# CHAPTER III

# EXPERIMENTALS

## A : MATERIALS

### EQUIPMENT

1. Melting point meter : BÜCHI 535
2. Ultraviolet-Visible spectrometer : Varian, Cary/1E/UV-Visible Spectrometer
3. Infrared spectrometer : Perkin Elmer FT-IR Spectrometer SPECTRUM 2000 and JASCO-FT/IR-5000
4. Nuclear magnetic resonance : JMR-A500 and ALPHA
5. Mass spectometer : Jeol FX 3000 double focusing

### CHEMICALS

1. Morin dihydrate : 95.0 % purity : Fluka
2. Acetic anhydride : AR : Riedel-deHaën
3. Palmitoyl chloride : 98.0 % purity : Aldrich Chemical
4. Nicotinic acid : Biochemistry grade : E. Merck
5. Thionyl chloride : General purpose reagent : BDH

6. Pyridine : A.C.S. reagent : Aldrich Chemical

7. Diethyl ether : AR : E. Merck

8. Chloroform : AR : Carlo Erba

9. n-Hexane : AR : Carlo Erba

10. Methanol : AR : BDH

11. Calcium chloride, anhydrous : AR : T.J. Baker

12. Sodium sulfate, anhydrous : 99% purity : Fluka

13. Sodium carbonate, anhydrous : AR : Riedel-deHaën

14. Dichloromethane : AR : Carlo Erba

15. Acetone : AR : Carlo Erba

16. Argon gas

## B : METHODS

### GENERAL

1. The reactions were carried out under argon atmosphere, excepted the reaction between nicotinic acid and thionyl chloride.

2. In preparative thin-layer chromatography, E. Merck silica gel 60 GF 254 was used as an absorbent.

3. Melting points were determined on a BÜCHI 535 melting point meter.

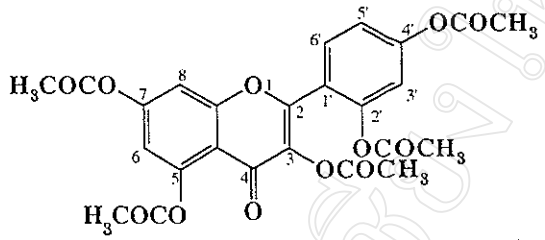
4. Ultraviolet-visible spectra were run on Varian, Cary/1E/UV-Visible Spectrometer, using chloroform as solvent.

5. Infrared spectra were taken on Perkin Elmer FT-IR Spectrometer SPECTRUM 2000 as potassium bromide pellet, excepted infrared spectra of morin-3,7,2′,4′-tetrapalmitate and nicotinoyl chloride were taken on JASCO-FT/IR-5000.

6. Proton nuclear magnetic resonance spectra were performed with a JEOL JNM-A-500 (500 MHz) and carbon-thirteen nuclear magnetic resonance spectrum of morin-3,5,7,2′,4′- pentaacetate was determined on ALPHA (500 MHz). The spectra were recorded in a deuterochloroform solution with tetramethylsilane added as the internal reference standard, chemical shift in ppm.

7. The electron-impact mass spectrum of morin-3,5,7,2′,4′-pentaacetate was acquired using a Jeol FX 3000 double focusing. The electron-impact conditions were 70 eV. The sample were introduced into the vacuum by direct insertion probe. And the mass spectra of morin-3,7,2′,4′-tetrapalmitate and morin-3,5,7,2′,4′-pentanicotinate were determined by using fast-atom bombardment technique.

## MORIN-3,5,7,2′,4′-PENTAACETATE (56)



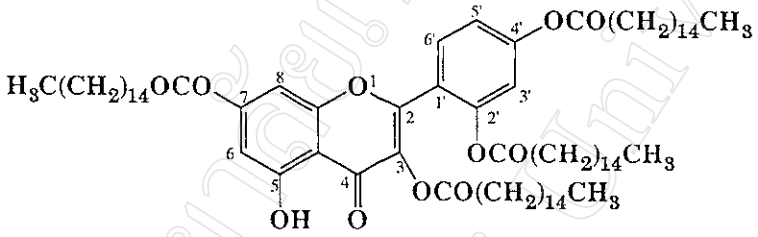
A mixture of morin dihydrate ( 100 mg, 0.30 mmol ), acetic anhydride (2.0 ml, 0.02 mol) and pyridine (2 drops) was stirred for 20 minutes at 90 °C. The reaction mixture was poured into 50.0 ml of ice water. Morin pentaacetate was extracted with 60.0 ml of diethyl ether and washed with 50.0 ml of 0.2 N hydrochloric acid , 2x50.0 ml of 0.2 N sodium carbonate and 2x50.0 ml of distilated water. Diethyl ether phase was dry with anhydrous sodium sulfate and evaporated under reduced pressure to give colorless amorphous morin-3,5,7,2′,4′- pentaacetate which was pured, showed by TLC [Stationary phase : 20x20 aluminum sheet E. Merck silica gel 60 GF 254 ; Mobile phase : chloroform-hexane-methanol (10-5-1) ; $R_f$ = 0.68], mp. 142.5-143.8 °C. Percentage yield was 66.8.

The structure of morin-3,5,7,2′,4′-pentaacetate was confirmed by ultraviolet-visible spectrum (see page 67), infrared spectrum (see page 71), proton nuclear magnetic resonance spectra (see page 76-78), carbon-thirteen nuclear magnetic resonance (see page 86-87) and mass spectrum

(see pages 89,90).

The reaction apparatuses were set up at shown in appendix F,page 91.

## MORIN-3,7,2′,4′-TETRAPALMITATE (56)



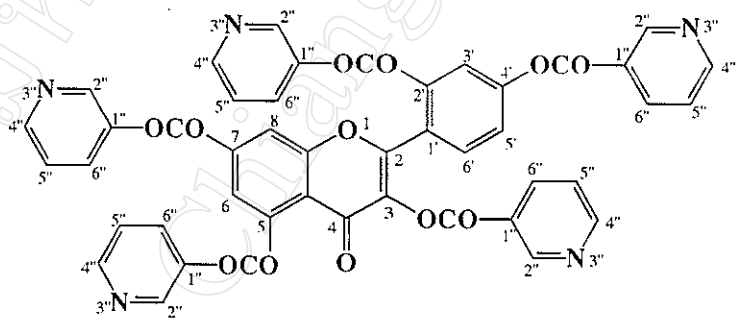

A mixture of morin dihydrate (100 mg, 0.30 mmol), palmitoyl chloride (546.0 mg, 1.99 mmol), dichloromethane (30.0 ml) and pyridine (2 drops) was refluxed for 2 hours at 90 °C. The reaction mixture was poured into 50.0 ml of ice water. The crude products were extracted with 60.0 ml of dichloromethane and washed with 2x50.0 ml of 0.2 N hydrochloric acid, 5x50.0 ml of 0.002 N sodium carbonate and 2x50.0 ml of distillated water. Dichloromethane phase was dried with anhydrous sodium sulfate and evaporated under reduced presure. Morin-3,7,2′,4′-tatrapalmitate was separated and purified by preparative thin-layer chromatography; using chloroform-hexane-methanol (10-5-1) as mobile phase and dichloromethane as extraction solvent. Dichloromethane phase was dried with anhydrous sodium sulfate and evaporated under reduced

pressure to give 23.5 percentage yield colorless amorphous morin-3,7,2′,4′-tetrapalmitate which was pured, showed by TLC [Stationary phase : 20x20 aluminum sheet E. Merck silica gel 60 GF 254 ; Mobile phase : chloroform-hexane-methanol (10-5-1) ; $R_f$ = 0.86], mp. 60.9-61.9 °C.

The structure of morin-3,7,2′,4′- tetrapalmitate was confirmed by ultraviolet-visible spectrum (see page 68), infrared spectrum (see page 72), proton nuclear magnetic resonance spectrum (see pages 79-81) and mass spectrum (see pages 91, 92)

The reaction apparatuses were set up at shown in appendix F, page 96.

## MORIN-3,5,7,2′,4′- PENTANICOTINATE (56)



Thionyl chloride (0.5 ml) was added dropwise into the stirred mixture of nicotinic acid (553.4 mg) and pyridine (1.0 ml) at room temperature. The mixture was gradually heated to and maintained at 90 °C for 1 hour, to the resulting nicotinoyl chloride (IR spectrum : see page 73).

The mixture was added a solution of morin dihydrate (109.4 mg, 0.31 mmol) in pyridine (5.0 ml), then the mixture was heated for 1 hour at 90 $^{\circ}$C . The reaction mixture was poured into 50.0 ml of ice water. The crude products were extracted with 60.0 ml of diethyl ether and washed with 3x50.0 ml of 0.2 N hydrochloric acid, 2x50.0 ml of 0.2 N sodium carbonate and 2x50.0 ml of distillated water. Diethyl ether phase was dried with anhydrous sodium sulfate and evaporated under reduced pressure. Morin-3,5,7,2$'$,4$'$- pentanicotinate was separated and purified by preparative thin-layer chromatography; using chloroform-hexane-methanol (7-4-1) as mobile phase and dichloromethane as extraction solvent. Dichloromethane phase was evaporated under reduced pressure to give 12.2 percentage yield colorless amorphous morin-3,5,7,2$'$,4$'$- pentanicotinate which was pured, showed by TLC [Stationary phase : 20x20 aluminum sheet E. Merck silica gel 60 GF 254 ; Mobile phase : chloroform-hexane-methanol (7-4-1) ; $R_f$ = 0.36], mp. 140.9-142.6 $^{\circ}$C.

The structure of morin-3,5,7,2$'$,4$'$-pentanicotinate was confirmed by Ultraviolet-Visible spectrum (see page 69), infrared spectrum (see page 74), proton nuclear magnetic resonance spectrum (see pages 82-85) and mass spectrum (see page 93).

The reaction apparatuses were set up at shown in appendix F, pages 97 and 98.