Chemical Constituents from The Roots of *Dalbergia Stipulacea*

องค์ประกอบทางเคมีจากรากมะขามเฒ่า

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ABSTRACT

The propose of this study was to extract and purify the chemical constituents from the crude methanol extract of the roots of *Dalbergia stipulacea* by chromatographic methods.The chemical investigation led to the isolation of six known compounds including two pterocarpan derivatives; medicarpin (**1**) and nitiducarpin (**2**), two isoflavone derivatives; formononetin (**3**) and biochanin A (**4**) and two isoflavane derivatives; nitidulan (**5**) and dalvelutinane A (**6**). Their structures were characterized by spectroscopic methods (IR, ^{1}H-NMR and ^{13}C-NMR) and by comparison with those of published compounds.

บทคัดย่อ

การศึกษานี้มีวัตถุประสงค์เพื่อสกัดแยกองค์ประกอบทางเคมีและทำให้บริสุทธิ์จากส่วนสกัดหยาบเมทานอลของรากต้นมะขามเฒ่าด้วยวิธีการทางโครมาโทกราฟี จากการแยกองค์ประกอบทางเคมีพบว่าได้สารประกอบที่มีการรายงานโครงสร้างแล้วทั้งหมด 6 สาร ประกอบด้วย อนุพันธ์เทอร์โรคาแพน 2 สาร คือ medicarpin (**1**) และ nitiducarpin (**2**) อนุพันธ์ของสารไอโซฟลาโวน 2 สาร คือ formononetin (**3**) และ biochanin A (**4**) และอนุพันธ์ของสารไอโซฟลาแวน 2 สาร คือ nitidulan (**5**) และ dalvelutinane A (**6**) พิสูจน์โครงสร้างของสารเหล่านี้โดยวิธีการทางสเปคโทรสโกปี (IR, ^{1}H-NMR และ ^{13}C-NMR) และเปรียบเทียบกับสารที่มีการรายงานโครงสร้างแล้ว

Keywords: *Dalberdia stipulacea*, Pterocarpan, Isoflavone

คำสำคัญ: ต้นมะขามเฒ่า เทอร์โรคาแพน ไอโซฟลาโวน

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Introduction

Dalbergia stipulacea (Fabaceae), a climbing shrub, was found in Thailand, Vietnam, Laos, southern China and eastern India. Its roots are used as fish poison (Bhatt et al. 1992). The plant is traditional medicine as infusion of the wood and roots is considered to be emmenagogue. The major compound from the roots of this plant was stipulin (Bhatt et al. 1992). Moreover, luteolin 4'-rutinoside and luteolin were found from its leaves (Borai and Dayal 1993). It was found many flavonoids and isoflavonoids from *Dalbergia* genus such as *D. parviflora* (Songsiang et al. 2009 and 2011; Umehara et al. 2009). Several geranylated flavanones, veluflavanones A-P, were discovered from the stems of *D. velutina* (Kaennakam et al. 2019). Many glycoside derivatives were also isolated from the same plant (Kaennakam et al. 2016). Isoflavones, isoflavone glycosides and neoflavones were isolated from *D. spinosa* (Radha et al. 2015). Moreover, coumarins, chalcones, flavonoids, isoflavonoids glycosides and aurones were found form *D. tonkinensis* (The Son et al. 2018). There is very few studies of chemical substances from *D. stipulacea*. In this study, the crude methanol extract of the roots of *D. stipulacea* was purified and six compounds were isolated.

Objectives of the study

1. To purify the chemical constituents from the crude methanol extract of the roots of *D. stipulacea* by chromatographic methods.

2. To identify the structures of pure compounds by spectroscopic analysis.

Materials and methods

General

The IR spectra were measured with a FTIR Spectrometer: BRUKER TENSOR 27. ^{1}H and ^{13}C-NMR spectra were recorded in $CDCl_3$ using a Varian 400 Plus Spectrometer. Column chromatography (CC) and flash column chromatography (FCC) were carried out on silica gel 60 (0.0063-0.200 and less than 0.0063 mm mesh). Preparative thin layer chromatography was examined on glass-supported silica gel plates using silica gel 60 PF_{254} for preparative layer chromatography. Thin layer chromatography (TLC) was carried out on MERCK silica gel 60 F_{254} TLC aluminium sheets. TLC spots were visualized by UV light (254 nm) or staining with anisaldehyde reagent.

Plant material

The roots of *D. stipulacea* were collected in February 2018 from Phuwieng District, Khon Kaen Province, Thailand. The plant was identified by Assoc. Prof. Suppachai Tiyaworanant, Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand. A voucher specimen (KKU012018) was deposited at the Department of Chemistry, Faculty of Science, Khon Kaen University, Thailand.

Extraction and isolation

The air-dried powdered roots of *D. stipulacea* (11.0 kg) were extracted with hexanes (3×20 L), EtOAc (3×20 L) and MeOH (3×20 L) at room temperature for three days. After evaporation, crude hexanes (162.0 g), EtOAc (368.2 g), and MeOH (375.7 g) extracts were obtained. The crude MeOH was subjected to silica gel CC, eluted with a gradient system of EtOAc: hexanes and EtOAc: MeOH. On the basis of their TLC profile, the fractions which contained the same major compounds were combined to give 11 fractions (MF1-MF11). Fraction MF2 was purified by silica gel CC and eluted with a gradient of 10% EtOAc: hexanes to give nine subfractions, MF2.1-MF2.9. Subfraction MF2.1 was purified on silica gel CC using 2% EtOAc:hexanes as eluent to obtain **1** (27.7 mg). Subfraction MF2.2 was purified on silica gel FCC and 5% acetone: hexanes as eluting solvent to give **2** (12.3 mg). Subfraction MF2.3 was purified by FCC and 5% EtOAc:hexanes as eluting solvent to afford **3** (10.5 mg). Subfraction MF2.4 was separated by FCC and 10% EtOAc:hexanes as eluent to obtain **5** (47.7 mg). Further purification of subfraction MF2.5 by preparative TLC (2% acetone:CH_2Cl_2) yielded **6** (6.5 mg). The solid in subfraction MF2.7 was recrystallized (EtOAc:hexanes) to give **4** (3.1 mg).

Results and discussion

Chromatographic separation of methanol extract of the roots of *D. stipulacea* yielded six known compounds (**1**-**6**). The structures of all isolated compounds were identified by spectroscopic techniques (IR, ^{1}H-NMR and ^{13}C-NMR) and compared with published values. They were (-)-medicarpin (**1**) (Cheng et al. 2019), nitiducarpin (**2**) (Charles and Gandhidasan 2006), formononetin (**3**) (Korbanjhon et al. 2017), biochanin A (**4**) (Songsiang et al. 2009), nitidulan (**5**) (Kaennakam et al. 2017) and dalvelutinane A (**6**) (Kaennakam et al. 2017). (Figure 1).

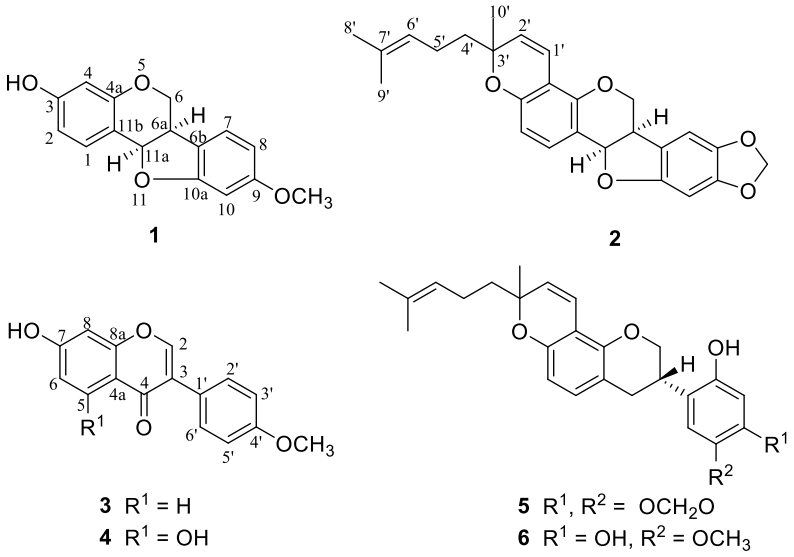

Figure 1 Structures of the isolated compounds from the roots of *D. Stipulacea*

Compound **1** was obtained as a yellowish powder, mp. 123–124 °C (Lit. = 128 °C (Sichaem et al. 2018)). The IR spectrum showed C-H and C-C stretching frequencies of aromatic at 3374 and 1618 cm^{-1}, respectively. The ^{1}H NMR displayed ABX spin system of H-1 (δ_H 7.30, d, *J* = 8.4 Hz), H-2 (δ_H 6.52, dd, *J* = 8.4, 2.4 Hz) and H-4 (δ_H 6.41, d, *J* = 2.4 Hz). The other ABX pattern of H-7 (δ_H 7.10, d, *J* = 8.0 Hz), H-8 (δ_H 6.36, dd, *J* = 8.0, 2.4 Hz) and H-10 (δ_H 6.39, d, *J* = 2.4 Hz) was evident. The ^{1}H-NMR signal of H-6α displayed as doublet of doublets signal at δ_H 4.17 (*J* = 10.8, 4.9 Hz) and H-6β showed triplet signal at δ_H 3.56 (*J* = 10.8 Hz). The signal at δ_H 5.44 (d, *J* = 6.7 Hz) was assigned as H-11a. The methoxyl group exhibited singlet signal at δ_H 3.72. The ^{13}C-NMR spectrum contained four oxygenated aromatic carbons at δ_C 161.0 (C-9), 160.6 (C-10a), 158.4 (C-3) and 156.5 (C-4a). In addition, an oxygenated methylene carbon C-6 exhibited at δ_C 66.5 while an oxygenated methine carbon C-11a showed at δ_C 78.8. The comparison of the ^{1}H and ^{13}C NMR spectra of **1** corresponded to (-)-medicarpin.

Compound **2** was found as a white amorphous solid, mp. 84-85 °C (Lit. = 84 °C (Heerden et al. 1978)). The ^{1}H and ^{13}C NMR spectra of **2** were similar to those of **1**, except for the presence of an additional geranyl group. Two doublet signals at δ_H 6.70 (*J* = 10.0 Hz, H-1′) and 5.54 (*J* = 10.0 Hz, H-2′) were observed. The olefinic proton H-6′ showed triplet signal at δ_H 5.12 with *J* = 6.0 Hz because it was coupled with methylene protons H-5′. Three singlet signals at δ_H 1.70, 1.60 and 1.41 were assigned as CH_3-8′, CH_3-9′ and CH_3-10′, respectively. In addition, the methylenedioxy group appeared at δ_H 5.90 and 5.88 and attached to carbon at δ_C 101.2. The ^{13}C NMR also showed additional four olefinic carbons at δ_C 131.5 (C-7′), 128.0 (C-2′), 124.1 (C-6′) and 117.0 (C-1′); two methylene carbons at δ_C 41.1 (C-4′) and 22.7 (C-5′); three methyl carbons at δ_C 26.3 (C-10′), 25.7 (C-8′) and 17.6 (C-9′). The oxygenated quaternary carbon at δ_C 78.8 was determined to C-3′. Thus compound **2** was identified as nitiducarpin.

Compound **3** was obtained as a amorphous solid, mp. 250–251 °C (Lit. = 250–251 °C (Sichaem et al. 2018)). The IR spectrum displayed the presence of carbonyl group at 1599 cm^{-1}. The ^{13}C NMR also showed the conjugated carbonyl carbon at δ_C 174.6 (C-4). Its ^{1}H and ^{13}C NMR spectra showed the characteristic signal of isoflavone flamework at δ_H/δ_C 8.35 (1H, s, H-2)/153.2 (C-2). The ABX pattern of protons on ring A displayed at δ_H 7.98 (1H, d, *J* = 8.7 Hz, H-5), 6.95 (1H, dd, *J* = 8.7, 2.1 Hz, H-6) and 6.88 (1H, d, *J* = 2.1, H-8). The *para* disubstituted aromatic pattern of ring B showed at δ_H 7.52 (2H, d, *J* = 8.5, H-2′ and 6′) and 7.00 (2H, d, *J* = 8.5, H-3′ and 5′). Three oxygenated carbons appeared at δ_C 162.6 (C-7), 159.0 (C-4′) and 157.5 (C-8a). The carbon signals at δ_C 130.1 (H-2′ and H-6′) and 113.6 (H-3′ and H-5′) were evident. A methoxyl group showed signals at δ_H/δ_C 3.80 (3H, s)/55.2 in the ^{1}H and ^{13}C NMR spectra. From all data, compound **3** was determined as formononetin.

Compound **4** was given as a white amorphous solid, mp. 213–214 °C (Lit. = 213–214 °C (Sichaem et al. 2018)). The carbonyl group of this compound showed absorption band at 1650 cm^{-1} in the IR experiment. In addition, the signal at δ_C 180.7 of carbonyl carbon was observed in the ^{13}C NMR data. A 1,4-disubstituted aromatic moiety was evident by showing two doublet signals at δ_H 7.37 (2H, d, *J* = 8.6, H-2′ and 6′) and δ_H 6.91

(2H, d, J = 8.6, H-3′ and 5′). Two *meta* coupling protons (J = 2.0 Hz) of H-6 (δ_H 6.25) and H-8 (δ_H 6.29) were detected. The low field signal at δ_H 12.87 was assigned as intramolecular hydrogen bonding of OH-5. An oxygenated methine carbon at δ_C 152.6 was identified as C-2, the characteristic signal of isoflavone derivative. The ^{13}C NMR also exhibited four oxygenated carbons at δ_C 164.0 (C-7), 162.1 (C-5), 159.6 (C-4′), and 158.2 (C-8a). The high field carbon signals at δ_C 99.2 and 94.0 were assigned to C-6 and C-8, respectively. The same as **3**, carbons C-2′/C-6′ and C-3′/C-5′ displayed at δ_C 130.0 and 114.0, respectively, in the ^{13}C NMR data. Thus, compound **4** was elucidated as biochanin A.

Compound **5** was obtained as a white amorphous solid, mp. 55-56 °C (Lit. = 55 °C (Heerden et al. 1978)). The IR data displayed O-H stretching band at 3728 cm^{-1}. In the ^{1}H NMR spectrum, two doublet signals (J = 8.2 Hz) at δ_H 6.81 and 6.36 were assigned as H-5 and H-6, respectively. Two singlet signals at δ_H 6.38 and 6.60 were assigned to H-3′ and H-6′, respectively. The prochiral methylene group at C-2 displayed at δ_H 4.34 (br d, J = 10.0 Hz, H-2a) and 3.99 (t, J = 10.0 Hz, H-2b). In addition, prochiral methylene group at C-4 position showed at δ_H 2.91 (dd, J = 15.0, 10.3 Hz, H-4a) and 2.80 (dd, J = 15.0, 6.7 Hz, H-4b). The multiplet signal at δ_H 3.50 (H-3) and broad singlet signal of hydroxy proton at δ_H 4.74 were found in the ^{1}H NMR data. A methylenedioxy group appeared at δ_H 5.89 and δ_H 5.88 and were attached on carbon at δ_C 101.1. Five oxygenated aromatic carbons at δ_C 152.1 (C-7), 149.6 (C-8a), 147.8 (C-2′), 146.4 (C-4′) and 141.9 (C-5′) were determined. The sp^3 methylene carbons at δ_C 69.9 (C-2) and 30.6 (C-4) as well as sp^3 methine carbon at δ_C 31.8 (C-3) were assigned. This compound contained geranyl moiety were similar to that of **2**. In this part, two doublet signals (J = 10.0 Hz) of H-1′′ (δ_H 6.67) and H-2′′ (δ_H 5.52) as well as triplet signal (J = 7.0 Hz) of H-6′′ were observed. An oxygenated quaternary carbon displayed at δ_C 77.0 (C-3′′) while olefinic carbons appeared at δ_C 124.2 (C-6′′) and 131.5 (C-7′′) in the ^{13}C NMR experiment. Thus, compound **5** was identified as nitidulan.

Compound **6** was found as a yellow viscous oil. The hydroxy group showed the characteristic band at 3389 cm^{-1} in the IR spectrum. The ^{1}H and ^{13}C NMR spectra were similar to those of **5**, except for the disappearance of methylenedioxy group. These spectra showed methoxyl group at δ_H/δ_C 3.78 (3H, s)/56.8. Two hydroxy group exhibited as broad singlet at δ_H 5.49 and 4.62. From all data, compound **6** was determined as dalvelutinane A.

Conclusions

The chemical investigation of crude methanol extract of *D. stipulacea* roots led to the isolation of six known compounds. From the structural identification by using spectroscopic methods and the comparison with the previous reported, the known compounds were (-)-medicarpin (**1**), nitiducarpin (**2**), formononetin (**3**), biochanin A (**4**), nitidulan (**5**) and dalvelutinane A (**6**).

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