STEREOCHEMICAL ASSIGNMENT OF PSEUDOINDOXYL ALKALOIDS‡

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Abstract --- The stereochemistries of the C2 and C3 positions in three pseudoindoxyl alkaloids, i.e., mitragynine pseudoindoxyl, yohimbine pseudoindoxyl, and β-yohimbine pseudoindoxyl, were elucidated by spectroscopic analyses. The CD spectra of these compounds and that of fluorocarpamine showed the antipodal-like curves in the long wavelength region.

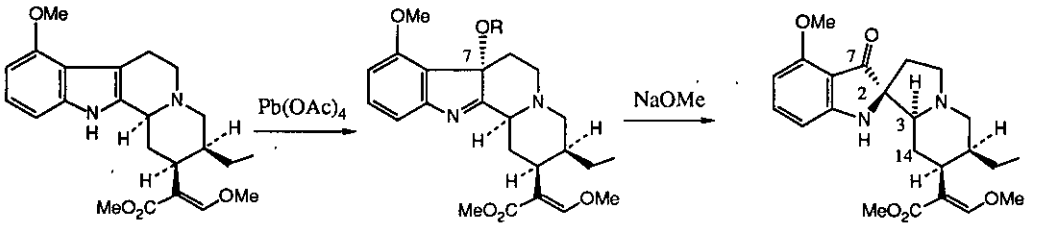
The leaves of *Mitragyna speciosa* Korth. have been known as an opium substitute in traditional use by Thai and Malay natives. A number of pharmacological studies of this plant have been carried out, but the principle as well as the mechanism of the biological activities of this folk medicine have not been completely elucidated up to now.[1] The major alkaloidal constituent in the leaves is an indole alkaloid, mitragynine (**1**), and several minor components have been isolated.[2] In 1974, Zarembo *et al.* reported that mitragynine pseudoindoxyl, which was obtained by the microbial transformation of mitragynine (**1**) by the fungus Helminthosporum sp., displayed analgesic activity in the D'Amour-Smith test almost ten-fold stronger than mitragynine (1) itself.[3] During the investigation of medicinally useful compounds from *Mitragyna* plants, we have had chemical and pharmacological interest in mitragynine pseudoindoxyl. Thus far many natural and semisynthetic pseudo-

‡This paper is dedicated to the memory of the late Professor Yoshio Ban.

indoxyl alkaloids have been reported, however, their stereochemical and spectroscopic studies were not completed. In this paper, we describe the chemical synthesis of mitragynine pseudoindoxyl and other pseudoindoxyl alkaloids as well as their spectroscopic analysis, which has clarified the hitherto obscure stereochemistry of pseudoindoxyl alkaloids.

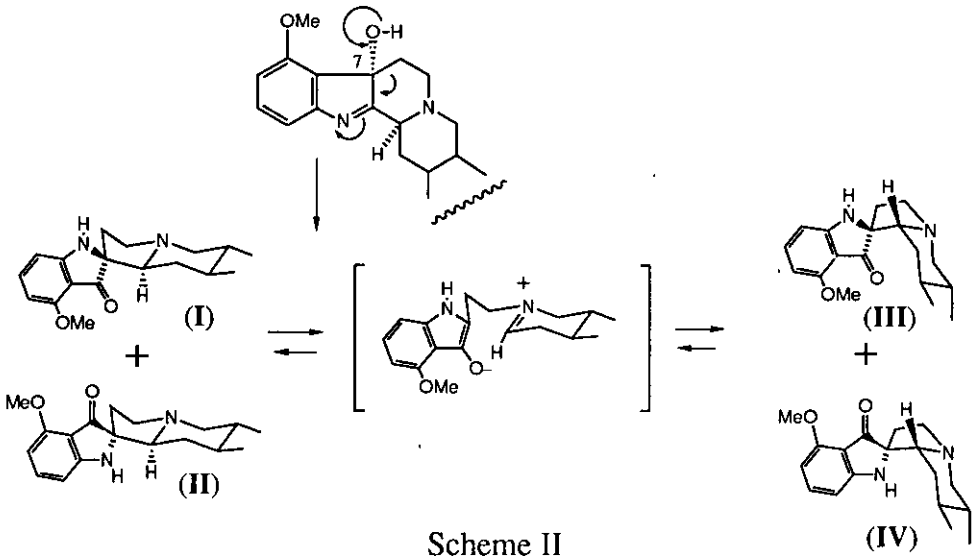
Mitragynine (**1**) was oxidized with one equivalent of lead tetraacetate [$Pb(OAc)_4$][4] in dry CH_2Cl_2 at 0 °C to afford the 7-acetoxyindolenine derivative (**2**) in 50% yield. The stereochemistry at the C7 position was determined by a comparison of the CD spectra of **2** and 7α-acetoxy-7*H*-yohimbine (**7**), whose absolute stereochemistry was confirmed by X-ray analysis.[5] The rearrangement reaction from 7-acetoxyindolenine (**2**) to the pseudoindoxyl derivative (**4**) was carried out by treatment with 1.7 equivalents of NaOMe in hot MeOH[5] for 4 h to yield pseudoindoxyl (**4**)[6a] (yield 11%) together with 7-hydroxy indolenine (**3**) (yield 39%), the latter of which was identical to the natural product reported by us.[2e] The hydroxyindolenine (**3**) could be converted to mitragynine pseudoindoxyl (**4**) by treatment with excess NaOMe in MeOH under reflux condition for 12 h. The physical and spectroscopic data were identical with those in the literature.[3] Although mitragynine pseudoindoxyl (**4**) was obtained as a single product from the above reaction, up to four diastereomeric pseudoindoxyls could have been generated from **2** or **3** based on the mechanistic considerations illustrated in scheme II.[5] After unambiguous assignment of all the protons and carbons using HH-COSY and CH-COSY spectra, differential NOE experiments were done to determine the configuration at the C2 position. The observation of NOE between the NH proton and 14β-H (δ 2.23, ddd, J=11.0, 11.0, 11.0 Hz in DMSO-d_6) confirmed the stereochemistry at C2 as the *S* form. The chemical shift of 3-H (δ 1.90) in **4** and the coupling constants ($J_{3\text{-}14\alpha}$=2.5 Hz, $J_{3\text{-}14\beta}$=11.0 Hz) are consistent with the trans relationship between 3-H and the lone pair of N_b,[7] meaning that mitragynine pseudoindoxyl (**4**) adapts the conformer (I). The CD spectrum of **4**, having the C2(*S*) configuration, exhibited a negative Cotton effect in the wavelength region of about 400 nm.

Previously, Finch and Taylor had prepared some pseudoindoxyls of yohimbinoid alkaloids and tentatively assigned their C2(*S*) configuration by the following consideration. The basicity of N_b is reduced when N_a is methylated by an effect that the introduction of the N_a-methyl group would produce steric hindrance to the protonation of N_b.[5] To give the obvious conclusion to the stereochemistry of the pseudoindoxyl derivatives of yohimbinoid alkaloids, we planned the preparation and spectroscopic analysis of these compounds. Yohimbine (**5**) and pseudoyohimbine (**6**), which is the 3-epimer of **5**, were oxidized with $Pb(OAc)_4$ to afford the 7-acetoxyindolenine derivatives (**7** and **8**), respectively. From the observation of NOE between the $OCOCH_3$ group and 3-H in **8** as well as the CD spectral comparison (see Figure 3), it was deduced that the acetoxy group

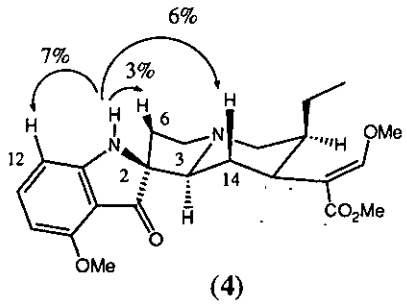


mitragynine (**1**) R=Ac (**2**) R=H (**3**) mitragynine pseudoindoxyl (**4**)

Scheme I



Scheme II



(**4**)

Figure 1

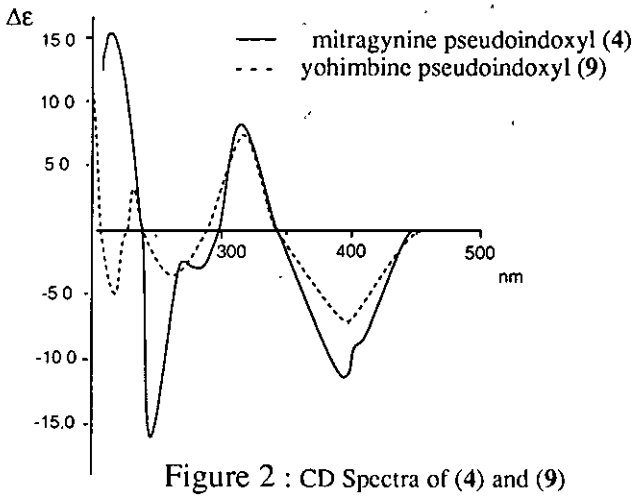
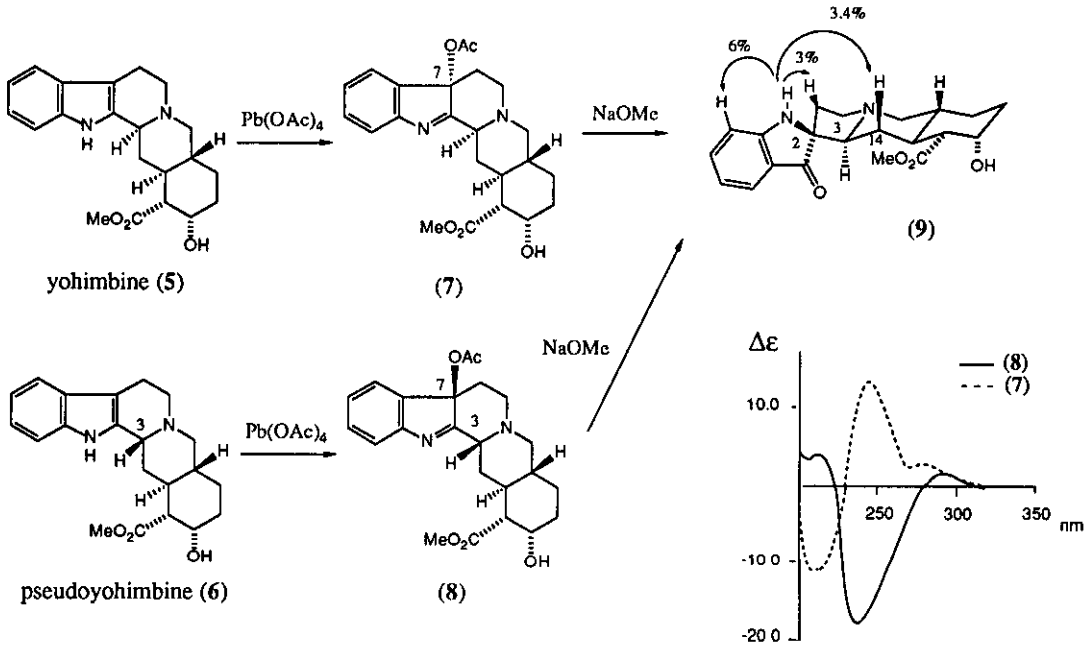
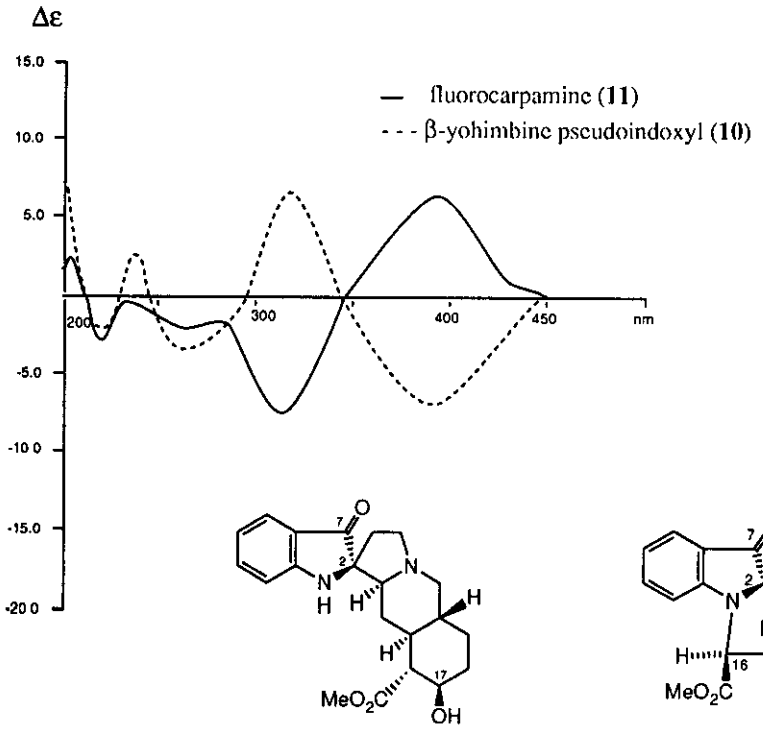


Figure 2 : CD Spectra of (**4**) and (**9**)



Scheme III

Figure 3 : CD spectra of (7) and (8)



β-yohimbine pseudoindoxyl (10)

fluorocarpamine (11)

Figure 4 : CD Spectra of β-Yohimbine pseudoindoxyl (10) and Fluorocarpamine (11)

rearrangement reaction by treatment with NaOMe in MeOH to give a single pseudoindoxyl derivative (**9**).[6b] The stereochemistries at C2 and C3 were clearly determined as (*S*) and (*S*), respectively, from the differential NOE data and CD spectrum (Figure 2). Through the reaction mechanism described above, a thermodynamically stable compound (**9**) would be produced from the different stereoisomers (**7** and **8**).

The stereochemistries at the C2 and C3 positions in β-yohimbine pseudoindoxyl (**10**), which had been isolated from *Aspidosperma oblongum*, have not been completely elucidated in the literature.[8] According to the reported method,[8] β-yohimbine was converted to **10** and its stereochemistry was explained to be C2(*S*) and C3(*S*) by the same procedure (see the CD spectrum of **10** in Figure 4) established in case of compounds (**4** and **9**).

During the chemical studies of *Hunteria zeylanica* (Apocynaceae) native to Thailand,[9] we have isolated a known pseudoindoxyl alkaloid, fluorocarpamine (**11**)[6c, 10] as one of the minor components. Interestingly, the CD spectrum of **11** (Figure 4) having a rigid cage structure showed the antipodal-like curve compared with those of mitragynine pseudoindoxyl (**4**) and yohimbinoid pseudoindoxyls (**9** and **10**).

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6. ^{13}C-Nmr data (in $CDCl_3$, δ) (a) mitragynine pseudoindoxyl (**4**); 75.2 (C2), 73.2 (C3), 53.2 (C5), 35.1 (C6), 199.5 (C7), 109.8 (C8), 162.1 (C9), 99.1 (C10), 138.6 (C11), 103.8 (C12), 158.6 (C13), 23.8 (C14), 38.4 (C15), 111.8 (C16), 160.2 (C17), 12.9 (C18), 19.3 (C19), 40.1 (C20), 54.8 (C21), 55.7 (9-OMe), 168.9 (CO_2), 51.1 (O_2Me), 61.4 (17-OMe). (b) yohimbine pseudoindoxyl (**9**); 74.4 (C2), 71.3 (C3), 52.7 (C5), 35.3 (C6), 202.0 (C7), 120.5 (C8), 124.5 (C9), 118.3 (C10), 137.2 (C11), 111.7 (C12), 160.4 (C13), 28.9 (C14), 35.8 (C15), 52.5 (C16), 66.5 (C17), 31.2 (C18), 23.4 (C19), 40.5 (C20), 58.6 (C21), 175.4 (CO_2), 51.6 (O_2Me). (c) fluorocarpamine (**11**); 76.2 (C2), 62.0 (C3), 55.3 (C5), 39.2 (C6), 205.3 (C7), 120.7 (C8), 124.3 (C9), 119.6 (C10), 137.3 (C11), 111.2 (C12), 163.7 (C13), 25.2 (C14), 30.7 (C15), 63.2 (C16), 12.5 (C18), 121.1 (C19), 134.1 (C20), 53.7 (C21), 172.6 (CO_2), 52.0 (O_2Me).

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