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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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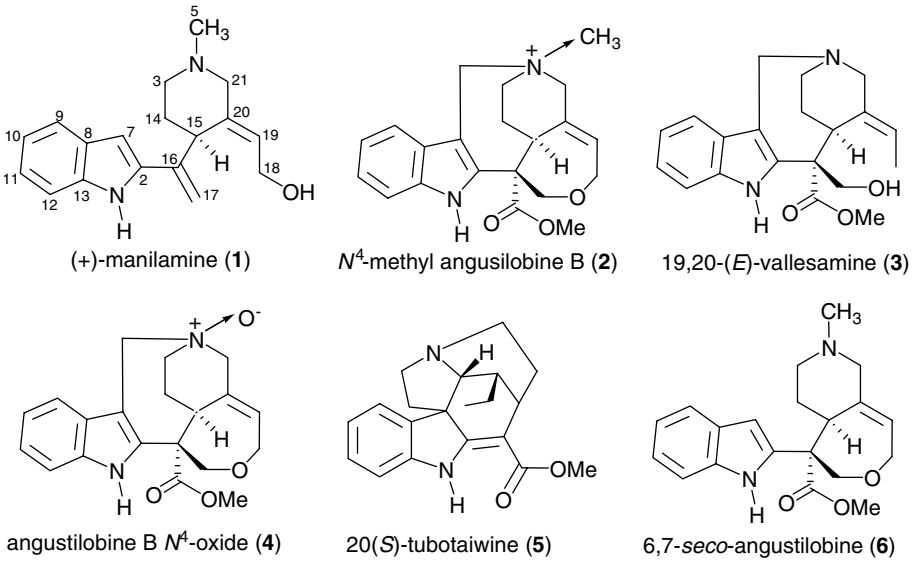

Fig. 1. Structures of alkaloids isolated from *Alstonia scholaris*.

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

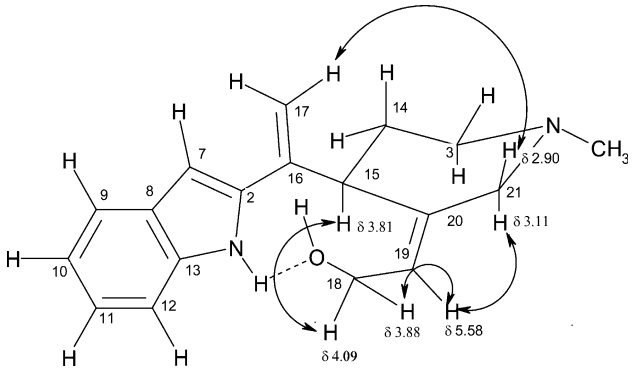

Fig. 2. Key NOESY correlations observed for manilamine (**1**).

in the monoterpene indole alkaloid biogenetic pathway (Cordell, 1981 and references therein). The NOESY correlation (Fig. 2) observed between H-15α and the protons resonating at δ 4.09 indicated proximity to H-18α. A distinction between the H_2-21 protons was made through the correlation of H-21β with one of the H_2-17. A correlation between H-19 and H-21α, coupled with the H-18β to H-19 correlation, revealed the $\Delta^{19,20}$-bond to have an *E* configuration. A *Z* geometric isomer is deduced if a spatial correlation is observed between the olefinic H-19 and H-15α (Mukhopadhyay et al., 1983). The structure of **1** is further evidenced by fragmentation based on several *m/z* observed values in the EIMS spectrum (Fig. 3).

Manilamine represents a new structural subgroup among the monoterpene indole alkaloids. Other *seco*-alkaloids isolated from this genus are biogenetically related to apparicine (Zeches et al., 1987). However, manilamine (**1**) appears to be derived from intermediate A (Fig. 4), which is postulated (Cordell, 1981 and references therein) as the branching point in uleine/apparicine biogenesis, from which either C-5 (apparicine pathway) or C-6 (uleine pathway) are lost. Manilamine (**1**) may therefore represent a third pathway from intermediate A in which C-5/N-4 reduction occurs, and thus blocks formation of both the uleine and apparicine skeleta. Structurally, manilamine (**1**) may also be regarded as a $\Delta^{19,20}$-7,21-*seco*-uleine derivative, carrying a C-18 hydroxy group, and an alternative name is 18-hydroxy-19,20-dehydro-7,21-*seco*-uleine.

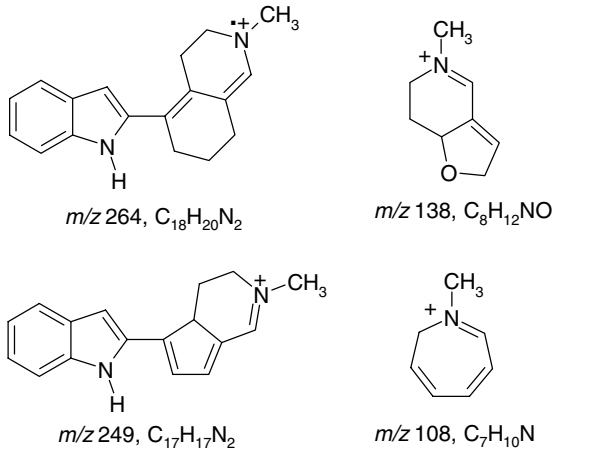

Fig. 3. Major fragments observed in the mass spectrum of manilamine (**1**).

Compound **2** was identified together with angustilobine B-N^4-oxide (**4**) from the flesh colored precipitate that separated out from the chloroform-soluble fraction (fraction 3) upon standing. This solid (m.p. 238–240 °C) was observed to be insoluble in most organic solvents except pyridine. The *m/z* values obtained from the LR-EIMS data showed characteristic fragment ions for angustilobine B (Zeches et al., 1987; Caron et al., 1989). These are the *m/z* values at 307, 294 (100%), 280, 263, 251, and 122. In addition to the fragment ion at *m/z* 307 for angustilobine B, additional peaks were observed at *m/z* 353 (18%) and 354 (10%) which signal the presence of molecular ion peaks corresponding to N^4-methyl and N^4-oxide derivatives. The ^{1}H NMR spectrum (pyridine-d_5) of this mixture showed poor resolution of resonances due to the observation of broad peaks, although close observation of these signals revealed chemical shifts reminiscent to angustilobine B (Zeches et al., 1987; Caron et al., 1989). Notable of these is the presence of an additional singlet at δ 3.54 for the N^4-methyl moiety, aside from the methyl ester protons at δ 3.85. In the ^{13}C NMR spectrum, the N^4-methyl group showed a peak at δ 50.5, which was further evidenced by its positive intensity in the DEPT-135 spectrum.

Previous investigations on a sample from Laguna, Philippines afforded the alkaloids, angustilobine B, angustilobine B acid, losbanine (6,7-*seco*-6-*nor*-angustilobine B) and 6,7-*seco*-angustilobine B (Yamauchi et al., 1990). Angustilobine alkaloids were also identified from other *Alstonia* species, such as *A. angustiloba* Miq., *A pneumatophora* Backer ex. L.G. Den Berger (Zeches et al., 1987) and *A. congensis* Engl. (Caron et al., 1989), where the latter species is closely related and resembled *A. scholaris* according to Monachino (Caron et al., 1989). To the best of our knowledge, this is the first report on the isolation, identification, and occurrence of N^4-methyl angustilobine B (**2**) and angustilobine B N^4-oxide (**4**) (Caron et al., 1989) from *A. scholaris*. Alkaloid **4** was earlier identified from *A. congensis*, while the identification of **2** from this genus and other plant species to date, has not been reported. The result of our investigation strengthens previous studies that angustilobine-type alkaloids are also largely confined to *A. scholaris* growing in the Philippines.

A report of the detailed inhibitory activity of the indole alkaloids against *M. tuberculosis* H_{37}Rv isolated from this plant is described elsewhere (Macabeo et al., submitted). The search for the more active constituents from the leaves of *A. scholaris* is continuing.

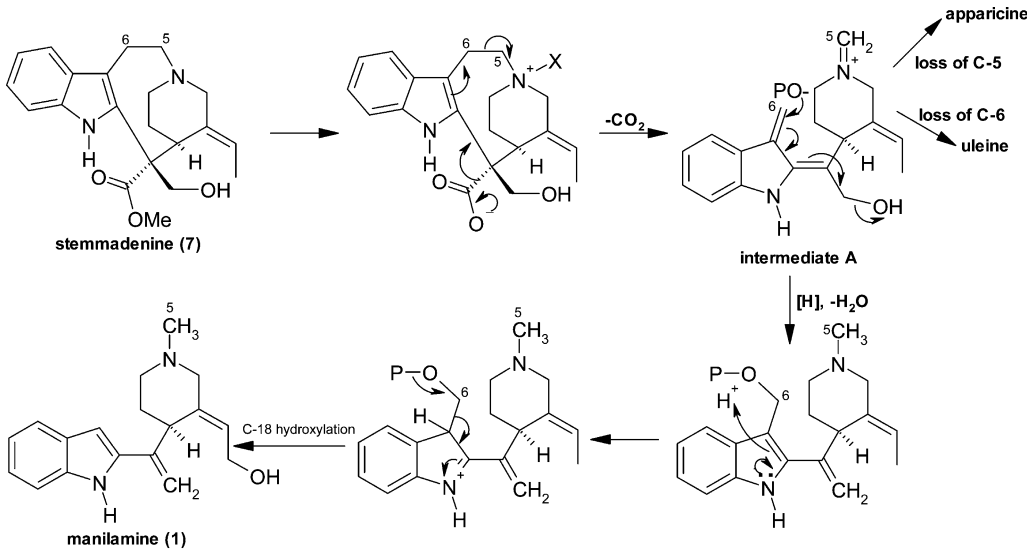

Fig. 4. Biogenesis of manilamine (**1**) from stemmadenine (**7**).

3. Experimental

3.1. General procedures and instrumentation

^{1}H and ^{13}C NMR were determined in MeOH-d_4 for **1**, $CDCl_3$ for **3**, **5**–**6**, and pyridine-d_5 for **2** and **4** with TMS as internal standard at either 300 or 400 MHz for ^{1}H and either 75 or 100 MHz for ^{13}C. EIMS: direct probe insertion at 70 eV; ESI-MS: octopole voltage set at 2.94 V, 300 °C. CC: silica gel 60 (230–400 mesh). TLC: precoated silica gel plates 60 F_{254} (0.25 mm thick). Melting point is uncorrected. Color reactions were observed by spraying TLC plates with Dragendorff's reagent, 3% $Ce(NH_4)_2SO_4$ in 85% H_3PO_4, or Ehrlich's reagent.

Alstonia scholaris (L.) R. Brown leaves were collected on the campus of the University of Santo Tomas (UST) in Espana, Manila in April, 2000. The sample was identified by Rosie S. Madulid, and herbarium specimens of the plants are deposited at the Botany Section of the Research Center for the Natural Sciences of UST (USTH4008).

3.2. Extraction and isolation of the alkaloids

Air-dried leaves (20 kg) were extracted with methanol to afford a green, syrupy extract (2 kg) which was subjected to acid–base extraction at pH 5 with EtOAc to give the crude alkaloid extract (45.6 g). The latter extract was subjected to silica HF_{254} vacuum liquid chromatography (gradient elution from EtOAc to MeOH, 20% increment) to give a fraction (3 g) which was purified by silica gel column chromatography twice by $CHCl_3$–MeOH–NH_4OH (9:1:0.1) followed by $CHCl_3$–MeOH–NH_4OH (9.5:0.5:0.01) to give 19,20-(*E*)-vallesamine (**3**) (8 mg). The third fraction from vacuum liquid chromatography gave a precipitate from chloroform and this was identified as a mixture of N^4-methyl angustilobine B (**2**) and angustilobine B N^4-oxide (**4**). The concentrated supernate (14.5 g) was column chromatographed using $CHCl_3$–MeOH–NH_4OH (9.5:0.5:0.01) to yield 20(*S*)-tubotaiwine (**5**) (9 mg), 6,7-*seco*-angustilobine B (**6**) (300 mg) and (+)-manilamine (**1**) (9.6 mg). The known alkaloids were identified through spectroscopic comparison with literature data (Zeches et al., 1987; Caron et al., 1989; Yamauchi et al., 1990).

3.2.1. (+)-Manilamine (*1*); 2-{4-[1-(1H-indol-2-yl)-vinyl]-1-methyl-piperidin-3-ylidene}ethanol)

Flesh-colored (beige) amorphous solid (9.6 mg); m.p. 88–92 °C. $[\alpha]_D$ + 15.5° (MeOH, *c* 0.50). UV (MeOH) λ_{max} (log ε): 223 (2.69), 282 (2.38) and 297 (2.49) nm; IR ν_{max} (KBr) cm^{-1}: 3400 (OH), 3250 (NH), 1625 (Ar >C=C<), 1600 (>C=C<); EIMS: *m*/*z* (rel. int.): $[M]^+$, 282 (59), 264 ($M^+ - H_2O$, 100), 249 (92), 141 (61), 138 (82), 108 (43). ESI-MS: 283.18033 ($C_{18}H_{22}N_2O + H^+$). ^{1}H NMR (300 MHz, MeOH-d_4): δ = 7.35 (*d*,*J* = 7.2, H-9), 7.19 (*d*, *J* = 7.2, H-12), 6.94 (*td*, *J* = 7.2, 1.0, H-11), 6.83 (*td*, *J* = 7.5, 1.0, H-10), 6.35 (*s*, H-7), 5.58 (*br t*, *J* = 6.0, H-19), 5.47 (*br s*, H-17), 4.94 (*br s*, H-17′), 4.09 (*dd*, *J* = 13.4, 7.0, H-18α), 3.88 (*ddd*, *J* = 13.4, 5.5, 1.6, H-18β), 3.81 (*br d*, *J* = 4.6, H-15α), 3.11 (*br d*, *J* = 12.0, H-21α), 2.90 (*br d*, *J* = 12.0, H-21β), 2.47 (*br d*, *J* = 12.0, H-3β), 2.15 (*s*, N–Me), 2.12 (*dd*, *J* = 12.0, 3.0, H-3α), 1.92 (*br d*, *J* = 12.0, H-14α), 1.83 (*br d*, *J* = 12.0, H-14β). ^{13}C NMR (75 MHz, MeOH-d_4): δ = 30.1 (*t*, C-14), 38.5 (*d*, C-15), 45.9 (*q*, C-5 or N–Me), 52.8 (*t*, C-3), 58.8 (*t*, C-18), 62.8 (*t*, C-21), 101.2 (*d*, C-7), 111.9 (*d*, C-12), 113.8 (*t*, C-17), 120.3 (*d*, C-10), 121.2 (*d*, C-9), 122.9 (*d*, C-11), 129.8 (*d*, C-19), 136.9 (*s*, C-16), 138.3 (*s*, C-13), 139.2 (*s*, C-2), 142.4 (*s*, C-20), (C-8 not detected).

3.2.2. Angustilobine B N^4-oxide (**2**) and N^4-methyl angustilobine B (**3**)

Flesh-colored amorphous solid (400.0 mg); m.p. 238–240 °C. IR ν_{max} (KBr) cm^{-1}: 3250 (NH), 1728 (>C=O ester), 1658 (Ar >C=C<), 1620 (>C=C<); EIMS: *m*/*z* (rel. int.): $[M]^+$, 353 (18), 354 (10) 307 (65), 294 (100), 280 (44), 263 (59), 251 (12), and 122 (40). ^1H NMR (400 MHz, pyridine-d_5): δ = 7.22–7.58 (*broad signals*, H-9 to H-12), 3.85–5.38 (*broad signal*s, H_2-6, H_2-17, H_2-18, H-19), δ 3.85 (*br s*, CO_2Me), δ 3.54 (*br s*, N^4-Me), 1.26–2.08 (*broad signals*, H_2-14). ^{13}C NMR (100 MHz, pyridine-d_5): δ = 30.7 (*t*, C-14), 44.7 (*d*, C-15), 48.9 (*t*, C-3), 50.5 (*q*, N^4-Me), 52.4 (*t*, C-6), 53.1 (methyl ester), 55.7 (*s*, C-16), 58.6 (*t*, C-21), 70.9 (*t*, C-18), 72.9 (*t*, C-17), 109.9 (*d*, C-12), 119.2 (*d*, C-9), 120.4 (*d*, C-10), 122.5 (*d*, C-11), 130.0 (*s*, C-8), 130.4 (*d*, C-19), 138.9 (*s*, C-13), 174.2 (>C=O ester) (C-2, C-7, and C-20 were not detected).

3.2.3. Anti-tuberculosis bioassay

The procedure for the MABA assay against *M. tuberculosis* $H_{37}Rv$ of the methanolic and (pH 5) alkaloid extracts was previously described by Collins and Franzblau (1997). For comparison, rifampin was used as a positive standard exhibiting 98% inhibition at 0.125 μg/ml.

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