Published in final edited form as:

Nat Prod Commun. 2009 July ; 4(7): 907–910.

Phytochemical Characterization of the Leaves of *Mitragyna speciosa* Grown in USA

Francisco León,

Eman Habib,

Jessica E. Adkins,

Edward B. Furr,

Christopher R. McCurdy,

Stephen J. Cutler

Department of Medicinal Chemistry & National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, MS 38677, USA

Abstract

Mitragyna speciosa (Rubiaceae) has traditionally been used in the tropical regions of Asia, Africa and Indonesia as a substitute for opium. Indole alkaloids are the most common compounds that have been isolated. We investigated the constituents of the leaves of *M. speciosa* that was grown at the University of Mississippi. Several alkaloids were isolated, including ajmalicine, corynantheidine, isomitraphylline, mitraphylline, paynantheine, isocorynantheidine, 7-hydroxymitragynine and mitragynine, but their percentages were lower than those in a commercial Thai sample of “kratom”. In addition, we isolated the flavonoid epicatechin, a saponin daucosterol, the triterpenoid saponins quinovic acid 3-*O*-β-D-quinovopyranoside, quinovic acid 3-*O*-β-D-glucopyranoside, as well as several glycoside derivatives including 1-*O*-feruloyl-β-D-glucopyranoside, benzyl-β-D-glucopyranoside, 3-oxo-α-ionyl-*O*-β-D-glucopyranoside, roseoside, vogeloside, and epivogeloside. This is the first report of the last group of compounds having been isolated from a *Mitragyna* species. Biological studies are currently underway to test these compounds for opioid activity.

Keywords

Mitragyna speciosa ; alkaloids; glycoside derivatives; triterpenoid saponins

Mitragyna (Rubiaceae) is a small Afro-Asian genus consisting of nine species; four African and five Asian. They are mainly arboreal trees with characteristic mitriform stigmatic lobes and globular flowering heads [1]. In Thailand and Malaysia, *M. speciosa*, *M. hirsuta*, *M. diversifolia*, *M. rotundifolia* are commonly seen [2], but the most known species is *M. speciosa* or “kratom” in Thailand and “biak-biak” in Malaysia. The leaves of *M. speciosa* have been habitually used by natives and laborers for their euphoric effects at low doses, either by chewing the leaves or by infusing them (decoction) to make tea [3]. In traditional

cutler@olemiss.edu .

medicine, the plant has been used to treat diarrhea, cough, hypertension, and to relieve muscle pain. It is also commonly used as a substitute for opium [4].

The use of *M. speciosa* has been forbidden in Thailand since 1946 due its narcotic effects. It is also illegal in Australia, Malysia, and Myanmar. However, the availability of *M. speciosa* on the internet reflects a high demand for this material. In the United States, the use of *Mitragyna* is still legal, however, the DEA has put it in a list of drugs and chemicals of concern since 2005 [5]. Kratom is currently purchased by approximately 40 million Americans to self-manage the symptoms associated with opium withdrawal [6].

Phytochemical studies on *M. speciosa* [7a] showed that mitragynine, an indole alkaloid, is the major alkaloid [7b], constituting around 50% of the total alkaloidal content, and is responsible for the opioid effects. Recent pharmacological studies reported antinociceptive effects in animal models for an analogous alkaloid, 7-hydroxymitragynine [7c,7d].

Environmental factors play a vital role in modifying the alkaloidal content or even the presence of other classes of metabolites in the same species. For example, *M. speciosa* from Thailand has higher content in mitragynine than *M. speciosa* from Malaysia [7b,7d].

The exceptional properties of this medicinal plant have attracted many researchers in recent years [4,7]. In the course of our project to find therapeutic agents for pain, anxiety, and drug addiction [3], we investigated the secondary metabolites in the MeOH extract of the leaves of *M. speciosa* growing in the gardens of the University of Mississippi. Herein we report the isolation and identification of eighteen compounds including eight alkaloids: ajmalicine [8a], corynantheidine [8b], isomitraphylline [8c], mitraphylline [8c], paynantheine [7d], isocorynantheidine [8d], 7-hydroxymitragynine [7b], and mitragynine [7b]; a flavonoid (−)-epicatechin [7e]; a saponin daucosterol [9a]; two triterpenoid saponins: quinovic acid 3-*O*-β-D-quinovopyranoside [9b] and quinovic acid 3-*O*-β-D-glucopyranoside [9a]; two monoaryl glycosides: 1-*O*-feruloyl-β-D-glucopyranoside [10a] and benzyl-β-D-glucopyranoside [10b]; two cyclohexenone glycosides: 3-oxo-α-ionyl-*O*-β-D-glucopyranoside [10c] and (6*S*, 9*R*) roseoside [10d]; and two secoiridoid glycosides: vogeloside [10e] and epivogeloside [10e]. The structures of all isolated compounds were identified by interpretation of their spectral data including ESI-MS, ^{1}H and ^{13}C NMR, as well as by comparison of their spectral data with those reported previously in the related literature (Figure 1).

The predominant alkaloid in *M. speciosa* leaves was mitraphylline, in contrast with Takayama *et al* [7b,7d] who reported mitragynine as the major alkaloid in *M. speciosa* from Thailand and Malaysia. Moreover, we observed less prevalence of C-9-methoxy indole alkaloids. These results reinforce the idea that there are different sub-varieties of *M. speciosa* from the viewpoint of plant chemotaxonomy, as proposed by Takayama *et al.* [7d]. On the other hand, Shellard [7e] proposed a biogenetic pathway in *M. speciosa* and reported that the amount of mitragynine is less in young plants than in old trees. This could be important because our samples were from trees younger than five years old, although environmental factors (temperature, soil composition, pressure, etc) could have had a major effect on the alkaloidal patterns. It is also worth mentioning that in our study we observed a significant decrease in the total amount of alkaloids compared to previous studies [7].

Although triterpenoid saponins are common in most of the Rubiaceae family [9c] and have been encountered in several members of the genus *Mitragyna* [9a,9d], this is the first report of their presence in *M. speciosa*.

It is rare to find secoiridoids or monoterpenes in Rubiaceae. Also there are few examples of compounds reported that belong to the vogeloside or roseoside chemical type [10f]. To the best of our knowledge there are no reports of monoaryl glycosides derivatives from *Mitragyna* species.

According to the above results, we suggest that our USA grown *M. speciosa* displays a different chemotype than the Asian-African plants.

Experimental

General Experimental Procedures:

^{1}H and ^{13}C NMR spectra were obtained on Bruker model AMX-500 and 400 NMR spectrometers with standard pulse sequences, operating at 500 MHz and 400 MHz in ^{1}H and 125 MHz and 100 MHz in ^{13}C. $CDCl_3$, Acetone-d_6, CD_3OD, and CD_3CN were used as solvents and TMS as internal standard. The High-Resolution Mass Spectra (HRMS) were recorded on a Micromass Q-Tof Micro mass spectrometer with a lock spray source. Column chromatography was carried out on silica gel (70–230 mesh, Merck) and Sephadex LH-20. Fractions obtained from column chromatography were monitored by TLC (silica gel 60 F_{254}) and preparative TLC was carried out on silica gel 60 $PF_{254+366}$ plates (20 × 20 cm, 1 mm thick).

Plant Material:

Young plants of *M. speciosa* were obtained in January of 2005, identified by Rita Moraes from the National Center for Natural Products Research, and grown in the gardens of the University of Mississippi, University, MS, USA. Fresh leaves were collected in August 2008 and extracted.

A voucher (MISS 76727) specimen has been deposited in the herbarium of the University of Mississippi.

Extraction and isolation:

Fresh leaves (70 g) were powdered and extracted with hot MeOH in a Soxhlet apparatus for 72 h. The extract was evaporated and gave a residue (18 g), which was subjected to column chromatography on Si gel using *n*-hexane-EtOAc/MeOH gradient to afford one hundred fractions. Fractions (60–85) contained alkaloids (as indicated by Dragendorff's reagent). These fractions were combined and chromatographed on Silica gel using hexane/EtOAc gradient until 100% EtOAc followed by EtOAc/MeOH gradient until 20%, to give 20 sub-fractions (200 mL/fraction). The sub-fractions were compared using analytical TLC ($CHCl_3$/Acetone 9:1 and 8:2) to yield four substantive fractions. Fraction 1 was chromatographed on Sephadex LH-20 using hexane/DCM/MeOH (1:1:1) and silica gel column using petroleum ether/acetone gradient until 50% to give 20 sub-fractions, the first sub-fractions (1–10)were combined and rechromatographed on preparative TLC with

$CHCl_3$/acetone (8:2), and toluene/acetone (9:1) (two elutions) to yield ajmalicine (3 mg, 1.6×10^{-4} %), mitragynine (8 mg, 4.4×10^{-4} %), corynantheidine (4 mg, 2.2×10^{-4} %). The last ten sub-fractions were combined and purified using preparative TLC using benzene/EtOAc (7:3) and $CHCl_3$/acetone (75:25) to yield the alkaloids 7-hydroxymitragynine (1.2 mg, 6.6×10^{-5} %), isomitraphylline (7 mg, 3.8×10^{-4} %), and mitraphylline (4 mg, 2.2×10^{-4} %). Fraction 2 was chromatographed by column chromatography using a silica gel column eluted with $CHCl_3$/acetone gradient to obtain 40 sub-fractions. Sub-fractions 1–8 were combined and chromatographed by preparative TLC eluted with DCM/acetone (8:2) to obtain paynantheine (2 mg, 1.1×10^{-4} %). The sub-fractions 9–20 were combined and a precipitate was formed and recrystallized to give mitraphylline (20 mg, total 24 mg, 1.3×10^{-3} %). Sub-fractions 21–25 were chromatographed using silica gel column with $CHCl_3$/MeOH gradient, followed with Sephadex LH-20 using DCM/MeOH (1:1) to give the compounds isocorynantheidine (3 mg, 1.6×10^{-4} %), and a mixture of saponins that was separated by Sephadex LH-20 using MeOH to yield daucosterol (30 mg, 1.6×10^{-3} %) as the major product, and quinovic acid 3-*O*-β-D-quinovopyranoside (15 mg, 8.3×10^{-4} %). In the last subfractions 26–40, there was a major product that was purified by Sephadex LH-20 using $CHCl_3$/MeOH (1:1) as mobile phase to yield epicatechin (12 mg, 6.6×10^{-4} %). Fraction 3 was chromatographed by silica gel column using EtOAc/MeOH gradient, to obtain twenty fractions which were combined in three fractions (**a-c**). The fraction (**a**) was rechromatographed by Sephadex LH-20, to yield quinovic acid 3-*O*-β-D-glucopyranoside (10 mg, 5.5×10^{-4}%) as a major product. Fraction (**b**) was purified by preparative TLC using toluene/IPA (8:2) and $CHCl_3$/IPA (7:3) to obtain 1-*O*-feruloyl-β-D-glucopyranoside (8 mg, 4.4×10^{-4} %). The last fraction (**c**) was chromatographed by Sephadex LH-20 and silica gel column using $CHCl_3$/IPA to obtain benzyl-β-D-glucopyranoside (7 mg, 3.8×10^{-4} %). Fraction 4 was chromatographed by column of silica gel using EtOAc with increasing polarity with MeOH to obtain twenty fractions. The sub-fractions 5–10 were chromatographed over Sephadex LH-20 using $CHCl_3$/MeOH (1:1), then further purified by preparative TLC using EtOAc/MeOH (10%) and $CHCl_3$/IPA (9:1) several times to yield the compounds (+)-3-oxo-α-ionyl-*O*-β-D-glucopyranoside (3.2 mg, 1.7×10^{-4} %), roseoside (4 mg, 2.2×10^{-4} %), epivogeloside (2 mg, 1.1×10^{-4} %), and vogeloside (5 mg, 2.7×10^{-4} %).

Acknowledgments-

This investigation was conducted in a facility constructed with support from research facilities improvement program C06 RR-14503-01 from the NIH National Center for Research Resources and research support by the NIH-NCRR 5P20RR021919. This paper's contents are solely the responsibility of the authors and do not necessarily represent the official view of NCRR or NIH.

References

[1]. Razafimandimbison SG, Bremer B. (2002) Phylogeny and classification of Naucleeae S.L. (Rubiaceae) inferred from molecular (ITS, *RBCL*, and *TRNT-F*) and morphological data. American Journal of Botany, 89, 1027–1041. [PubMed: 21665704]

[2]. Sukrog S, Zhu S, Ruangrusgsi N, Phadungcharoen T, Palanuvej C, Komatsu K. (2007) Molecular analysis of the genus *Mitragyna* existing in Thailand based on rDNA ITS sequences and its applications to identify a narcotic species: *Mitragyna speciosa*. Biological & Pharmaceutical Bulletin, 30, 1284–1288. [PubMed: 17603168]

[3]. Babu KM, McCurdy CR, Boyer EW. (2008) Opioid receptors and legal highs: *Salvia divinorum* and Kratom. Clinical Toxicolgy, 46, 146–152.

[4]. Jansen KL, Prast CJ. (1988) Ethnopharmacology of kratom and the *Mitragyna* alkaloids. Journal of Ethnopharmacology, 23, 115–119. [PubMed: 3419199]

[5]. U.S. Department of Justice, Drug Enforcement Administration, (2006) Microgram Bulletin, 39, 30–32. (www.usdoj.gov/dea/programs/forensicsci/microgram/mg0306/mg0306.html)

[6]. Boyer E, Babu K, Macalino G, Compton W. (2007) Self-treatment of opioid withdrawal with a dietary supplement, kratom. American Journal on Addictions, 16, 352–356. [PubMed: 17882605]

[7]. (a)Shellard EJ. (1974) The alkaloids of *Mitragyna* with special reference to those of *Mitragyna speciosa*, Korth. Bulletin on Narcotics, 26, 41–55; [PubMed: 4607551] (b)Takayama H, Kurihara M, Kitajima M, Said IM, Aimi N. (1998) New indole alkaloids from the leaves of Malaysian *Mitragyna speciosa*. Tetrahedron, 54, 8433–8440;(c)Ponglux D, Wongseripipatana S, Takayama H, Kikuchi M, Kurihara M, Kitajima M, Aimi N, Sakai SI. (1994) A new indole alkaloid, 7α-hydroxy-7H-mitragynine, from *Mitragyna speciosa* in Thailand. Planta Medica, 60, 580–581; [PubMed: 17236085] (d)Matsumoto K, Horie S, Takayama H, Ishikawa H, Aimi N, Ponglux D, Murayama T, Watanabe K. (2005) Antinociception, tolerance and withdrawal symptoms induced by 7-hydroxymitragynine, an alkaloid from the Thai medicinal herb *Mitragyna speciosa*. Life Sciences, 78, 2–7; [PubMed: 16169018] (e)Houghton PJ, Said IM. (1986) 3-dehydromitragynine: an alkaloid from *Mitragyna speciosa*. Phytochemistry, 25, 2910–2912;(f)Shellard E6J, Houghton PJ, Resha M. (1978) The *Mitragyna* species of Asia, part XXXII. Planta Medica, 34, 253–263.

[8]. (a)Wenkert E, Chang CJ, Chawla HPS, Cochran DW, Hagaman EW, King JC, Orito K. (1974) General methods of synthesis of indole alkaloids. Short routes of construction of Yohimboid and ajmalicinoid alkaloid system and their ^{13}C Nuclear magnetic resonance spectral analysis. Journal of the American Society, 98, 3645–3655;(b)Staerk D, Lemmich E, Christensen J, Kharazmi A, Olsen CE, Jaroszewski JW. (2000) Leishmanicidal, antiplasmodial and cytotoxic activity of indole from *Corynanthe pachyceras*. Planta Medica, 66, 531–536; [PubMed: 10985079] (c)Seki H, Takayama H, Aimi N, Sakai SI, Ponglux D. (1993) A nuclear magnetic resonance study on the eleven stereoisomers of heteroyohimbine-type oxindole alkaloids. Chemical & Pharmaceutical Bulletin, 41, 2077–2086;(d)Lounasmaa M, Jokela R, Laine C, Hanhinen P. (1998) Preparation of (±)-hirsutine and (±)-isocorynantheidine. Heterocycles, 49, 445–450.

[9]. (a)Bishay DW, Che CT, Gonzalez A, Pezzuto JM, Kinghorn AD, Farnsworth NR. (1988) Further chemical constituents of *Mitragyna inermis* stem bark. Fitoterapia, 59, 397–398;(b)Ahmad VU, Uddin S, Bano S. (1990) Saponins from *Zygophyllum propinquum*. Journal of Natural Products, 53, 1193–1197;(c)Quin GW. (1998) Some progress on chemical studies of triterpenoid saponins from Chinese medicinal plants. Current Organic Chemistry, 2, 613–625;(d)Kang W, Hao X. (2006) Triterpenoid saponins from *Mitragyna rotundifolia*. Biochemical Systematics and Ecology, 34, 585–587.

[10]. (a)Baderschneider B, Winterhalter P. (2001) Isolation and characterization of novel benzoates, cinnamates, flavonoids, and lignans from Riesling wine and screening for antioxidant activity. Journal of Agricultural and Food Chemistry, 49, 2788–2798; [PubMed: 11409967] (b)DeRosa S, DeGuilio A, Tommonaro G. (1996) Aliphatic and aromatic glycosides from the cell cultures of *Lycopersicon esculentum*. Phytochemistry, 42, 1031–1034; [PubMed: 8688181] (c)Cui B, Nakamura M, Kinjo J, Nohara T. (1993) Chemical constituents of *Astragali semen*. Chemical & Pharmaceutical Bulletin, 41, 178–181;(d)Yamano Y, Ito M. (2005) Synthesis of optically active vomifoliol and roseoside stereoisomers. Chemical & Pharmaceutical Bulletin, 53, 541–546; [PubMed: 15863927] (e)Recio-Iglesias MC, Marston A, Hostettmann K. (1992) Xanthones and secoiridoid glucosides of *Halenia campanulata*. Phytochemistry, 31, 1387–1389;(f)Kitajima M, Fujii N, Yoshino F, Sudo H, Saito K, Aimi N, Takayama H. (2005) Camptothecins and two new monoterpene glucosides from *Ophiorrhiza liukiuensis*. Chemical & Pharmaceutical Bulletin, 53, 1355–1358. [PubMed: 16205003]

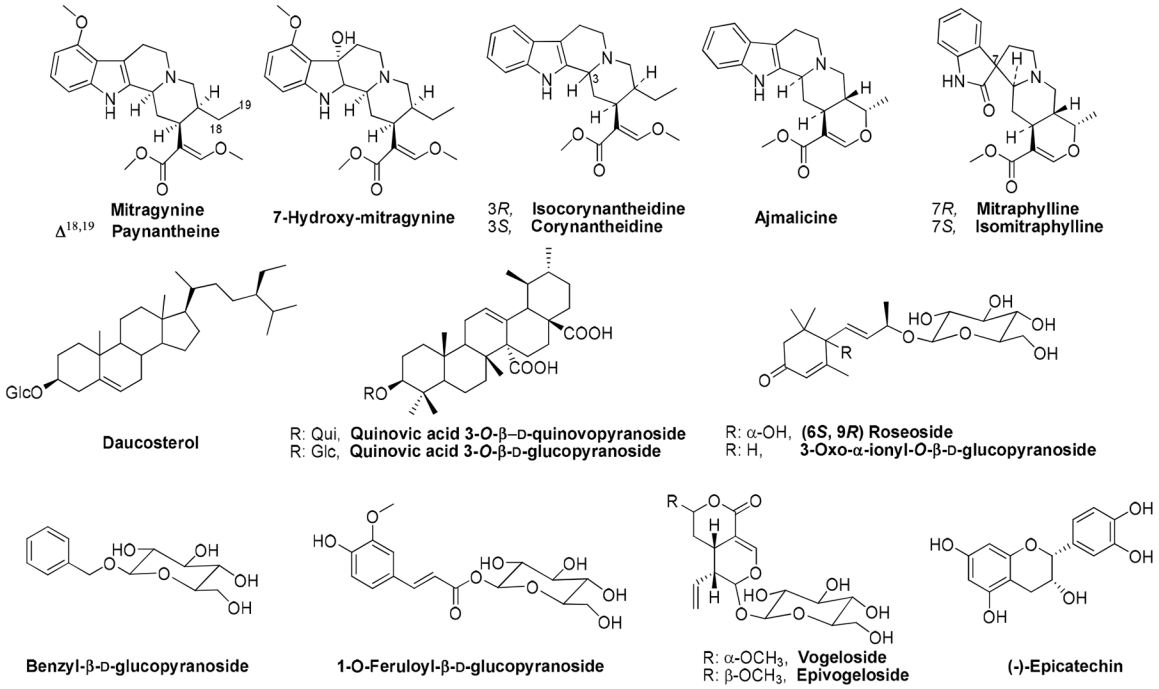

Figure 1:
Chemical structures of compounds isolated from the leaves of *Mitragyna speciosa*.