# MATARANINE A AND B: A NEW DIASTOMERIC INDOLE ALKALOID FROM *Alstonia scholaris* R.Br. OF LOMBOK ISLAND

**Surya Hadi**

*Study Program of Chemistry, University of Mataram, Jl. Majapahit 62, Mataram-NTB 83125*

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## ABSTRACT

*Alstonia scholaris R. Br., (Apocynaceae) is widely distributed in Indonesia and in Lombok Island, the plant locally known as "lolon nita" has been used to treat malaria. To locate potential bioactive compounds, acid-base extraction was carried out. From the base fraction, two new indole alkaloids with diastomeric structure, named Mataranine A and B, were isolated. The structures of the two alkaloids were elucidated on the basis of UV, NMR and mass spectral data.*

***Keywords***: *alkaloids, Alstonia scholaris, Lombok, mataranine*

## INTRODUCTION

The plant *Alstonia scholaris* is the tallest tree belonging to the family Apocynaceae and grows to a height of 20-25 m and a diameter of 40-80 cm. This tree, the most widely distributed species of the genus, occurs in the region from India to South China and the south Ryukyu islands through Indonesia, the Philippines and Solomon islands. *Alstonia scholaris*, known as "nita" in Lombok, is common in areas up to 900 m above sea level. Initial investigations of the constituents of *Alstonia* species were stimulated by knowledge that extracts of the plant were commonly used as a cure for malaria. Until the second review of *Alstonia* alkaloids in 1970 [1], there had been no reports suggesting that *Alstonia* extracts or its pure isolated alkaloids were effective antimalarial agents. In 1999 the Keawpradub group [2], reported that the methanol extract of the root bark of *A. macrophylla*, collected from Thailand, exhibited antiplasmodial activity. They observed that pronounced antiplasmodial activity occurred mainly among the bisindole alkaloids.

In Lombok, the concentrated aqueous extracts of the leaves or bark of young trees (3-5 years) have been used to treat malaria. A particular point of interest is that young leaves from young trees are used specifically. Antimalarial testing by Yamauchi and Abe [3] with *Plasmodium falciparum*, revealed that the alkaloids obtained from leaves of *A. scholaris* from Lombok were active, but no specific mention was made of the age of the trees from which the alkaloids were isolated. Therefore, there was good reason for a thorough investigation of the young trees of this species. Moreover, during the course of this project, it was found that the alkaloids are distributed through the entire young plant including leaves, bark, roots, and fruit. In this study, the investigation of antimalarial agents is focused only on the leaves from which the two new diastomeric indole alkaloids reported were isolated.

## EXPERIMENTAL SECTION

### Material

*Alstonia scholaris* R. Br. was collected from Narmada, West Lombok with permission of the local government and in collaboration with the University of Mataram, Lombok, Indonesia. The plant collection and identification were carried out by botanists from the University of Mataram and the Research and Development Centre for Biology, Bogor, Indonesia. A voucher specimen (voucher KWL01) was deposited at the Laboratory of Biology, the University of Mataram.

### Instruments

CI (reactant gas: isobutene) and EI (at 70 eV) mass spectra were obtained on a Shimadzu QP-5000 by the direct insertion technique. HRCIMS were run on a Fisons/VG Autospec-oa-TOF Mass Spectrometer; relative intensities of peaks are given in brackets after the m/z values. $^{1}$H, gCOSY, NOESYID, selective decoupling, gHSQC, and gHMBC NMR spectra were recorded on a Varian Inova-500 MHz NMR spectrometer, unless otherwise stated. $^{13}$C-NMR and DEPT spectra were collected on a Varian Unity 300 spectrometer running at 75.42 MHz. The UV absorption spectra (solvent corrected) were recorded on a Shimadzu UV-265 spectrophotometer. Preparative TLC was performed on plates made from Merck silica gel 60 $PF_{254}$, 0.3 mm thick and bands were observed under UV light (λ 360 nm). All solvents were re-distilled before

* Corresponding author. Tel/Fax : +62-370-634110
Email address : sur_hadi@telkom.net

use and their ratios are v/v. The 3D-structure modelling was performed by minimizing conformation energies on Spartan Version 4.1.

**Procedure**

Finely-powdered, air-dried young leaves (1.5 kg) from young trees (up to 2.0 m high) were extracted with cold MeOH (3 x 3.0 litres) with occasional swirling. Methanol extraction was discontinued when the residual plant material gave a negative result for Mayer's test for alkaloids. After filtration, the solvent was removed under reduced pressure at 40 °C yielding a dark-blue MeOH extract (218.7 g). The crude alkaloid mixture was then separated from neutral and acidic material, and water solubles, by initial extraction with aqueous acetic acid (300 mL; 5%, v/v) followed by dichloromethane (DCM) extraction (3 x 500 mL) of the aqueous acid extract, which was then basified with aqueous sodium carbonate solution (10%) to pH 10, and then extracted with dichloromethane (3 x 200 mL). The solvent was evaporated under reduced pressure to give a crude alkaloid residue (753.6 mg) which was chromatographed (PTLC) on silica gel (DCM:MeOH:conc. $NH_4OH$/17:2.5:0.5) with multiple development resulting in the isolation of Mataranine A and B as a mixture (28.3 mg; major*).*

***Mataranine A 1***

A light yellow oil mixed with mataranine B (approximate ratio A:B from $^1$H-NMR 1:1); **UV** $\lambda_{max}$nm ($CHCl_3$), 283, 291; **$^1$H-NMR** ($CDCl_3$, 500 MHz), 1.80 (m, 1H, H-14), 2.19 (d, 1H, $J_{18,\ 19}$ = 7.5, H-18), 2.25 (bd, 1H, H-16), 2.80 (m, 1H, H-6β), 2.91 (m, 1H, H-6α), 3.59 (m, 1H, H-5β), 3.64 (s, 3H, COOC$\underline{H}_3$), 3.70 (m,1H, H-5α), 4.02 (bd, 1H, H-3β), 4.25 *(*dd*,* 1H*,* $J_{3\alpha,\ 3\beta}$ = 12, $J_{3\alpha,\ 14}$ = 4.5-H-3α), 6.55 (q, 1H, H-17), 7.11 (t, 1H, H-11), 7.16 (t, 1H, H-10), 7.30 (d, 1H, $J_{12,\ 11}$ = 8.5, H-12), 7.47 (d, 1H, $J_{9,\ 10}$ = 8.0, H-9), 7.76 (s, 1H, H-20), 8.20 (bs, 1H, NH), 10.28 (s, 1H, C$\underline{H}$O); **$^{13}$C-NMR** ($CDCl_3$, 75.42 MHz), 15.3 (C-18), 22.3 (C-6), 30.5 (C-14), 47.7 (C-16), 50.9 (COO$\underline{C}H_3$), 51.3 (C-3), 51.3 (C-5), 108.7 (C-7), 111.3 (C-12), 118.3 (C-9), 120.0 (C-10), 122.4 (C-11), 126.5 (C-13), 127.9 (C-8), 136.4 (C-19), 143.7 (C-17), 143.8 (C-2), 146.2 (C-15), 147.7 (C-20), 153.1 ($\underline{C}OOCH_3$), 196.1 ($\underline{C}$HO). **LRCIMS**, m/z 351 ($MH^+$); **LREIMS**, m/z (relative intensity, %), 350 (43), 335 (11), 322 (46), 321 (13), 307 (31), 291 (61), 279 (100), 265 (39), 264 (26), 263 (71), 249 (20), 238 (20), 221 (58), 209 (31), 208 (26), 193 (14), 184 (23), 169 (50), 156 (30), 155 (21), 154 (25), 145 (23), 144 (23), 143(22), 130 (21), 129 (33), 128 (21); **HRCIMS**, $C_{21}H_{22}N_2O_3$ (measured 351.1711, calc. 351.1709, for $MH^+$)

***Mataranine B 2***

**$^1$H-NMR** ($CDCl_3$, 500 MHz), 1.92 (m, 1H, H-14), 2.09 (d, 1H, $J_{18,\ 19}$ = 7.5, H-18), 2.18 (bd, H-16), 2.80 (m, 1H, H-6β), 2.91 (m, 1H, H-6α), 3.59 (m, 1H, H-5β), 3.64 (s, 3H, COOC$\underline{H}_3$), 3.70 (m, 1H, H-5α), 4.02 (bd, 1H, H-3β), 4.48 (bd, 1H, $J_{3\alpha,\ 3\beta}$ = 12, $J_{3\alpha,\ 14}$ = 4.5, H-3α), 6.58 (q, 1H, H-17), 7.11 (t, 1H, H-11), 7.16 (t, 1H, H-10), 7.30 (d, 1H, $J_{12,\ 11}$ = 8.5, H-12), 7.48 (d, 1H, $J_{9,\ 10}$ = 8.0, H-9), 7.68 (s, 1H, H-20), 8.14 (bs, 1H, NH), 9.37 (s, 1H, C$\underline{H}$O); **$^{13}$C-NMR** ($CDCl_3$, 75 MHz), 13.5 (C-18), 22.3 (C-6), 28.6 (C-14), 49.5 (C-16), 50.9 (COOC$\underline{H}_3$), 51.3 (C-3), 51.3 (C-5), 108.7 (C-7), 111.3 (C-12), 118.3 (C-9), 120.0 (C-10), 122.4 (C-11), 126.5 (C-13), 128.9 (C-8), 136.4 (C-19), 140.0 (C-2), 143.7 (C-17), 147.7 (C-20), 152.0 (C-15), 168.4 ($\underline{C}OOCH_3$), 190.6 ($\underline{C}$HO).

**RESULT AND DISCUSSION**

These new alkaloids, that the author has called mataranine A **1** and mataranine B **2**, were obtained as a light yellow oil in the form of a 1:1 diastereomeric mixture, which could not be separated fully by PTLC. However, separating the top and bottom portions of the band on the TLC plate changed the ratio of the resultant two compounds (after extraction from the silica gel), which assisted in differentiating their spectra and hence elucidating their structures. The mixture of **1** and **2** showed absorbance maxima at 283 and 291 nm in the UV spectrum consistent with an indole chromophore being present. The presence of the aldehyde and methyl ester (COOMe) groups was suggested by the LREIMS spectrum with peaks at m/z 321 ($M^+$-29), and 291 ($M^+$-59) respectively. LRCIMS gave an ion of m/z 351 $[MH]^+$ and the LREIMS spectrum showed the $[M]^+$ ion at m/z 350. The empirical formula was established by HRCIMS as $C_{21}H_{21}N_2O_3$ (measured 351.1711, calc. 351.1709, for $MH^+$).

To elucidate the structure of the mataranines, $^1$H- and $^{13}$C-NMR spectra were collected and proton-proton connectivity was deduced from a gCOSY spectrum. The proton-carbon connectivity was assigned by recording gHSQC and gHMBC experiments and these experiments were complemented by DEPT spectra. These results are summarised in Tables 1 and 2.

From the $^1$H-NMR spectrum, the significant difference between the two compounds was the chemical shift of the aldehyde proton, which appeared to be dependant on the nature of the stereogenic centre H-16. Mataranine A **1** was assigned with the C-16 proton being *alpha* (α) relative to the C-14 stereogenic centre and in mataranine B **2** it was ascribed to the *beta* position relative to the C-14 stereogenic centre. Computer-derived 3D-mataranine models (Figure 1; modelling based on the Spartan

**Table 1.** $^{1}$H-NMR data of mataranine A **1** and B **2**

| Protons | Mataranine A | | | Mataranine B | | |
|---|---|---|---|---|---|---|
| | δ (ppm) multiplicity | Integration | *J* (Hz) | δ (ppm) multiplicity | Integration | *J* (Hz) |
| H-3α | 4.25 dd | 1H | $J_{3\alpha,\ 3\beta}$ = 12, $J_{3\alpha,\ 14}$ = 4.5 | 4.48 dd | 1H | $J_{3\alpha,\ 3\beta}$ = 12, $J_{3\alpha,\ 14}$ = 4.5 |
| H-3β | 4.02 bd | 1H | $J_{3\beta,\ 3\alpha}$ = 12 | 4.02 bd | 1H | $J_{3\beta,\ 3\alpha}$ = 12 |
| H-5α | 3.70 m | 1H | | 3.70 m | 1H | |
| H-5β | 3.59 m | 1H | | 3.59 m | 1H | |
| H-6α | 2.91m | 1H | | 2.91 m | 1H | |
| H-6β | 2.80 m | 1H | | 2.80 m | 1H | |
| H-9 | 7.47 d | 1H | $J_{9,\ 10}$ = 8.0 | 7.48 d | 1H | $J_{9,\ 10}$ = 8.0 |
| H-10 | 7.16 t | 1H | | 7.16 t | 1H | |
| H-11 | 7.11 t | 1H | | 7.11 t | 1H | |
| H-12 | 7.30 d | 1H | $J_{12,\ 11}$ = 8.5 | 7.30 d | 1H | $J_{12,\ 11}$ = 8.5 |
| H-14 | 1.80 m | 1H | | 1.92 m | 1H | |
| H-16 | 2.25 bd | 1H | | 2.18 bd | | |
| H-17 | 6.55 q | 1H | | 6.58 q | | |
| H-18 | 2.19 d | 3H | $J_{18,\ 19}$ = 7.5 | 2.09 | 3H | $J_{18,\ 19}$ = 7.5 |
| H-20 | 7.76 s | 1H | | 7.68 | 1H | |
| COOMe | 3.64 s | 3H | | 3.64 s | 3H | |
| CHO | 10.28 s | 1H | | 9.37 s | 1H | |
| NH | 8.20 bs | 1H | | 8.14 bs | 1H | |

**Table 2**. $^{13}$C-NMR Data of Mataranine A **1** and B **2**

| Carbon No. | Mataranine A | | Mataranine B | |
|---|---|---|---|---|
| | Chemical shift | Carbon type (DEPT) | Chemical shift | Carbon type (DEPT) |
| 2 | 143.8 | -C- | 140.03 | -C- |
| 3 | 51.3 | $CH_2$ | 51.3 | $CH_2$ |
| 5 | 51.3 | $CH_2$ | 51.3 | $CH_2$ |
| 6 | 22.3 | $CH_2$ | 22.3 | $CH_2$ |
| 7 | 108.7 | -C- | 108.7 | -C- |
| 8 | 127.9 | -C- | 128.9 | -C- |
| 9 | 118.3 | CH | 118.3 | CH |
| 10 | 120.0 | CH | 120.0 | CH |
| 11 | 122.4 | CH | 122.4 | CH |
| 12 | 111.3 | CH | 111.3 | CH |
| 13 | 126.5 | -C- | 126.5 | -C- |
| 14 | 30.5 | CH | 28.6 | CH |
| 15 | 146.2 | -C- | 152.0 | -C- |
| 16 | 47.7 | CH | 49.5 | CH |
| 17 | 143.7 | CH | 143.7 | CH |
| 18 | 15.3 | $CH_3$ | 13.5 | $CH_3$ |
| 19 | 136.4 | -C- | 136.4 | -C- |
| 20 | 147.7 | CH | 147.7 | -C- |
| COOMe | 153.1 | | 168.4 | |
| COOMe | 50.9 | $CH_3$ | 50.9 | $CH_3$ |
| CHO | 196.1 | CH | 190.6 | CH |

program) indicated that with the C-16 proton in the *alpha* position, the aldehyde proton is closer to the lone pair of electrons on N-1, than is the case with the epimer at C-16. This is consistent with a downfield shift of the signal ascribed to aldehyde proton (δ10.28 vs δ9.37) in the former case. The position of the aldehyde functionality (CHO) was determined by the gHMBC spectrum. In mataranine A, the CHO signal at δ10.28 showed cross peaks to signals at δ143.8 (C-2) and δ30.5 (C-14).

While the following discussion focuses on the structure elucidation of mataranine A **1**, a similar analysis of the spectral data of mataranine B **2** was also carried out; this data can be seen in Tables 1 and 2. In the aromatic region of the $^{1}$H-NMR spectrum, six distinct groups of peaks were discerned from which five were readily assigned to the indole nucleus, based on gCOSY and gHSQC spectra. Two doublets centred around δ7.47 and 7.30 were assigned to the H-9 and H-12 protons of the benzo group respectively. Two

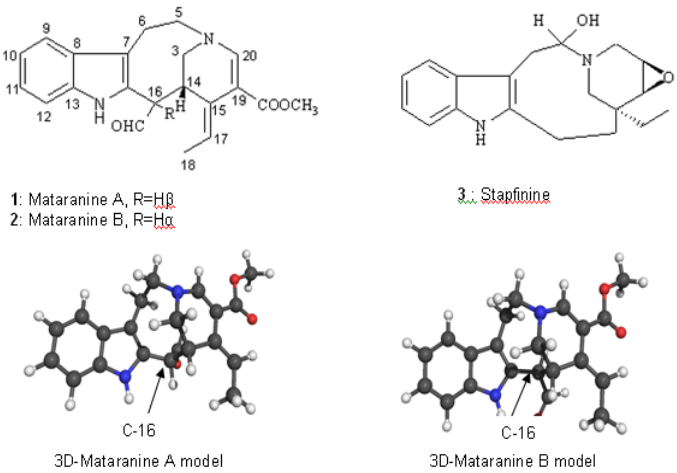


**Figure 1**. Computer-Derived 3D-Mataranine Models

triplets centred around δ7.16 and 7.11 were assigned to H-10 and H-11 respectively. A broadened singlet was observed at δ8.20, consistent with an indolic NH proton. Another singlet, at δ7.76, was assigned to H-20 with cross peaks in the gHMBC spectrum to δ136.4 (C-19), 50.9 (OMe), 47.7 (C-16), and 28.6 (C-14), which is consistent with the proposed structure.

The methyl ester appeared as a three-proton singlet at δ3.64. The observed broad doublet signal at δ2.25 was ascribed to H-16. From the gCOSY spectrum, H-16 (δ2.25) was coupled to a multiplet at δ1.80 (H-14) that also coupled to two broadened downfield doublets at δ4.25 (H-3α) and 4.02 (H-3β), suggesting the presence of a (–CH-CH-$CH_2$-N-) moiety. The ethylidene group was identified by the observation of a one-proton quartet at δ6.55 (H-17) coupled to a three-proton doublet at δ2.19 (H-18, $J_{18,\ 17}$ = 7.5 Hz). The position of the ethylidene group was confirmed by the gHMBC spectrum, which showed a weak cross peak from δ6.55 (H-17) to δ196.1 (aldehyde) and 30.5 (C-14). The (*Z*) stereochemistry about the double bond was indicated by an NOE observed between H-17 (δ6.55) and the aldehyde proton. Four distinct multiplets centred around δ3.70, 3.59, 2.91, and 2.80 were assigned to the H-5α, H-5β, H-6α, and H-6β protons respectively.

The mataranines are representative of a new indole alkaloid skeleton, which has C-16 linked to C-14 to form an eight membered ring. They may be derived from a cleavamine-velbanamine alkaloid type (e.g. stapfinine **3**), which have been isolated from three main species belonging to the family Apocynaceae: *Tabernaemontana eglandulose* [4], *Ervatamia coronaria* [5], and *Rhazya stricta* [6].

## CONCLUSION

A new diastomeric indole alkaloid, named Mataranine A and B, were successfully isolated in young leaves of *Alstonia scholaris* collected from Lombok Island. All measured spectral data are consistent with the proposed chemical structure of mataranine A and B.

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