Title in English Bioactive constituents from the leaves of *Clinacanthus nutans* Lindau

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Abstract The leaves of *Clinacanthus nutans* Lindau have long been traditionally used in Thailand as an anti-inflammatory drug for the treatment of insect bites, herpes infection and allergic responses. The crude chloroform extract was separated by column chromatography and further purified by preparative thin-layer chromatography to give six pure compounds. Structure elucidation of the isolated compounds was carried out on the basis of spectral analysis, including DEPT, COSY, NOESY, HMQC and HMBC. Five of these were identified as novel compounds related to chlorophyll a and chlorophyll b; they are 13^2-hydroxy-(13^2-S)-chlorophyll b, 13^2-hydroxy-(13^2-R)-phaeophytin b, 13^2-hydroxy-(13^2-S)-phaeophytin b, purpurin 18 phytyl ester, phaeophorbide a. The other was stigmasteryl-3-O-β-D-glucopysanoside, Which was not previously reported in this species.

Keywords (≤5) *Clinacanthus nutans* Lindau, chlorophyll a and chlorophyll b related compounds

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Graduate School, Kasetsart University

BIOACTIVE CONSTITUENTS FROM THE LEAVES OF *CLINACANTHUS NUTANS* LINDAU

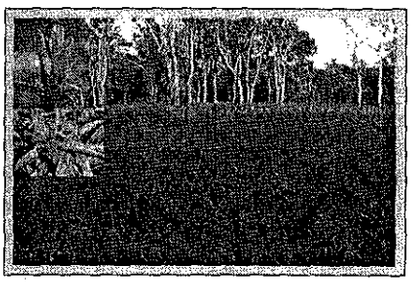
Presented by
Asst. Prof. Dr.Santi Saiudarat
Mr. Ketthai Punyakom

School of Chemistry
Institute of Science
Suranaree University of Technology

Outline

- Introduction
- Research Objectives
- Experimental
- Results and Discussion
- Conclusions

Introduction



Scientific Name: *Clinacanthus nutans* (Burm. f.) Lindau

Family: *Acanthaceae*

Common Name: Slaed Pang Pon (Tua mia), Phayaa Yo, Phak Man Kai, Phak Lin Khiat (Chiang Mai), Phayaa Plong Thong, Phayaa Plong Khom (Lampang), Pho-so-chuang (Karen-Mae Hong Son).

Phayaa Yo

•The leaves of Thai medicinal plant are traditionally used in the treatment of snake and insect bites.

•The plant is promoted for treatment of herpes simplex, herpes zoster, and skin pruritus in primary health care and public health care units across the country.

Pharmacological Study

•*C. nutans* extract was shown to possess anti-inflammatory (Sangkitporn et al.,1993) and virucidal activities against herpes simplex virus type 2 *in vitro* (Jayavasu et al., 1992).

•Clinical evaluations of extract in human subjects also gave satisfactory results in both patients suffering from genital herpes and herpes zoster by shortening the duration of the diseases and reducing the associated pain (Jayavasu et al., 1992).

Pharmacological Study

- Its therapeutic ability surpassed even that of acyclovir cream. Moreover, toxicological study indicated the extract to be quite safe (Sangkitporn et al.,1993).

- The plant thus has strong potential to be developed as a novel source of medicine to treat herpes infections.

medicinal products

Research Objectives

To extract and isolate the chemical constituents from the leaves of *C. nutans*

To purify and identify the structural formula of the isolated substances

To study bioactivity of the extracts from the leaves of *C. nutans*

Experimental

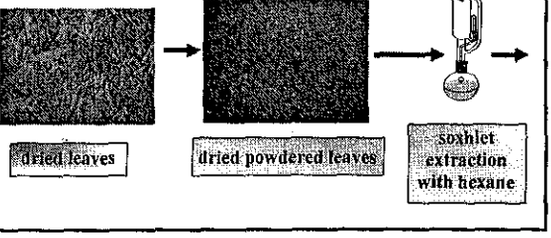
- Materials
  - The leaves of *C. nutans*
  - Chloroform
  - Hexane
  - Ethyl acetate
  - Methanol
  - Thin Layer Chromatography (TLC)
  - Preparative Thin Layer Chromatography

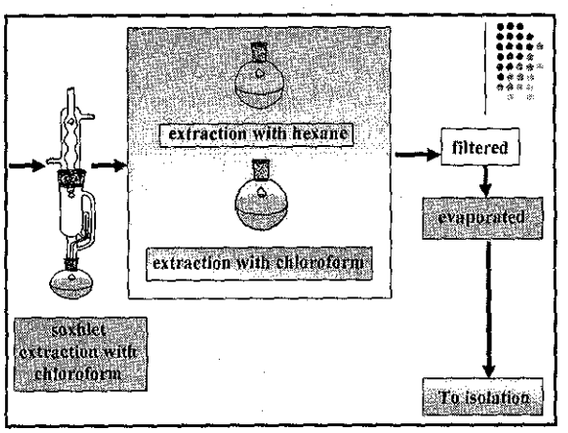
Experimental

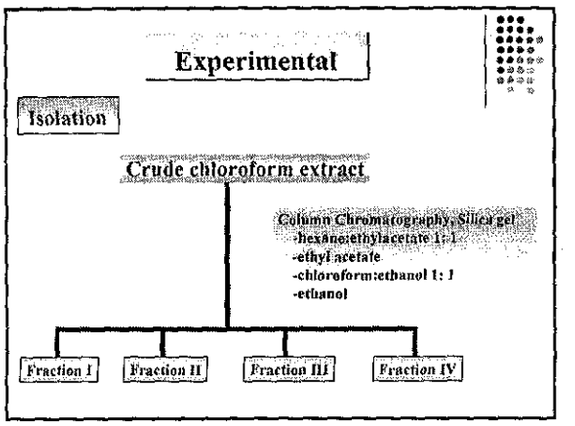
- Apparatus
  - Rotary evaporator (Buchi R-114 Vacuum System)
  - Spectrophotometer (Camag UV-Cabinet)
  - NMR (Varian: INOVA 300)
  - FT-IR (Model Spectrum GX, Perkin-Elmer)

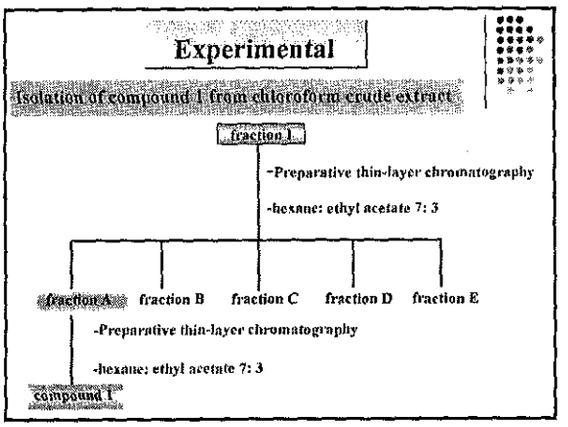
Experimental

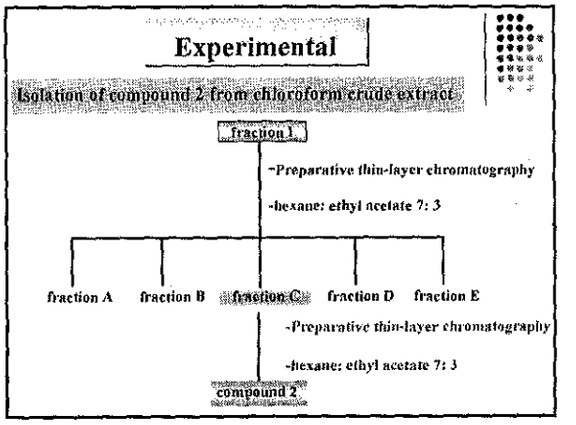
Extraction

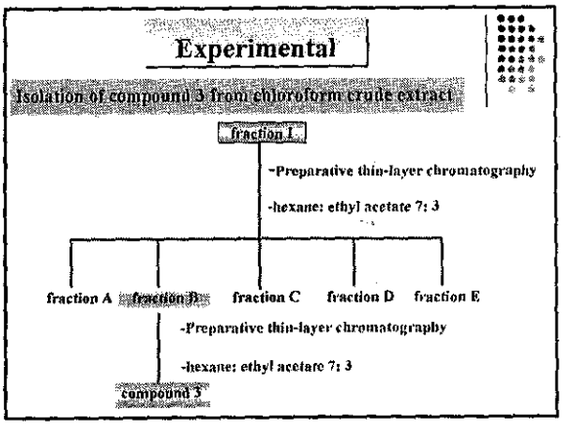


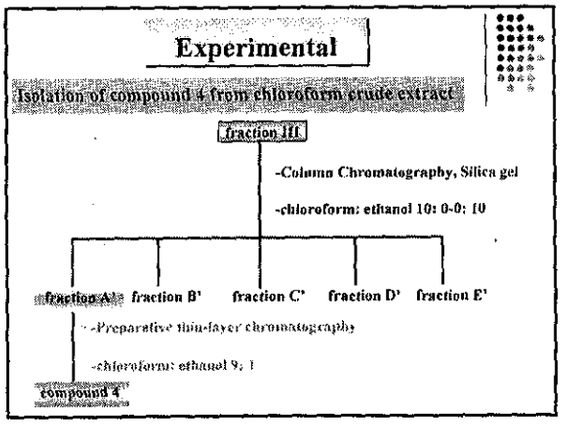

extraction with hexane
filtered
evaporated
extraction with chloroform
soxhlet extraction with chloroform
To isolation


Experimental
Isolation
Crude chloroform extract
Column Chromatography, Silica gel
-hexane:ethylacetate 1: 1
-ethyl acetate
-chloroform:ethanol 1: 1
-ethanol
Fraction I
Fraction II
Fraction III
Fraction IV


Experimental
Isolation of compound 1 from chloroform crude extract
fraction I
-Preparative thin-layer chromatography
-hexane: ethyl acetate 7: 3
fraction A
fraction B
fraction C
fraction D
fraction E
-Preparative thin-layer chromatography
-hexane: ethyl acetate 7: 3
compound 1


Experimental
Isolation of compound 2 from chloroform crude extract
fraction I
-Preparative thin-layer chromatography
-hexane: ethyl acetate 7: 3
fraction A
fraction B
fraction C
fraction D
fraction E
-Preparative thin-layer chromatography
-hexane: ethyl acetate 7: 3
compound 2


Experimental
Isolation of compound 3 from chloroform crude extract
fraction I
-Preparative thin-layer chromatography
-hexane: ethyl acetate 7: 3
fraction A
fraction B
fraction C
fraction D
fraction E
-Preparative thin-layer chromatography
-hexane: ethyl acetate 7: 3
compound 3


Experimental
Isolation of compound 4 from chloroform crude extract
fraction III
-Column Chromatography, Silica gel
-chloroform: ethanol 10: 0-0: 10
fraction A'
fraction B'
fraction C'
fraction D'
fraction E'
-Preparative thin-layer chromatography
-chloroform: ethanol 9: 1
compound 4

Experimental

Isolation of compound 5 from chloroform crude extract

fraction III

-Column Chromatography, Silica gel

-chloroform: ethanol 10: 0-0: 10

fraction A' fraction B' fraction C' fraction D' fraction E'

-Preparative thin-layer chromatography

-chloroform: ethanol 9: 1

compound 5

Experimental

Isolation of compound 6 from chloroform crude extract

fraction IV

-Column Chromatography, Silica gel

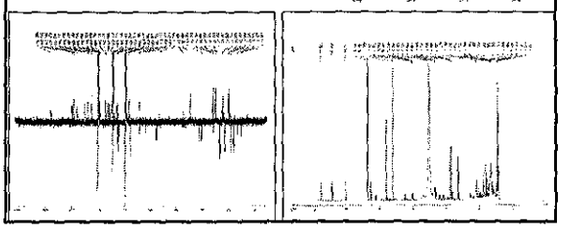
-chloroform: methanol 10: 0-0: 10

compound 6

Results and Discussion

^{13}C-NMR and ^{1}H Spectrums of Compound 1

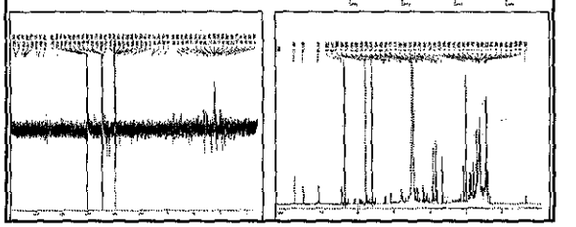
13^2-hydroxy-(13^2-S)-chlorophyll b



Results and Discussion

^{13}C-NMR and ^{1}H Spectrums of Compound 2

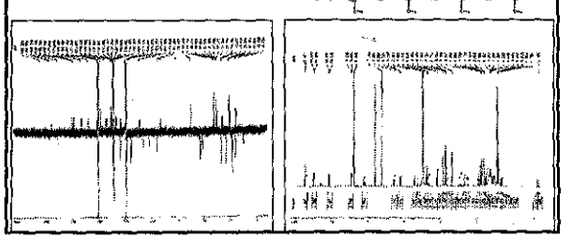
13^2-hydroxy-(13^2-R)-phaeophytin b



Results and Discussion

^{13}C-NMR and ^{1}H Spectrums of Compound 3

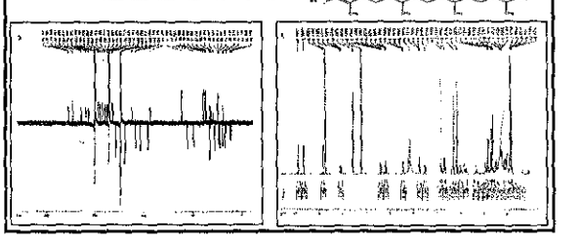
13^2-hydroxy-(13^2-S)-phaeophytin b



Results and Discussion

^{13}C-NMR and ^{1}H Spectrums of Compound 4

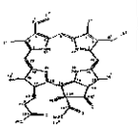
purpurin 18 phytyl ester

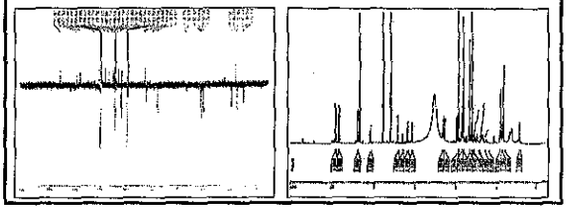


Results and Discussion

^{13}C-NMR and ^{1}H Spectrums of Compound 5

phaeophorbide a

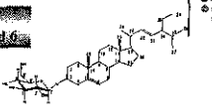


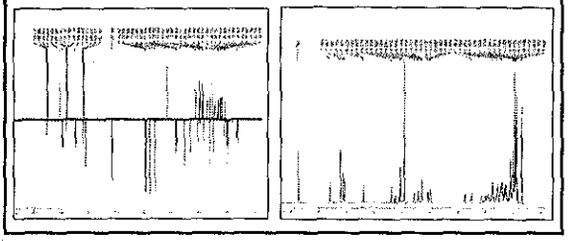


Results and Discussion

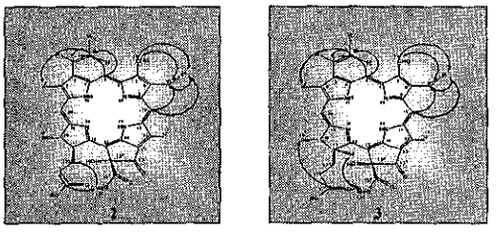
^{13}C-NMR and ^{1}H Spectrums of Compound 6

stigmastery1-3-O-β-D-glucopysanoside



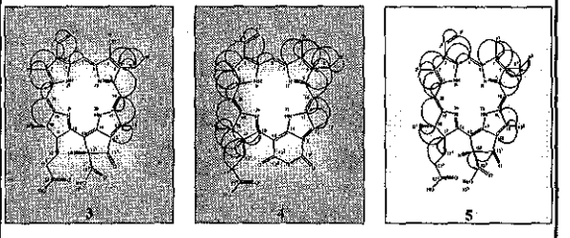


Results and Discussion



The NOESY correlations of compounds 2 and 3

Results and Discussion



The HMBC correlations in compounds 3, 4, and 5

Conclusions

The dried powdered leaves of *C. nutans* (Burm. f.) Lindau were extracted with hexane and chloroform, respectively. The crude chloroform extract was separated by column chromatography and further purified by preparative thin-layer chromatography to give six pure compounds.

Structure elucidation of the isolated compounds was carried out on the basis of spectral analysis, including DEPT, COSY, NOESY, HMQC, and HMBC.

Conclusions

Five of these were identified as novel compounds related to chlorophyll a and chlorophyll b; they are

13²-hydroxy-(13²-S)-chlorophyll b,
13²-hydroxy-(13²-R)-phaeophytin b,
13²-hydroxy-(13²-S)-phaeophytin b,
purpurin 18 phytyl ester,
phaeophorbide a.

The other was stigmastery1-3-O-β-D-glucopysanoside, which was not previously reported in this species.

Acknowledgements

I would like to thank Asst. Prof. Dr. Sumil Sakdarat for allowing me to work in his group and learn more about the area of natural product chemistry. I would also like to thank all the members of the Sakdarat group for their help and guidance.

Thank you

Question please.