q), 33.6 (t), 33.4 (t), 28.8 (t), 28.7 (t), 28.6 (t), 28.0 (q), 27.9 (q), 22.0 (q), 18.2 (q), 10.3 (q) (Figure 113, page 252).

GS-Y3-2

Orange-red powder (acetone). Melting point 144-147°C. Soluble in chloroform, ethyl acetate and acetone. $[\alpha]_D^{-78^\circ}$ ($c = 0.18$, CHCl_3).

UV λ_{max} , (MeOH), nm (log ϵ) : 253 (3.75) sh, 412 (3.76) (Figure 118, page 260).

IR ν_{max} , film, cm^{-1} : 3300-3000, 2925, 1728, 1699, 1641, 1444 (Figure 119, page 260).

EIMS 70 eV, m/z (rel. int.) : 450 $[\text{M}]^+$ (34) 436 (14), 267 (7), 253 (28), 241 (24), 227 (18), 202 (43), 201 (100), 200 (26), 43 (73) (Figure 120, page 261).

^1H NMR (500 MHz, CDCl_3) : 6.97 (1H, *dd*, $J = 7.2, 1.4$ Hz), 6.93 (1H, *br s*), 6.48 (1H, *d*, $J = 1.4$ Hz), 6.27 (1H, *d*, $J = 7.2$ Hz), 3.09 (1H, *dd*, $J = 16.5, 6.7$ Hz), 2.97 (1H, *dd*, $J = 16.5, 2.1$ Hz), 2.85 (1H, *ddd*, $J = 14.3, 4.3, 2.1$ Hz), 2.27 (1H, *dd*, $J = 6.7, 2.1$ Hz), 2.20 (1H, *m*), 2.19 (3H, *s*), 1.85 (1H, *td*, $J = 13.4, 4.0$ Hz), 1.80 (1H, *td*, $J = 13.4, 4.3$ Hz), 1.76 (1H, *m*), 1.65 (1H, *td*, $J = 14.3, 5.2$ Hz), 1.58 (1H, *ddd*, $J = 14.3, 5.2, 2.1$ Hz), 1.57 (3H, *s*), 1.55 (1H, *td*, $J = 14.3, 4.3$ Hz), 1.48 (3H, *s*), 1.45 (3H, *s*), 1.35 (3H, *s*), 0.29 (3H, *s*) (Figure 121, page 262)

^{13}C NMR (125 MHz, CDCl_3) : 212.9 (*s*), 209.4 (*s*), 178.4 (*s*), 166.3 (*s*), 163.8 (*s*), 146.1 (*s*), 133.2 (*d*), 127.9 (*s*), 119.8 (*d*), 118.7 (*d*), 117.0 (*s*), 82.7 (*s*), 46.0 (*s*), 45.5 (*d*), 43.9 (*s*), 42.5 (*s*), 40.1 (*s*), 37.9, 35.0 (*t*), 33.2 (*t*), 29.6 (*q*), 29.3 (*t*), 29.3 (*q*), 28.8 (*t*), 28.6 (*t*), 21.6 (*q*), 17.6 (*q*), 10.2 (*q*) (Figure 122, page 264).

GS-Y4-1

Orange-red powder (acetone). Melting point 120-125°C. Soluble in chloroform, ethyl acetate and acetone. $[\alpha]_{\text{D}}^{-24^\circ} - +24^\circ$ ($c = 0.10, \text{CHCl}_3$).

UV λ_{max} , (MeOH), nm (log ϵ) : 254 (3.86) sh, 420 (3.79) (Figure 127, page 271).

IR ν_{max} , film, cm^{-1} : 3300-2800, 2925, 1714, 1590, 1444 (Figure 128, page 271).

EIMS 70 eV, m/z (rel. int.) : 452 $[\text{M}]^+$ (34), 438 (6), 437 (6), 434 (6), 420 (4), 419 (5), 267 (8), 253 (21), 241 (78), 227 (33), 202 (18), 201 (75), 43 (100) (Figure 129, page 272).

^1H NMR (500 MHz, CDCl_3) : 7.04 (1H, *dd*, $J = 7.0, 1.2$ Hz), 6.50 (1H, *d*, $J = 1.2$ Hz), 6.32 (1H, *d*, $J = 7.0$ Hz), 2.56 (1H, *d*, $J = 12.5$ Hz), 2.52 (1H, *m*), 2.50 (1H, *m*), 2.31 (1H, *d*, $J = 17.4$ Hz), 2.23 (3H, *s*), 2.23 (3H, *s*), 2.17 (1H, *m*), 2.15 (1H, *m*), 2.11 (1H, *d*, $J = 12.5$ Hz), 1.95 (1H, *td*, $J = 13.4, 5.9$ Hz), 1.85 (1H, *td*, $J = 13.5, 3.7$ Hz), 1.79 (1H, *td*, $J = 13.4, 6.0$ Hz), 1.75 (1H, *td*, $J = 13.5, 4.3$ Hz), 1.58 (1H, *br d*, $J = 13.5$ Hz), 1.46 (3H, *s*), 1.44 (3H, *s*), 1.02 (3H, *s*), 0.94 (1H, *dd*, $J = 13.4, 5.9$ Hz), 0.58 (3H, *s*) (Figure 130, page 273).

^{13}C NMR (125 MHz, CDCl_3) : 206.9 (*s*), 178.4 (*s*), 176.8 (*s*), 169.0 (*s*), 164.9 (*s*), 146.1 (*s*), 134.0 (*d*), 127.6 (*s*), 119.8 (*d*), 117.6 (*d*), 117.5 (*s*), 44.0 (*s*), 43.4 (*d*), 42.8 (*s*), 41.1 (*t*), 40.8 (*s*), 40.5 (*t*), 39.2 (*q*), 35.8 (*s*), 33.8 (*t*), 32.2 (*t*), 30.1 (*q*), 29.4 (*t*), 28.8 (*q*), 28.4 (*t*), 21.7 (*q*), 15.7 (*q*), 10.3 (*q*) (Figure 131, page 275).

GS-T-1-ACID

Colorless needle crystals (EtOAc). Melting point 180-184°C. Soluble in chloroform, ethyl acetate and acetone. $[\alpha]_D -5^\circ$ ($c = 0.06$, CHCl_3).

UV λ_{max} , (MeOH), nm ($\log \epsilon$) : 253 (4.71) , 306 (3.81) (Figure 136, page 282).

IR ν_{max} , film, cm^{-1} : 3600-3100, 2928, 1705 (Figure 137, page 282).

^1H NMR (500 MHz, CDCl_3) : 6.72 (1H, *s*), 6.46 (1H, *d*, $J = 9.9$ Hz), 6.31 (1H, *d*, $J = 9.9$ Hz), 5.57 (1H, *d*, $J = 6.7$ Hz), 5.21 (1H, *br s*), 5.08 (1H, *br s*), 4.60 (1H, *s*), 3.66 (1H, *br s*), 2.71 (1H, *ddq*, $J = 12.6, 6.8, 6.8$ Hz), 2.22 (3H, *s*), 2.16 (1H, *dd*, $J = 17.4, 6.7$ Hz), 2.13 (1H, *m*), 2.13 (1H, *m*), 2.12 (1H, *m*), 2.06 (1H, *dd*, $J = 14.8, 6.8$ Hz), 1.92 (1H, *d*, $J = 7.4$ Hz), 1.76 (1H, *ddd*, $J = 14.8, 12.6, 7.4$ Hz), 1.51 (1H, *td*, $J = 15.0, 3.5$ Hz), 1.43 (1H, *dt*, $J = 13.1, 3.5$ Hz), 1.32 (3H, *s*), 1.07 (3H, *s*), 1.07 (3H, *d*, $J = 6.8$ Hz), 1.04 (3H, *s*), 0.81 (3H, *s*) (Figure 138, page 283).

GS-T-2-ACID

Colorless plate crystals (EtOAc). Melting point 254-255°C. Soluble in chloroform, ethyl acetate and acetone. $[\alpha]_D +1^\circ$ ($c = 0.06$, CHCl_3).

UV λ_{max} , (MeOH), nm ($\log \epsilon$) : 252 (4.89) , 306 (3.99) (Figure 139, page 285).

IR ν_{max} , film, cm^{-1} : 3100-3600, 2938, 1694 (Figure 140, page 285).

^1H NMR (500 MHz, CDCl_3) : 6.72 (1H, *s*), 6.46 (1H, *d*, $J = 10.1$ Hz), 6.31 (1H, *d*, $J = 10.1$ Hz), 5.58 (1H, *d*, $J = 6.1$ Hz), 5.21 (1H, *br s*), 5.08 (1H, *br s*), 2.99 (1H, *d*, $J = 13.6$ Hz), 2.56 (1H, *ddq*, $J = 12.6, 6.3, 6.3$ Hz), 2.22 (3H, *s*), 2.21 (1H, *dd*, $J = 17.1, 6.1$ Hz), 2.07 (1H, *dd*, $J = 14.3, 6.3$ Hz), 2.06 (1H, *td*, $J = 13.9, 3.7$ Hz), 1.89 (1H, *d*, $J = 17.1$ Hz), 1.80 (1H, *td*, $J = 13.9, 3.7$ Hz), 1.79 (1H, *d*, $J = 13.6$ Hz), 1.77 (1H, *d*, $J = 7.0$ Hz), 1.74 (1H, *ddd*, $J = 14.3, 12.6, 7.0$ Hz), 1.42 (1H, *dt*, $J = 13.9, 3.7$ Hz), 1.35 (1H, *dt*, $J = 13.9, 3.7$ Hz), 1.32 (3H, *s*), 1.06 (3H, *s*), 1.03 (3H, *s*), 1.00 (3H, *d*, $J = 6.3$ Hz), 0.96 (3H, *s*) (Figure 141, page 286).

GS-Y1-1-ACID

Colorless needle crystals (EtOAc). Melting point 238-240°C. Soluble in chloroform, ethyl acetate and acetone. $[\alpha]_D +78^\circ$ ($c = 0.06$, CHCl_3).

UV λ_{max} , (MeOH), nm ($\log \epsilon$) : 253 (4.71) , 307 (3.81) (Figure 142 page 288).

IR ν_{\max} , film, cm^{-1} : 3100-3600, 2924, 1689 (Figure 143, page 288).

^1H NMR (500 MHz, CDCl_3) : 6.71 (1H, *s*), 6.44 (1H, *d*, $J = 9.5$ Hz), 6.31 (1H, *d*, $J = 9.5$ Hz), 5.54 (1H, *d*, $J = 6.1$ Hz), 5.49 (1H, *br s*), 5.16 (1H, *br s*), 3.40 (1H, *br s*), 3.08 (1H, *d*, $J = 14.1$ Hz), 2.31 (1H, *dd*, $J = 15.9, 9.5$ Hz), 2.21 (3H, *s*), 2.14 (1H, *dd*, $J = 15.9, 2.1$ Hz), 2.08 (1H, *m*), 2.06 (1H, *m*), 2.03 (1H, *m*), 1.93 (1H, *d*, $J = 14.1$ Hz), 1.86 (1H, *d*, $J = 16.8$ Hz), 1.78 (1H, *td*, $J = 13.6, 4.1$ Hz), 1.51 (1H, *dt*, $J = 14.3, 4.1$ Hz), 1.44 (1H, *dt*, $J = 13.6, 4.1$ Hz), 1.40 (3H, *s*), 1.21 (3H, *s*), 1.06 (3H, *s*), 1.05 (3H, *s*), 1.04 (3H, *s*) (Figure 144, page 289).

7. Determination of lipophilicity

Lipophilic character of the isolated compounds was determined by means of R_m values which were obtained from the method modified from that of Boya and Milborrow¹⁵¹, and Biagi *et al*¹⁵² as followed.

The TLC plates, prepared from glass plates (20 x20 cm) and coated with 0.2 mm thick layer of silica gel G (Merck), were impregnated with 10% v/v of liquid paraffin in diethyl ether by developing method. Distilled water with acetone in the concentration range of 0-80% saturated with liquid paraffin, were used as aqueous mobile phase. The R_m values was calculated from the resulting R_f values by the equation :

$$R_m = \log (1/R_f - 1).$$

The range of acetone concentration which showed linear relationship to R_m values of each compound was defined and used to determine the extrapolated R_m value in accordance with the equation :

$$\text{Extrapolated } R_m = a (\text{acetone concentration}) + b.$$

Whereas a and b were slope and intercept, respectively. The intercept (b = R_m) was used to compare lipophilicity among tested compounds.

8. Determination of bioactivity

8.1) Brine-shrimp lethality bioassay (BSL assay)

The bioassay was modified from the method of Maeyer *et al*¹⁵³.

8.1.1) Hatching the brine shrimps

The eggs of brine shrimps (*Artemia salina* Leach) (Sigma Union Inc., USA) were hatched in a shallow rectangular box filled with artificial sea water, which was prepared from a commercial salt mixture (Aqua Marine, Jor Charoen aquarium, Thailand). The box was divided into 2 compartments by a perforated divider. The eggs were sprinkled into the compartment which was shielded from light by aluminium foil cover. The other compartment was illuminated. After 48 hours, the brine-shrimp nauplii which had hatched and moved to the lighted side were selected to use in the experiment.

8.1.2) Sample preparation

Test samples were dissolved in methanol or ethyl acetate according to their solubility to proper concentrations. Then, appropriate amounts of the solution were transferred to small test tubes and allowed to dry in vacuum overnight. Concentration of the samples in each test tube was calculated, when 3 ml of artificial sea water was added, as 100, 10, 1 and 0.1 µg/ml. All determination were duplicated with the solvent as control.

8.1.3) Procedure

All samples were dissolved in 1-2 ml of artificial sea water. Sonication was performed to give the best solubility. Fifteen brine shrimps were transferred to each sample tube and the test solutions were immediately adjusted to 3 ml with artificial sea water. The test tubes were maintained under illumination. After 24 hours, the number of dead brine shrimps in each sample concentration were counted and the results were reported in the term of LD₅₀ values (BSLD₅₀), calculated by Finney computer program. The LD₅₀ value of 100 µg/ml was set as positive criteria of this investigation.

8.2) Antimicrobial activity

This bioassay procedure was carried out by aseptic technique. All glasswares were sterilized before used with hot-air oven at 180°C for 1 hour. All sample concentrations were dublicately assayed with DMSO as control. Tetracycline HCl and tolnaftate were utilized as positive control for bacteria and fungi, respectively.

8.2.1) Microorganisms, media and incubation

The microorganisms used for the assay were listed below.

- Gram positive bacteria *Bacillus cereus* ATCC 11778
 Bacillus subtilis ATCC 6633
 Sarcina lutea ATCC 9341
 Staphylococcus aureus ATCC 6538P
- Gram negative bacteria *Escherichia coli* ATCC 25922
 Klebsiella pneumoniae ATCC10031
 Pseudomonas aeruginosa ATCC 9721
 Salmonella typhimurium ATCC 14028
- Yeasts *Candida albicans* ATCC 10230
 Saccharomyces cerevisiae ATCC 9763
- Fungi *Microsporium gypseum* (pathological strain)
 Tricophyton rubrum (pathological strain)

Muller Hinton (MH) media (Difco Laboratory) was utilized for all bacteria cultures, whereas Sabouraud dextrose (S) media (Difco Laboratory) was employed for yeasts and fungi. The media were sterilized by autoclaving at 121°C for 15 minutes before used. Results of the assay were determined after 24 hours incubation at 37°C for bacteria inocula, 2 days at 37°C for yeasts and 5 days at 30°C for fungi.

8.2.2) Susceptibility test

The susceptibility assay was, by means of the agar diffusion technique, modified from the method of Moujir, *et al*³⁴ and Cleeland and Grunberg¹⁵⁴, and carried out with the pure compounds to determine for their spectra of activity.

8.2.2.1) Inocula

8.2.2.1.1) Bacterial inocula

Each bacterium was inoculated to MH broth. After incubation at 37°C for 24 hours, turbidity of the inoculum was adjusted to be equal to that of MacFarland suspension No. 0.5 (0.5% BaCl₂ in 0.36 N H₂SO₄ v/v, equivalent to 10⁸ cells/ml).

8.2.2.1.2) Yeast inocula

Growth of yeast from 2-day culture on S agar slant incubated at 30°C was suspended in sterilized normal saline. The suspension turbidity was adjusted as that of the bacteria.

8.2.2.1.3) Fungal inocula

The 2-3 week growths of fungus on S agar slant at 30°C was scraped and washed from the agar surface with sterilized normal saline. The resulting suspension was further filtered through a gauze sheet to remove mycelial fragment. The filtrate containing fungal spore was diluted to give MacFarland No. 0.5 turbidity.

8.2.2.2) Sample preparation

Each pure compound was dissolved in DMSO to the concentration of 50 and 100 µg/ml. Aliquot method might be used owing to the small amount required.

8.2.2.3) Procedure

The inoculum was mixed with melted agar medium (45-50°C) to the concentration of 1% v/v. The mixture was poured into 100 mm glass petri dishes (20 ml/dish) and allowed to harden. The 6 mm diameter holes were made in the inoculated agar (4-6 holes/dish) and filled with 50 µl of sample solution. The visible inhibition zones were measured after the incubation period as previously mentioned.

8.2.3) Determination of Minimum inhibitory concentration (MIC)

Antimicrobial potency of active compounds from the preliminary assay was shown as the MIC against the susceptible microorganisms. The assay was performed using either the agar dilution or broth dilution techniques described by Cleeland and Grunberg¹⁵⁴.

8.2.3.1) Inocula

Microorganism inocula were prepared by the same method as that of susceptibility test.

8.2.3.2) Sample preparation

Serial 2-fold dilution of each tested sample were prepared in DMSO to give concentrations 20-fold higher than the final desired concentrations (0.125-64 µg/ml or 0.125-8 µg/ml for compounds with limited quantities).

8.2.3.3) Procedure

7.2.3.3.1) Agar dilution method

Each plate was prepared by mixing 1 ml of sample dilution with 19 ml of melted agar medium (45-50°C). The mixture was poured into 100 mm round glass petri dish and allowed to harden. Microorganism inocula were transferred into the seed tray of Steers-Foltz replicator and inoculated to the sample dilution dishes by a replicator. Several kinds of microorganisms were inoculated at the same time.

7.2.3.3.2) Broth dilution method

The mixtures were prepared from 0.25 ml of each sample dilution and 4.5 ml of broth media in the 18 x 150 mm test tube. All tubes were inoculated with 0.25 ml of the microorganism inocula and incubated.

After the period of incubation as mentioned above, the MIC values of both methods were defined as the lowest concentration of samples that visibly inhibited growth of the microorganisms.

9. Molecular mechanic calculations

The molecular mechanic studies were performed on the Chem3D version 3.5.1 computer program. The most stable conformation of the molecules were energy-minimized using the MM2 force field calculation.