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Indole alkaloids from the leaves of Philippine *Alstonia scholaris*

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Abstract

The first *seco*-uleine alkaloids, manilamine (**1**) (18-hydroxy-19,20-dehydro-7,21-*seco*-uleine) and N^4-methyl angustilobine B (**2**), were isolated from the (pH 5) alkaloid extract of Philippine *Alstonia scholaris* leaves together with the known indole alkaloids 19,20-(*E*)-vallesamine (**3**), angustilobine B N^4-oxide (**4**), 20(*S*)-tubotaiwine (**5**), and 6,7-*seco*-angustilobine B (**6**). The structure of the alkaloids was established from MS and NMR experiments.

Keywords: *Alstonia scholaris*; Apocynaceae; Manilamine; N^4-Methyl angustilobine B; Mass spectrometry; NMR spectroscopy; Monoterpene indole alkaloid biogenesis

1. Introduction

Alstonia scholaris (L.) R. Brown (Apocynaceae) (“dita” in Filipino) is a popular Philippine medicinal plant, where its root bark is known for its antimalarial properties (Quisumbing, 1978). Earlier phytochemical studies on indole alkaloids of *A. scholaris* thriving in India, Pakistan, Thailand, the Philippines, Malaysia and Indonesia resulted in the isolation of several indole alkaloids bearing different structural skeleta. Samples from continental countries (India, Pakistan and Thailand) contain the picrinine-type indole alkaloids, while those from Indonesia and the Philippines predominantly contain alkaloids bearing the angustilobine skeleton (Abe et al., 1989, 1990; Yamauchi et al., 1990; Kam et al., 1997; Salim et al., 2004). Trees cultivated in Indonesia show alkaloidal diversity. For example, leaf extracts collected in Java (Cianjur) give scholaricine, while leaf extracts from Lombok contained tubotaiwine (Yamauchi and Abe, 1998). Moreover, only a few studies have been reported on the biological activity of these alkaloids (Gandhi and Vinayak, 1990; Kamarajan et al., 1991; Keawpradub et al., 1997, 1999; Saraswathi et al., 1998). As part of a continuing effort to discover secondary metabolites from local medicinal plants active against *Mycobacterium tuberculosis* $H_{37}Rv$, the major constituents of the antitubercular fraction from the alkaloid extract of *A. scholaris* obtained at pH 5 were investigated. This paper reports the isolation of several known indole alkaloids 19,20-(*E*)-vallesamine (**3**), angustilobine

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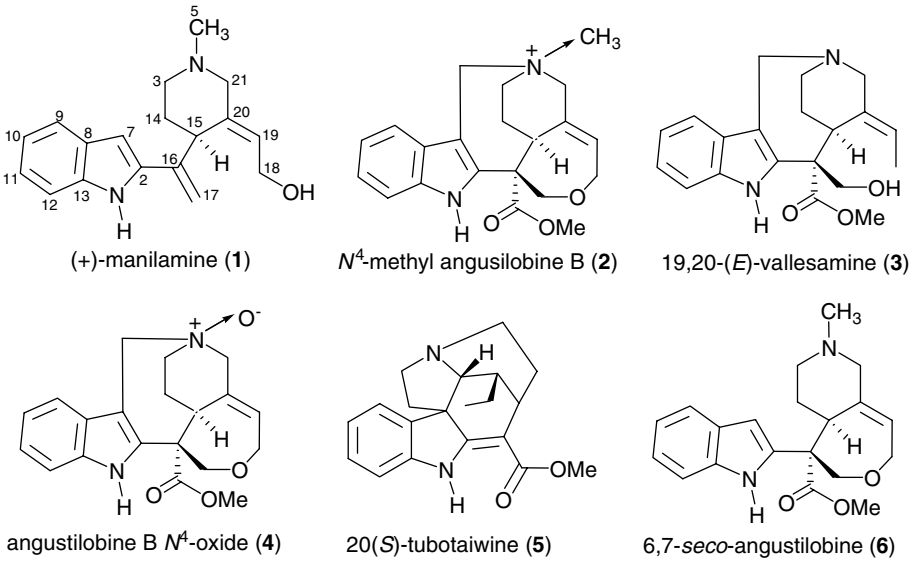

Fig. 1. Structures of alkaloids isolated from *Alstonia scholaris*.

B N^4-oxide (**4**), 20(*S*)-tubotaiwine (**5**), and 6,7-*seco*-angustilobine B (**6**), and in particular the structure identification of the new indole alkaloids, manilamine (**1**), a new *seco*-uleine alkaloid derivative and N^4-methyl angustilobine B (**2**) (Fig. 1).

2. Results and discussion

The crude methanol extract of the powdered, air-dried leaves was found to be moderately active against *M. tuberculosis* $H_{37}Rv$, using the MABA assay (Collins and Franzblau, 1997) (89% inhibition at 50 μg/ml). The alkaloids were extracted at pH 5. Group separation (separation of alkaloids on the basis of polarity) using vacuum liquid chromatography yielded five fractions. Purification of the first fraction gave alkaloid **3** (Zeches et al., 1987), while subjection of fraction three to silica gel 60 column chromatography afforded the new alkaloid **1**, along with the other known indole alkaloids **5** and **6** (Fig. 1). The latter two alkaloids were identified by comparison of their spectral data (Yamauchi et al., 1990).

Manilamine (**1**) was isolated as a flesh-colored solid ($[\alpha]_D^{20} + 15.5°$, MeOH *c* 0.5). Chemical characterization by TLC using ceric ammonium sulfate/H_3PO_4 and Erhlich's spray reagents gave a gray spot and pink color, respectively, suggesting the presence of a C-2 substituted vallesamine indole alkaloid. The UV spectrum displayed the characteristic absorptions of an indole chromophore conjugated with a vinylic residue (i.e., brafouedine), showing maximum absorptions at 223, 282, and 297 nm (Tillequin et al., 1993). The FTIR-DRS spectrum showed absorptions at 3400 (OH) and 3250 cm^{-1} (NH). The EI-mass spectrum displayed the $[M]^+$ at *m*/*z* 282, which was verified by ESI-mass spectrometry (MS). The molecular formula was established by high resolution ESI-MS as $C_{18}H_{22}N_2O + H^+$ (measured 283.18033, calc. 283.18049) and suggests nine degrees of unsaturation in the structure.

The ^1H NMR spectrum of **1**, recorded in MeOH-d_4, showed similar signal patterns as observed for 6,7-*seco*-angustilobine B (**6**) (Yamauchi et al., 1990; Zeches et al., 1987). The main differences were the absence of the signals for a methyl ester and for the geminal C-17 protons (–CH_2–O–R), and the presence of signals for an *exo*-methylene group (>C=CH_2) at δ 4.94 (H-17, *d*, J = 12 Hz) and δ 5.47 (H-17′), corroborated by an *exo*-methylene carbon atom at δ 113.8. Further analysis of the ^{13}C NMR spectral data suggested a C-2 monosubstituted indole alkaloid skeleton, typical of those isolated from several species of the genus *Alstonia* (*A. angustiloba* Miq., *A. pneumatophora* Backer ex L.G. Den Berger, and *A. scholaris*) (Yamauchi et al., 1990; Zeches et al., 1987).

The HMQC spectrum showed the presence of four pairs of diastereotopic protons connected to C-3, C-14, C-18, and C-21. The ^1H–^1H COSY spectrum revealed notable spin systems belonging to a 1,2-disubstituted benzenoid ring, a >N–CH_2–CH_2–CH< group, and an ethylidene moiety. Likewise, a long-range, *W*-type coupling (J = 1.2 Hz) was deduced from the correlation between H-7 and the indole N–H. An isolated AB spin system for the H_2-21 protons was shown by the cross-speak between δ 3.11 (H-21α) and δ 2.90 (H-21β). These protons were attached to a carbon at δ 62.8 bonded to both a tertiary nitrogen atom and an olefinic residue.

Analysis of the 2D NOESY was initiated at H-15 (δ 3.81). This is a convenient starting point from which conformational and configurational assignments can be made since, based on the absolute configuration of secologanin, it is α in alkaloids earlier than the secodines