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# A New 9-Methoxyyohimbine-Type Indole Alkaloid from *Mitragyna africanus*

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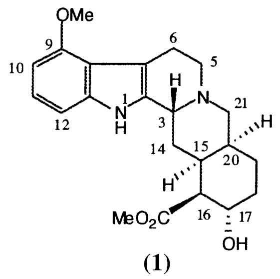
**A new yohimbine-type indole alkaloid (1) was isolated from the stem bark of *Mitragyna africanus* (Willd.) collected in Nigeria, along with known seven Corynanthe-type oxindole alkaloids, two secoiridoids, three lignans, and a quinovic acid derivative. Their structures were elucidated by spectroscopic analyses.**

**Key words** *Mitragyna*; alkaloid; indole; NMR

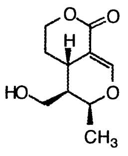
The bark and leaves of *Mitragyna africanus* (Willd.), a rubiaceous plant, have been traditionally used in West Africa for the treatment of bacterial infection, mental disorder and epilepsy. A recent pharmacological investigation disclosed that the aqueous methanolic extract of the stem bark of this plant exhibits potent depressant effects on the central nervous system.[1)] However, little is known about the active principles of this medicinal plant. On the other hand, we have demonstrated that the alkaloidal constituents of *Mitragyna speciosa* Korth, native to Southeast Asia, exhibit potent analgesic activity through opioid receptors.[2—6)] In this regard, we started a phytochemical investigation of *M. africanus*.

From the aqueous methanolic extract of the stem bark of *M. africanus* collected in Nigeria, seven Corynanthe-type oxindole alkaloids, *i.e.*, rhynchophylline,[7)] isorhynchophylline,[7)] corynoxeine,[8)] isocorynoxeine,[8)] ciliaphylline,[8)] rhynchociline,[8)] and isospecionoxeine,[8)] were isolated. In addition, three lignans, *i.e.*, (+)-dihydrodehydrodiconiferyl alcohol,[9)] (+)-isolariciresinol,[10)] and (+)-isolariciresinol-3$\alpha$-$O$-$\beta$-D-glucopyranoside,[11)] two secoiridoids, *i.e.*, sweroside[12)] and dihydroepinaucledal **2**[13)] (first example as natural product), scopoletin, and quinovic acid 3$\beta$-$O$-$\beta$-6-deoxy-D-glucopyranosyl-28-$\beta$-D-glucopyranosyl ester[14)] were obtained. Furthermore, a new indole alkaloid (**1**) was isolated as a minor component and its structure was elucidated as follows.

The new compound **1** was obtained as an amorphous powder. High-resolution FAB-MS analysis gave $m/z$ 385.2120 $[M+H]^+$ ($\Delta$ $-0.7$ mmu) and established the molecular formula as $C_{22}H_{28}N_2O_4$. Its UV spectrum displayed absorptions [224 nm ($\log \varepsilon=4.27$), 270 nm ($\log \varepsilon=3.60$)] typical of the 4-methoxyindole nucleus. This was supported by the presence of an ABX pattern for the three aromatic protons in the $^1$H-NMR spectrum, which resonated at $\delta$ 6.48 (d, $J=7.9$ Hz), 7.04 (dd, $J=7.9$, 8.1 Hz), and 6.93 (d, $J=8.1$ Hz), as well as by the observation of an NOE between the aromatic proton at $\delta$ 6.93 (H-12) and the indolic NH proton. The IR absorptions implied the presence of hydroxyl (3475 cm$^{-1}$) and ester carbonyl (1722 cm$^{-1}$) groups. $^1$H- and $^{13}$C-NMR as well as distortionless enhancement by polarization transfer (DEPT) spectra suggested the presence of nine $sp^2$ carbons including one ester carbonyl, five $sp^3$ methines (one of which has a hydroxyl function), six $sp^3$ methylenes, and two methyl groups. $^1$H–$^1$H correlation spectroscopy (COSY) and $^1$H-detected heteronuclear multiple quantum coherence (HMQC) spectra indicated three fragments: –$CH_2CH_2$–(C5–C6), –CHCHCH–(C10–C12), and –$CHCH_2CHCHCHCH_2CH_2CHCH_2$–(C3–C14–C21). All of these data indicated that **1** possesses a yohimbine skeleton having a methoxy group at the C-9 position.



(**1**)



(**2**)

It is well known that yohimbine alkaloids are classified into four types on the basis of the relative configurations of the C-3, C-15, and C-20 chiral centers: *normal*, *pseudo*, *allo*, and *epiallo*.[15)] The following findings, *i.e.*, the $^1$H-NMR signal of H-3 appearing at $\delta$ 4.41 (broad singlet) and the IR spectrum exhibiting no Bohlmann bands,[16)] indicated a *C*/*D* *cis*-quinolizidine ring junction in **1**, implying that **1** is a *pseudo*- or an *epiallo*-type compound (Fig. 1). *Epiallo*-type compounds have three possible conformers, two of which possess a *C*/*D* *cis*-quinolizidine form. Therefore, three possible stereostructures, *i.e.*, *pseudo*, *epiallo*-I, and *epiallo*-II, were surmised for **1**. The coupling constants of the protons at C-21 provided information on the stereochemistry at C-20, based on which the mode of *D*/*E* ring junction was concluded. The $H_2$-21 signals, unambiguously assigned by $^1$H-detected heteronuclear multiple bond connectivity (HMBC) from the carbon signals of C-3 and C-5, exhibited a broad doublet ($\delta$ 2.48, $J=11.8$ Hz) and a doublet of doublets ($\delta$ 3.03, $J=11.8$, 3.8 Hz), proving that H-20 was equatorial in ring *D* and thus had $\alpha$-orientation. Based on this analysis, **1** was concluded to be an *epiallo*-I-type compound. The stereochemistry of the two substituents at C-16 and C-17 in ring *E* was also elucidated from the coupling constants of the concerned protons. The signal for H-17 appearing as a doublet of triplets ($J=4.2$, 11.2 Hz) at $\delta$ 4.07 indicated that H-17 and H-16 are trans diaxially oriented, and that for H-16 appearing

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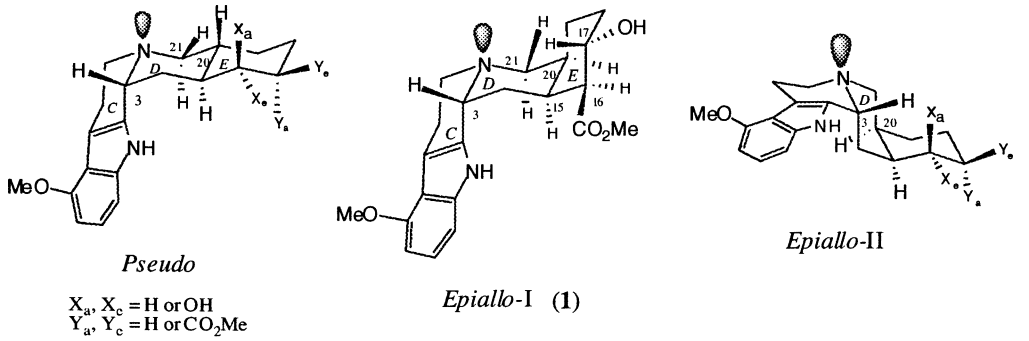

Fig. 1

as a doublet of doublets ($J$=4.0, 11.2 Hz) at $\delta$ 2.41 indicated a *cis* relation to H-15, as depicted in Fig. 1. Comparison of the $^{13}$C-NMR spectra of **1** with those of 3-*epi*-$\alpha$-yohimbine[17] and 10-methoxy-3-*epi*-$\alpha$-yohimbine[18] revealed their close similarity. The signals of the carbons of the non-aromatic part of **1** were almost superimposable on those of known alkaloids. In the CD spectrum, a negative Cotton effect in the longer wavelength region between 230—300 nm was observed, revealing C3-*R* (C3-$\beta$H) in **1**.[19] These data clearly indicated that the structure of the new alkaloid is 9-methoxy-3-*epi*-$\alpha$-yohimbine.

### Experimental

**General** UV: recorded in MeOH on a JASCO V-560 instrument. IR: recorded on a JASCO FT/IR-230 spectrophotometer. $^1$H- and $^{13}$C-NMR spectra: recorded on a JEOL JNM A-400, JNM A-500, JNM ECP-400, or JNM ECP-600 spectrometer, where $J$ values are given in Hz. EI-MS: direct probe insertion at 70 eV recorded on a JEOL JMS GC-mate spectrometer. FAB-MS: recorded on a JEOL JMS-HX110 mass spectrometer. CD: recorded on a JASCO J-720WI spectrometer. TLC: precoated Kieselgel 60 $F_{254}$ plates (Merck, 0.25 mm thick). Column chromatography: Kieselgel 60 [Merck, 70—230 (for open chromatography) and 230—400 mesh (for flash chromatography)], medium pressure liquid column chromatography: silica gel prepacked column, Kusano CPS-HS-221-05.

**Plant Material** The stem bark of *Mitragyna africanus* Willd. was collected in Borno State in Nigeria and identified by Mr. S. A. Sanusi, a plant taxonomist in the Biological Sciences Department, University of Maiduguri. A voucher specimen was deposited at the Herbarium of the Department of the Biological Sciences, University of Maiduguri, Maiduguri.

**Extraction and Isolation** One hundred and fifty grams of the powdered stem bark was extracted with 50% aqueous methanol under reflux and the extract was filtered. The filtrates were concentrated under reduced pressure to give the crude extract (92.23 g), which was then suspended in 10% acetic acid (2.5 l) and filtered through Celite. The aqueous filtrate was extracted with *n*-hexane (1.5 l), rendered basic with $Na_2CO_3$ (pH 10), and then extracted with 5% MeOH/$CHCl_3$ (4.0 l). The organic layer was dried over $MgSO_4$ and evaporated to give the crude alkaloidal fraction (1.25 g). The aqueous layer was again extracted with *n*-butanol (2 l), and the extract was concentrated under vacuum to give the *n*-butanol extract (9.75 g). A portion of the crude alkaloidal fraction (1.16 g) was roughly separated by silica gel column chromatography using 60% AcOEt/*n*-hexane to AcOEt gradient, 10% MeOH/$CHCl_3$ and then MeOH to give six fractions (A—F). The 60% AcOEt/*n*-hexane eluate (fraction B) was purified by $SiO_2$ column chromatography using 5% acetone/$CHCl_3$ to afford 113 mg of isorhynchophylline and 67 mg of isocorynoxeine. The AcOEt eluate (fraction C) was purified by $SiO_2$ column chromatography using 3% MeOH/AcOEt to afford 82 mg of rhynchophylline, 29 mg of corynoxeine, and 23 mg of isospecionoxeine. The AcOEt eluate (fraction D) was rechromatographed over $SiO_2$ column chromatography using 2% MeOH/$CHCl_3$ and then MeOH to give three fractions (D1—D3). From fraction D2, 2.3 mg of iridoid (**2**) was obtained. The 2% MeOH/$CHCl_3$ eluate (fraction D1) was further purified by $SiO_2$ column chromatography using 2% MeOH/AcOEt to afford 31 mg of ciliaphylline. The 2% MeOH/$CHCl_3$ eluate (fraction D3) was further purified by $SiO_2$ column chromatography using 3% MeOH/AcOEt to afford 4.7 mg of (+)-dihydrodehydrodiconiferyl alcohol and 4.1 mg of (+)-isolariciresinol. The 10% MeOH/$CHCl_3$ eluate (fraction E) was purified by aminosilica gel column chromatography using 3% MeOH/AcOEt to afford 10.4 mg of rhynchociline. The MeOH eluate (fraction F) was purified by $SiO_2$ column chromatography using 10% MeOH/$CHCl_3$ to afford 6.9 mg of 9-methoxy-3-*epi*-$\alpha$-yohimbine (**1**). A portion of the crude *n*-BuOH fraction (9.4 g) was roughly separated by silica gel column chromatography using 10% MeOH/$CHCl_3$ to 30% MeOH/$CHCl_3$ gradient, 50% MeOH/$CHCl_3$ and then MeOH to give four fractions (G—J). The 10% MeOH/$CHCl_3$ eluate (fraction G) was purified by $SiO_2$ column chromatography using 50% AcOEt/*n*-hexane to afford 6.8 mg of scopoletin. The 30% MeOH/$CHCl_3$ eluate (fraction H) was rechromatographed over $SiO_2$ column chromatography using 10% MeOH/AcOEt and then MeOH to give three fractions (H1—H3). The 10% MeOH/AcOEt eluate (fraction H2) was further purified by aminosilica gel column chromatography using 5% MeOH/$CHCl_3$ to afford 53 mg of sweroside. The 50% MeOH/$CHCl_3$ eluate (fraction I) was rechromatographed over $SiO_2$ column chromatography using 15% MeOH/AcOEt and then MeOH to give two fractions (I1—I2). The 15% MeOH/AcOEt eluate (fraction I1) was further purified by $SiO_2$ column chromatography using 15% EtOH/$CHCl_3$ to afford 133 mg of quinovic acid 3$\beta$-*O*-$\beta$-6-deoxy-D-glucopyranosyl-28-$\beta$-glucopyranosyl ester and 92 mg of (+)-isolariciresinol-3$\alpha$-*O*-$\beta$-D-glucopyranoside.

9-Methoxy-3-*epi*-$\alpha$-yohimbine (**1**): Colorless amorphous powder; UV (MeOH) $\lambda_{\max}$ (log $\varepsilon$) 270 (3.60), 224 (4.27); CD (0.26 mmol, MeOH, 23 °C) nm ($\Delta\varepsilon\lambda$) 308 (0), 267 (−4.1), 243 (−2.7), 234 (−4.7), 227 (0), 220 (+5.4), 209 (0), 200 (−3.5); IR ($CHCl_3$) $\nu_{\max}$ 3475, 2961, 1722, 1261, 1099, 1015 cm$^{-1}$; $^1$H-NMR ($CDCl_3$, 500 MHz) $\delta$: 7.58 (1H, br s, H-1), 7.04 (1H, dd, $J$=7.9, 8.1 Hz, H-11), 6.93 (1H, d, $J$= 8.1 Hz, H-12), 6.48 (1H, d, $J$=7.9 Hz, H-10), 4.41 (1H, br s, H-3), 4.07 (1H, ddd, $J$=11.2, 11.2, 4.2 Hz, H-17), 3.89 (3H, s, $CO_2Me$), 3.84 (3H, s, C9-OMe), 3.17 (1H, m, H-6), 3.15 (2H, m, $H_2$-5), 3.03 (1H, dd, $J$=11.8, 3.8 Hz, H-21), 2.81 (1H, m, H-6), 2.48 (1H, d, $J$=11.8 Hz, H-21), 2.41 (1H, dd, $J$=11.2, 4.0 Hz, H-16), 2.16 (1H, m, H-18), 2.14 (1H, m, H-19), 2.13 (1H, m, H-14), 2.10 (1H, m, H-15), 1.64 (1H, m, H-14), 1.56 (1H, m, H-20), 1.55 (1H, m, H-19), 1.35 (1H, m, H-18); $^{13}$C-NMR ($CDCl_3$, 125 MHz) $\delta$: 174.5 (C-22), 154.0 (C-9), 136.9 (C-13), 130.0 (C-2), 122.1 (C-11), 117.9 (C-8), 108.2 (C-7), 104.3 (C-12), 99.8 (C-10), 66.0 (C-17), 55.2 ($CO_2\underline{Me}$), 53.9 (C-16), 53.8 (C-3), 51.9 (C9-OMe), 51.3 (C-5), 49.6 (C-21), 35.9 (C-20), 33.3 (C-18), 32.4 (C-15), 24.3 (C-14), 23.7 (C-19), 18.5 (C-6); EI-MS (%) *m/z* 384 ([M]$^+$, 100), 369 (11), 353 (9), 325 (10), 214 (14), 199 (18); HR-FAB-MS (NBA/PEG) *m/z* 385.2120 (M+H; Calcd for $C_{22}H_{29}O_4N_2$, 385.2127).

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