A New Indole Alkaloid, 7α-Hydroxy-7*H*-mitragynine, from *Mitragyna speciosa* in Thailand

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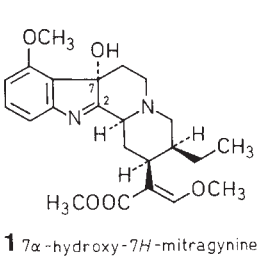
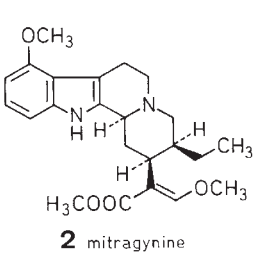
The leaves of *Mitragyna speciosa* Korth. (Rubiaceae) are the origin of the Thai traditional drug "Kratom". Because of its unique medicinal profiles, such as stimulation like coca and depressive effects like opium, many chemical (1–4) and pharmacological (5, 6) studies on this plant have been carried out over the last fifty years. However, the principles as well as the mechanisms of the biological activities of this drug have not been completely elucidated up to now. In the course of our chemical study on the Thai medicinal plants (7), we reinvestigated the alkaloidal constitutents of leaves of *Mitragyna speciosa*. Mitragynine (**2**) was the main component (66% based on the crude base) of the young big leaves and a new alkaloid **1** was also obtained as a minor constituent (1.6%). The heteroyohimbine-type oxindole alkaloids, such as mitraphylline and isomitraphylline, could not be detected from this plant (8).



1 7α-hydroxy-7*H*-mitragynine



2 mitragynine

The high-resolution positive FAB-MS spectrum of **1** displayed a protonated molecular ion at *m/z* = 415.2233, corresponding to the formula $C_{23}H_{31}N_2O_5$. This contains one more oxygen than that of the main alkaloid, mitragynine (**2**). The EI-MS fragmentation pattern [M^+ (91%), $M^+ - OH$ (100%)] indicated the presence of a hydroxy group in **1**. Although the ^{1}H-NMR spectral pattern of **1** was very similar to that of **2**, the UV spectrum was different from the 4-methoxyindole chromophore in **2**. The ^{13}C-NMR spectrum of **1** indicated the presence of ten sp^2 and thirteen aliphatic carbons. Unambiguous assignments of all the carbons and protons were obtained by using HH-COSY, CH-COSY and COLOC spectra. The signal of C-2 in **1** was observed at lower field by 50.6 ppm as compared with the corresponding signals of **2** (3), on the contrary, that of C-7 in **1** was shifted to upper field (δ = 80.88). These data indicated that **1** was a 7-hydroxyindolenine derivative of mitragynine. With the purpose of establishing the structure, laboratory synthesis of the new compound **1** starting from mitragynine was carried out. Mitragynine (**2**) was treated with iodosobenzene diacetate (9) in aqueous CH_3CN to afford the indolenine derivative in 29% yield. The identity of the semisynthetic compound with natural product was fully confirmed by comparison of their chromatographic behaviors, UV, ^{1}H and ^{13}C-NMR, mass, and CD spectra. The CD spectra of **1** and 7α-acetoxy-7*H*-yohimbine, whose stereochemistry at the C-7 position was confirmed by X-ray analysis (10), displayed very similar Cotton curves. From these data, the structure of the new alkaloid **1** was concluded to be 7α-hydroxy-7*H*-mitragynine.

The auto-oxidative transformation of indole alkaloids to the corresponding 7-hydroxyindolenine derivatives is known in the literature (11), but, on standing in chloroform for several days or by passing through silica gel column chromatography, the transformation of the alkaloid **2** into **1** could not be observed at all. Then, the possibility that the alkaloid **1** is the artefact from **2** would be slight.

Materials and Methods

Plant material

The leaves of *M. speciosa* were collected in the campus of the Faculty of Pharmaceutical Sciences, Chulalongkorn University, in December 1991; Voucher sample deposited in the Herbarium of Faculty of Pharmaceutical Sciences, Chulalongkorn University.

Isolation of alkaloids

The dried, powdered, young big leaves (165.5 g) were extracted with hot MeOH five times. Concentration of the solution under reduced pressure gave a crude extract (53.5 g), a part of which (50.3 g) was dissolved in 10% aqueous AcOH solution. The aqueous layer was basified with Na_2CO_3 at 0 °C and extracted with $CHCl_3$. The organic layer was washed with water, dried over $MgSO_4$, and evaporated to give the crude base (2.43 g), which was purified by SiO_2 column chromatography (6 × 17 cm) using a $CHCl_3$/AcOEt and AcOEt/MeOH gradients. The solvent gradient used was $CHCl_3$/AcOEt = 9 : 1 (370 ml), $CHCl_3$/AcOEt = 4 : 1 (240 ml), $CHCl_3$/AcOEt = 1 : 1 (320 ml), AcOEt (80 ml), MeOH/AcOEt = 1 : 19 (120 ml), and MeOH/AcOEt = 1 : 4 (160 ml). The elution order of the alkaloids was mitragynine (**2**) (1343 mg, 66% based on the crude base), a fraction containing the alkaloid **1**, paynantheine (178 mg, 8.9%), speciogynine (132 mg, 6.6%), and speciociliatine (15 mg, 0.8%). The $CHCl_3$/AcOEt = 1 : 1 eluate from the first column chromatography of the crude base was subjected again to SiO_2 flash column chromatography (3 × 17 cm) (*n*-hexane/AcOEt = 1 : 5) to give 40 mg of **1**.

*7α-Hydroxy-7*H*-mitragynine* (1)

Amorphous powder; $[\alpha]_D^{23}$: +47.9°C (*c* = 0.55, $CHCl_3$); UV λ_{max}^{EtOH} 220.5 (log ε 4.36), 245 (sh, log ε 4.06), 305 (sh, log ε 3.43) nm; IR $\nu_{max}^{CHCl_3}$ 3590 (OH), 2850, 2820, 2750 (Bohlmann band), 1700 (ester) cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 7.43 (1H, s, 17-H), 7.27 (1H, t, *J* = 8.0 Hz, 11-H), 7.19 (1H, d, *J* = 8.0 Hz, 12-H), 6.71 (1H, d, *J* = 8.0 Hz,10-H), 3.85 (3H, 3, Ar-OCH_3), 3.80 (3H, s, OCH_3), 3.68 (3H, s, CO_2CH_3), 3.12 (1H, dd, *J* = 10.9, 2.5 Hz, 3-H), 3.04 (1H, dd, *J* = 11.4, 2.2 Hz, 21-H), 3.00 (1H, dt, *J* = 13.7, 3.4 Hz,

15-H), 2.48 (1H, dd, J = 11.4, 2.7 Hz, 21-H), 1.87 (1H, br. d, J = 13.7 Hz, 14-H), 1.68 and 1.24 (each 1H, m, 19-H_2), 0.82 (3H, t, J = 7.3 Hz, 18-H_3); ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 184.33 (C-2), 61.41 (C-3), 49.98 (C-5), 35.64 (C-6), 80.88 (C-7), 126.51 (C-8), 155.85 (C-9), 108.79 (C-10), 130.55 (C-11), 114.10 (C-12), 154.98 (C-13), 25.99 (C-14), 39.25 (C-15), 111.18 (C-16), 160.65 (C-17), 12.75 (C-18), 18.87 (C-19), 40.44 (C-20), 58.10 (C-21), 55.37 (Ar-OMe), 61.69 (OMe), 169.22 (CO), 51.19 (O_2Me); EI-MS m/z (%): 414 (M^+, 91), 397 (100), 383 (38), 367 (39); High-resolution FAB-MS Calcd for $C_{23}H_{31}N_2O_5$: 415.2233. Found: 415.2235. CD (MeOH) $\Delta\varepsilon$ (nm) 0 (336), 4.3 (303), 0.9 (282), 6.4 (258), 0 (240), −6.4 (228), 0 (214).

Chemical synthesis of **1** *from mitragynine* (**2**)

A mixture of **2** (41.5 mg) and iodosobenzene diacetate (33.8 mg) in aqueous CH_3CN (1.1 ml) was stirred at 0 °C under argon for 3 h. The reaction mixture was diluted with chilled water, basified with 10% $NaHCO_3$ solution, and then extracted with $CHCl_3$. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified by SiO_2 flash column chromatography (1 × 5 cm) (*n*-hexane/AcOEt = 1 : 1) to afford 12.5 mg (29%). The product thus obtained was identical with **1**, on the basis of their chromatographic behaviour, and UV, MS, CD, and NMR comparisons.

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Tetra- and Pentacyclic Triterpenes from the Aerial Parts of *Euphorbia piscatoria*

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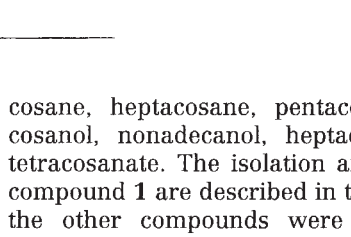
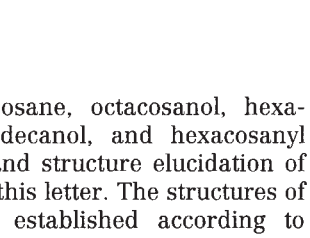
Euphorbia piscatoria Ait. (Euphorbiaceae) is a shrub endemic to the Madeira archipelago. The plant contains a large amount of latex which has an irritant effect on the skin and mucous membranes. It is used by the fishermen for fishing because of its paralyzing action.

The aerial parts of *Euphorbia piscatoria* have been investigated and the results are reported herein. From the heptane extract was isolated a new natural triterpenic ketone, obtusifolione (**1**). Eleven other triterpenes were also identified as 24-methylenecycloartanone, cycloartenol acetate, cycloartenol, butyrospermol, 24-methylenecycloartanol, obtusifoliol (**2**), simiarenone (**3**), simiarenol (**4**), glutinol, α- and β-amyrin, β-sitosterol and the linear compounds tritriacontane, hentriacontane, nonacosane, heptacosane, pentacosane, octacosanol, hexacosanol, nonadecanol, heptadecanol, and hexacosanyl tetracosanate. The isolation and structure elucidation of compound **1** are described in this letter. The structures of the other compounds were established according to spectral data and comparison with published reported data (1–10). Copies of the original spectra are obtainable from the author of correspondence.



1 R = O
2 R = H, β-OH
2a R = H, β-OAc



3 R = O
4 R = H, β-OH

The molecular formula, $C_{30}H_{48}O$, of obtusifolione (**1**) was deduced by mass spectrometry (molecular ion peak at m/z = 424) and elemental analysis. Its IR spectrum showed characteristic bands for a carbonyl group (1705 cm^{-1}) and for a terminal double bond (3070, 1635, and 880 cm^{-1}). The ^{1}H-NMR spectrum showed three singlets, attributed to tertiary methyl groups, four secondary methyl groups, and two olefinic protons at δ = 4.67 and 4.72 ppm as evidence for a terminal methylene group. The nature of the carbonyl group was confirmed in the ^{13}C-NMR spectrum by a signal at δ = 213.52 ppm. The peaks at δ = 132.47 and 135.64 ppm revealed the presence of the double bond C(8)–C(9) (8). The existence of a terminal double bond was also confirmed in the ^{13}C-NMR spectrum by the resonances at δ = 156.79 and 106.00 ppm corresponding to the two sp^2 carbons. The fragmentation