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Chemical Constituent from the MeOH extract of the stem of *Cananga latifolia*

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Abstract

Five juvenile hormone III (JH III), (2*E*,6*E*,10*R*)-10,11-epoxy-3,7,11-trimethyldodeca-2,6-dienoic acid methyl ester (**1**), (2*E*,6*E*,10*R*)-10-hydroxy-3,7,11-trimethyldodeca-2,6,11-trienoic acid methyl ester (**2**), (2*E*,6*E*)-11-hydroxy-3,7,11-trimethyldodeca-10-one-2,6-dienoic acid methyl ester (**3**), (2*E*,6*E*,10*R*)-10-acetoxy-11-hydroxy-3,7,11-trimethyl dodeca-2,6-dienoic acid methyl ester (**4**) and (2*E*,6*E*,10*R*)-10,11-dihydroxy-3,7,11-trimethyldodeca-2,6-dienoic acid methyl ester (**5**), have been isolated from the MeOH extract of the stem of *C. latifolia*. Their structures were elucidated on the basic of spectroscopy methods and by comparison with spectroscopic data in the literature.

Keywords: *Cananga latifolia*, juvenile hormone III, sesquiterpenes

1. Introduction

Cananga latifolia is known as "Sakae Saeng" in Thailand, and it is a tree, up to 15 m in height, growing widely in Cambodia, Vietnam, Laos, Myanmar and Thailand.[1] The stems are used as an antipyretic and the stem barks are used as nasal polyposis and antifungal activity against *Pythium insidiosum* .[2],[3],[4] The roots and seeds of this plant are used for the treatment of infectious diseases in early childhood, antirheumatism, antimalarial and antidiarrhoeal.[5],[6] In previous investigations of *C. latifolia* was found that alkaloids, fatty acids, flavonoids, flavonoid glycosides and juvenile hormone III analogues were isolated from the stem barks and roots .[7],[8] In this work, we now report the isolation and structural illumination of five juvenile hormone III analogues from the MeOH extract of the stem of *C. latifolia*.

Juvenile hormones (JHs) are sesquiterpeniods that are vitally important in many functions to all insects. They regulate insect development, reproduction, metamorphosis, polyphenism, diapauses and caste differentiation.[9] The most commonly found JH is JH III, (2*E*,6*E*)-10,11-epoxy-3,7,11-trimethyldodeca-2,6-dienoic acid methyl ester which regulates the biological functions of insects.[10] JH III analogues are harmless to non-arthropods, but selectively effective on insects so they can be used as natural insecticides. JH derivatives have been found in plants such as *Cyperus iria* and *Cyperus aromatics* and, recently, *Cananga latifolia*.[11],[12]

2. Materials and Methods

2.1 General experiment procedures

UV spectra were measured using a JASCO J-810 apparatus. IR spectra were obtained using Bruker Tenser 27 spectrophotometer. NMR spectra were recorded on a Varian Mercury plus spectrometer operating at 400 MHz (^{1}H) and at 100 MHz (^{13}C). Thin layer chromatography (TLC) was carried out on Merck silica gel 60 F_{254} TLC aluminium sheet. Preparative layer chromatography (PLC) was carried out on glass supported silica gel plates using silica gel 60 PF_{254} for preparative layer chromatography. Column chromatography was done with silica

gel 0.063-0.200 mm or less than 0.063 mm and the packing material for molecular sieve column chromatography was Sephadex LH-20. All solvents were routinely distilled prior to use.

2.2 Plant material

The stems of *C. latifolia* were collected in November 2015 from Phuwieng District, Khon Kaen Province, Thailand. The plant was identified by Dr. Pranom Chantaranothai, Faculty of Science, Khon Kaen University, Thailand

2.3 Extraction and isolation

The stems of *C. latifolia* (5.5 kg) were extracted at room temperature for three days with hexane (2 × 10 L), EtOAc (2 × 10 L), and MeOH (2 × 10 L). The extracts were evaporated *in vacuo* to obtain three dry extracts, crude hexane (23.5 g), EtOAc (135.3 g), and crude MeOH (330.7 g).The MeOH extract was separated by silica gel flash column chromatography (FCC) and the column was eluted with a gradient system hexane/EtOAc, EtOAc, EtOAc/MeOH, MeOH. The eluents were collected and monitored by thin layer chromatography (TLC) resulting in 9 groups of eluting fraction which were designated as F_1 to F_9. Fraction F_4 was purified by silica gel FCC using 5% EtOAc:hexane as eluent to give three subfractions, $F_{4.1}$-$F_{4.3}$. Subfraction $F_{4.1}$ was purified by PLC and 10% EtOAc:hexane was used as developing to afford 1 (1.2 g, 0.0218%). Purification of subfraction $F_{4.2}$ by PLC using 15% EtOAc:hexane as developing solvent to yield 2 (14.2 mg,0.0003%). Fraction F_6 was purified by silica gel FCC and eluted with a gradient system of EtOAc:hexane to give eight subfractions, $F_{6.1}$-$F_{6.8}$. Purification of subfraction $F_{6.5}$ with gel filtration (Sephadex LH-20) and eluting with MeOH to give three subfractions, $F_{6.5.1}$-$F_{6.5.3}$. Subfraction $F_{6.5.1}$ was purified by PLC and 50% EtOAc:hexane was used as developing solvent to afford 3 (25.1 mg, 0.0005%). Subfraction $F_{6.7}$ and $F_{6.8}$ were subjected on a column of Sephadex LH-20, using MeOH as eluent and then by PLC (40% EtOAc:hexane) to give 4 (15.7 mg, 0.0003%) and 5 (1.9 g, 0.0345%), respectively.

2.4 Spectroscopic Data of Compounds

(2*E*,6*E*,10*R*)-10,11-epoxy-3,7,11-trimethyl-dodeca-2,6-dienoic acid methyl ester (1): colorless oil; IR (Neat) ν_{max} 3463, 2973, 1716, 1647, 1221, 1149 cm^{-1}; ^1H ($CDCl_3$, 400 MHz) and ^{13}C NMR data ($CDCl_3$, 100 MHz), see Tables 1 and 2

(2*E*,6*E*,10*R*)-10-hydroxy-3,7,11-trimethyl-dodeca-2,6,11-trienoic acid methyl ester (2): colorless oil; IR (Neat) ν_{max} 3467, 2928, 1721, 1650, 1224, 1152 cm^{-1}; ^1H ($CDCl_3$, 400 MHz) and ^{13}C NMR data ($CDCl_3$, 100 MHz), see Tables 1 and 2

(2*E*,6*E*)-11-hydroxy-3,7,11-trimethyldodeca-10-one-2,6-dienoic acid methyl ester (3): colorless oil; IR (Neat) ν_{max} 3486, 2975, 1713, 1648, 1224, 1148 cm^{-1}; ^1H ($CDCl_3$, 400 MHz) and ^{13}C NMR data ($CDCl_3$, 100 MHz), see Tables 1 and 2

(2*E*,6*E*,10*R*)-10-acetoxy-11-hydroxy-3,7,11-trimethyl dodeca-2,6-dienoic acid methyl ester (4): colorless oil; IR (Neat) ν_{max} 3464, 2977, 1715, 1648, 1227, 1148 cm^{-1}; ^1H ($CDCl_3$, 400 MHz) and ^{13}C NMR data ($CDCl_3$, 100 MHz), see Tables 1 and 2

(2*E*,6*E*,10*R*)-10,11-dihydroxy-3,7,11-trimethyldodeca-2,6-dienoic acid methyl ester (5): colorless oil; IR (Neat) ν_{max} 3426, 2948, 1717, 1647, 1222, 1144 cm^{-1}; ^1H ($CDCl_3$, 400 MHz) and ^{13}C NMR data ($CDCl_3$, 100 MHz), see Tables 1 and 2

3. Results and Discussions

A MeOH extract of dried stem of *C. latifolia* was accomplished by repeated column chromatography and Sephadex LH-20 followed by PLC to give five known compounds, (2*E*,6*E*,10*R*)-10,11-epoxy-3,7,11-trimethyldodeca-2,6-dienoic acid methyl ester (**1**)[13], (2*E*,6*E*,10*R*)-10-hydroxy-3,7,11-trimethyldodeca-2,6,11-trienoic acid methyl ester (**2**)[8], (2*E*,6*E*)-11-hydroxy-3,7,11-trimethyldodeca-10-one-2,6-dienoic acid methyl ester (**3**)[4], (2*E*,6*E*,10*R*)-10-acetoxy-11-hydroxy-3,7,11-trimethyl dodeca-2,6-dienoic acid methyl ester (**4**)[14],[15] and (2*E*,6*E*,10*R*)-10,11-dihydroxy-3,7,11-trimethyldodeca-2,6-dienoic acid methyl ester (**5**).[16] All isolated compounds were

juvenile hormone III and the structural elucidation of all compounds were identified on the basic of spectroscopy method. The ^{1}H and ^{13}C NMR spectra of all compounds exhibited Table 1 and 2, respectively. The biological activity of compounds **1**, **2** and **5** exhibited antifungal activity against *Pythium insidiosum* by showing inhibition zone with diameters of 16.0, 14.0 and 24.0 mm (Phatchana et al., 2015), respectively.

4. Conclusions

The isolation and purification of Thai traditional medicine plant, *Cananga latifolia* have been studied. The chromatographic separation from the crude MeOH extract from the stems of *C. latifolia* afforded five compounds (Figure 1). They were five known juvenile hormone III analogues, (2*E*,6*E*,10*R*)-10,11-epoxy-3,7,11-trimethyldodeca-2,6-dienoic acid methyl ester (**1**), (2*E*,6*E*,10*R*)-10-hydroxy-3,7,11-trimethyldodeca-2,6,11-trienoic acid methyl ester (**2**), (2*E*,6*E*)-11-hydroxy-3,7,11-trimethyldodeca-10-one-2,6-dienoic acid methyl ester (**3**), (2*E*,6*E*,10*R*)-10-acetoxy-11-hydroxy-3,7,11-trimethyl dodeca-2,6-dienoic acid methyl ester (**4**) and (2*E*,6*E*,10*R*)-10,11-dihydroxy-3,7,11-trimethyldodeca-2,6-dienoic acid methyl ester (**5**). Their structures were elucidated on the basis of spectroscopic method, including UV, IR, ^{1}H NMR and ^{13}C NMR). Compounds **1**, **2** and **5** exhibited antifungal activity against *Pythium insidiosum* by showing inhibition zone with diameters of 16.0, 14.0 and 24.0 mm, respectively.

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6. References

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Table 1 ^{1}H NMR (400 MHz, $CDCl_3$) spectral data of compounds **1-5** (*J* in ppm).

position	1	2	3	4	5
2	5.66, s	5.67, s	5.66, s	5.68, s	5.67, s
4	2.17, m	2.13, m	2.16, m	2.17, m	2.13, m
5	2.17, m	2.18, m	2.16, m	2.17, m	2.18, m
6	5.13, br s	5.06, br s	5.10, br s	5.07, br s	5.09, br s
8	2.15, m	2.13, m	2.28, t (7.6)	1.93, m	2.00, m
9	1.64, m	1.654, m	2.60, t (7.6)	1.71, m	1.60 m
10	2.69, t (6.2)	4.08, t (6.2)		4.77, dd (12.0,4.0)	3.25, dd (9.2,3.3)
12	1.25, s	H_a 4.82, s H_b 5.96, s	1.36, s	1.18, s	1.09, s
13	1.29, s	1.60, s	1.38, s	1.19, s	1.14, s
14	1.62, s	1.68, s	1.62, s	1.59, s	1.56, s
15	2.17, s	2.16, s	2.17, s	2.16, s	2.14, s
2'				2.11, s	
OMe	3.68, s	3.68, s	3.68, s	3.68, s	3.68, s

Table 2 ^{13}C NMR (100 MHz, $CDCl_3$) spectral data of compounds **1-5**.

position	1	2	3	4	5
1	167.1 C	167.2 C	167.3 C	167.4 C	167.3 C
2	115.3 CH	115.3 CH	115.6 CH	115.5 CH	115.3 CH
3	159.8 C	159.9 C	159.9 C	160.0 C	159.9 C
4	40.8 CH_2	40.8 CH_2	40.8 CH_2	40.9 CH_2	40.7 CH_2
5	25.9 CH_2	25.9 CH_2	26.0 CH_2	25.0 CH_2	25.8 CH_2
6	123.5 CH	123.3 CH	123.7 CH	123.8 CH	123.4 CH_3
7	135.3 C	135.8 C	135.0 C	135.3 C	135.9 C
8	36.3 CH_2	35.6 CH_2	33.8 CH_2	36.2 CH_2	36.6 CH_2
9	27.4 CH_2	33.1 CH_2	34.6 CH_2	28.0 CH_2	29.7 CH_2
10	64.1 CH	75.5 CH	215.2 C	79.7 CH	77.9 CH
11	58.2 C	147.5 C	76.5 C	72.6 C	72.6 C

12	18.8 CH_3	110.9 CH_2	26.5 CH_3	25.0 CH_3	23.1 CH_3
13	24.8 CH_3	17.6 CH_3	26.5 CH_3	26.8 CH_3	26.3 CH_3
14	15.9 CH_3	15.9 CH_3	16.0 CH_3	16.1 CH_3	15.9 CH_3
15	18.7 CH_3	18.8 CH_3	18.8 CH_3	19.0 CH_3	18.7 CH_3
1'				171.3 C	
2'				23.0 CH_3	
OMe	50.7 CH_3	50.8 CH_3	50.9 CH_3	50.9 CH_3	50.8 CH_3

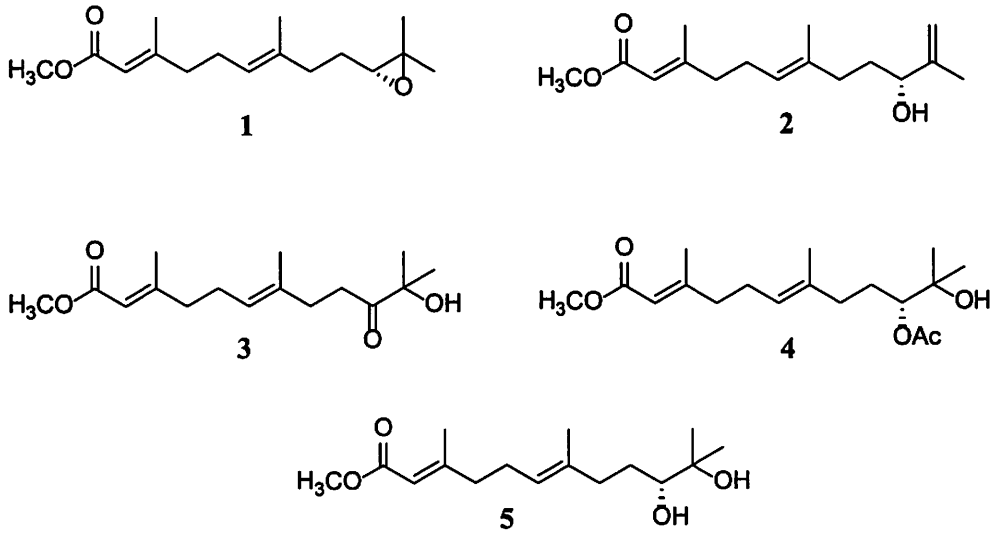

Figure 1 Structures of isolated compounds from the MeOH extract of stem of *C. latifolia*