

## Alstoctazine, a Novel Bisindole Alkaloid from *Alstonia macrophylla*

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**Abstract** : Alstoctazine, a new bisindole alkaloid, together with villalstonine were isolated from the stem-bark of *Alstonia macrophylla*. The structures were elucidated by spectroscopic methods.

**Abstrak** : Alstoctazine, satu bisindole alkaloid baru dan villalstonine diasingkan dari kulit tumbuhan *Alstonia macrophylla*. Strukturnya ditentukan secara kaedah spektroskopi.

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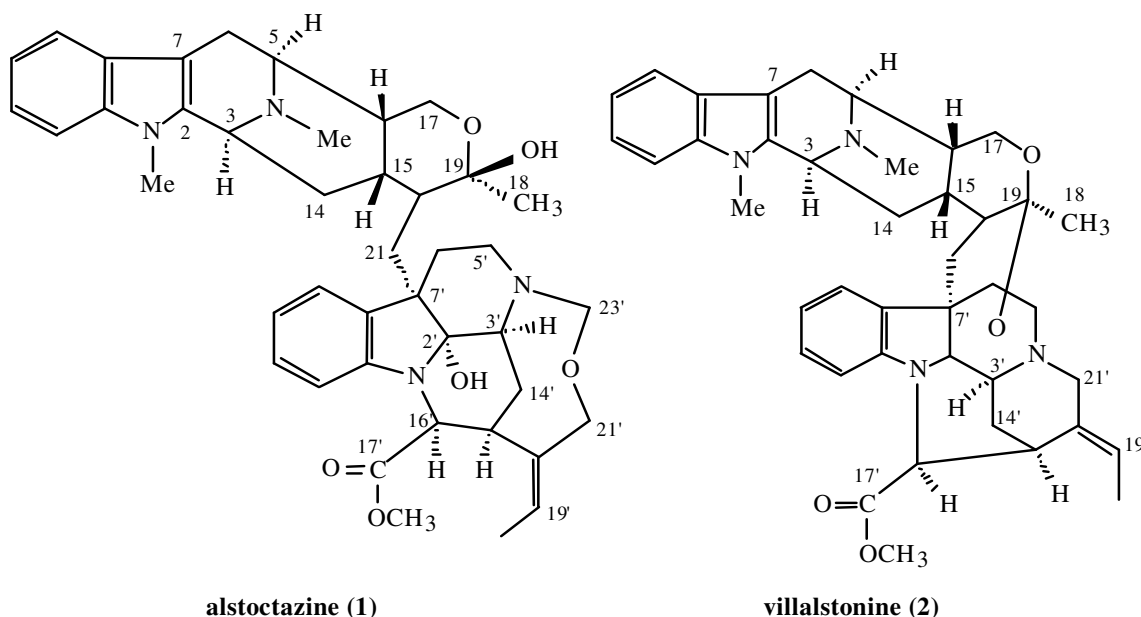
### Introduction

In our earlier study on alkaloids from the *A. macrophylla* Wall. (Apocynaceae), we reported the structures of two new oxindole alkaloids, *N*<sub>b</sub>-demethylalstophyllal oxindole and alstonal.<sup>1</sup> In the present study, I report the structural elucidation of a new bisindole alkaloid, alstoctazine (**1**), which displays a different entity to that of villalstonine (**2**), in which the ether-linkage has been cleaved to form an oxy-methylene bridge and an hydroxyl group.

### Results and Discussion

Alstoctazine (**1**) was isolated as a white amorphous solid. The mass spectral value of 709.3887 (calc. MH<sup>+</sup> 709.3965) obtained by HR-FAB-MS was consistent with the molecular formula C<sub>42</sub>H<sub>52</sub>N<sub>4</sub>O<sub>6</sub>. The presence of 42 carbon atoms was confirmed by the <sup>13</sup>CNMR data. The presence of 8 aromatic protons in the <sup>1</sup>HNMR spectrum *viz* four doublets and four triplets of doublet (see Table 1) indicated that **1** is an unsubstituted bisindole. Typical signals observed in the <sup>1</sup>HNMR spectrum for the macroline unit were: two three-proton singlets δ 3.61 and δ 2.32 were assigned to N<sub>1</sub> and

N<sub>4</sub> methyl groups; one-proton triplet (*J* = 12 Hz) and doublet of doublet (*J* = 12, 6.6 Hz) resonate at δ 3.98 and δ 3.85 (H-17) respectively; one-proton doublet (*J* = 6.6 Hz) at δ 2.97 (H-5); one-proton doublet (*J* = 16.7 Hz) at δ 2.60 and a doublet of doublet (*J* = 16.7, 6.6 Hz) at δ 3.28 assigned to protons H-6. The presence of a pleiocarpamine type unit<sup>2</sup> was confirmed by its typical NMR spectral data including a methoxy group at δ 52.45 and ester carbonyl at δ 169.67; a characteristic ethylidene group with E-configuration at δ 130.26 (C-19') and one-proton quartet at δ 5.88 (H-19', *J* = 6.8 Hz) along with the three-proton doublet resonated at δ 1.68 (18'-Me, *J* = 6.8 Hz); methylene protons at C-21' (δ 59.26) resonated as an isolated AB system (doublet) with geminal coupling of 14 Hz at δ 5.23 and δ 4.24 whereas C-5' (δ 52.87) methylene protons resonated as broad triplet and doublet at δ 4.07 (*J* = 13.4 Hz) and δ 3.80 (*J* = 13.4 Hz) respectively. Detailed structural elucidation of **1** was done by 2DNMR experiments including <sup>1</sup>H-<sup>1</sup>H COSY,



HETCOR and HMBC measurements.  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of **1** showed connectivities of H-3', H-14', H-15' and H-16'. The resonance at  $\delta$  57.40 is attributed to C-16' adjacent to a carbonyl function and is correlated to the doublet at  $\delta$  4.48 (H-16') which shows cross peak with H-15' ( $\delta$  3.36) and C-15' ( $\delta$  30.49) in the  $^1\text{H}$ - $^1\text{H}$  COSY and HMBC spectra, respectively.

As different from the villalstonine<sup>3-5</sup> having a double junction, the C2'-O-C19 bond is cleaved in the new dimer **1** while retaining the single linkage between the two monomers at C-7' ( $\delta$  42.48) and C-21; reduction of the double bond between C-2' and C-7' of the indole moiety is supported by the  $^{13}\text{C}$ NMR data, and the presence of hydroxyl group is evident by FTIR spectrum showing a broad peak at  $3370\text{ cm}^{-1}$ . The DEPT and HETCOR results indicated that the signal at  $\delta$  67.21 is an oxy-

methylene group assigned to C-23'; its geminal hydrogens resonate as two doublets at low field  $\delta$  5.93 ( $J = 10\text{ Hz}$ ) and  $\delta$  5.83 ( $J = 10\text{ Hz}$ ) suggesting the methylene was an isolated unit between two hetero atoms. This AB system does not show decoupling or COSY interaction with other protons. However, NOE interactions of the H-23' with H-14' and H-21' were observed in the NOESY spectrum.

In addition, the  $J^3$  connectivities of the H-23' with C-5' and H-21' with C-23' inferred that C-23' was attached to N<sub>4</sub>' atom as an oxy-methylene group present in the pleiocarpamine unit and thus gave rise to an eight-membered ring. The presence of such eight-membered ring consisting of a N-CH<sub>2</sub>-O-CH<sub>2</sub> function<sup>6</sup> is unprecedented in dimeric alkaloid.

**Table 1** :  $^1\text{H}$  (400 MHz) and  $^{13}\text{C}$  (100 MHz) NMR Spectral Data of Dimers **1** and **2**\*

| Position                        | <b>1</b>         |   | <b>2</b> | Position           | <b>1</b>         |  | <b>2</b> |
|---------------------------------|------------------|---|----------|--------------------|------------------|--|----------|
|                                 | $\delta\text{C}$ | $\delta\text{H}$                                      |          |                    | $\delta\text{C}$ | $\delta\text{C}$                                   |          |
| 2                               | 132.39           | -   | 135.9    | 2'                 | 91.34            | -  | 92.1     |
| 3                               | 53.31            | 3.88 <i>d</i> (4.3)                                   | 53.4     | 3'                 | 57.59            | 4.97 <i>br s</i>                                   | 51.8     |
| 5                               | 54.30            | 2.97 <i>d</i> (6.6)                                   | 54.4     | 5'                 | 52.87            | 4.07 <i>br t</i> (13.4)<br>3.80 <i>br d</i> (13.4) | 47.4     |
| 6                               | 22.72            | 3.28 <i>dd</i> (16.7, 6.6)<br>2.60 <i>d</i> (16.7)    | 22.9     | 6'                 | 27.12            | 2.47 <i>br t</i> (13.4)<br>1.71 <i>m</i> †         | 31.3     |
| 7                               | 106.97           | -   | 106.6    | 7'                 | 42.48            | -  | 44.1     |
| 8                               | 126.47           | -   | 126.4    | 8'                 | 132.73           | -  | 132.9    |
| 9                               | 118.49           | 7.56 <i>d</i> (7.3)                                   | 118.1    | 9'                 | 121.57           | 6.96 <i>d</i> (7.3)                                | 120.8    |
| 10                              | 118.94           | 7.13 <i>td</i> (7.3, 1)                               | 120.9    | 10'                | 120.66           | 6.84 <i>td</i> (7.3, 1)                            | 118.1    |
| 11                              | 120.98           | 7.22 <i>td</i> (7.3, 1)                               | 118.9    | 11'                | 127.81           | 7.08 <i>td</i> (7.3, 1)                            | 126.4    |
| 12                              | 108.66           | 7.32 <i>d</i> (7.3)                                   | 108.8    | 12'                | 110.16           | 6.20 <i>d</i> (7.3)                                | 109.3    |
| 13                              | 136.99           | -   | 137.0    | 13'                | 145.13           | -  | 146.9    |
| 14                              | 32.46            | 2.44 <i>dd</i> (11.2, 4.3)<br>1.45 <i>br d</i> (11.2) | 32.5     | 14'                | 21.80            | 2.91 <i>d</i> (15.2)<br>2.10 <i>m</i> †            | 27.5     |
| 15                              | 31.56            | 1.66 <i>m</i> †                                       | 32.4     | 15'                | 30.49            | 3.36 <i>d</i> (3.5)                                | 31.8     |
| 16                              | 36.32            | 1.20 <i>dd</i> (12.0, 6.6)                            | 37.9     | 16'                | 57.40            | 4.48 <i>d</i> (3.5)                                | 57.8     |
| 17                              | 65.99            | 3.98 <i>t</i> (12.0)<br>3.85 <i>dd</i> (12.0, 6.6)    | 65.6     | 17'                | 169.67           | -  | 171.0    |
| 18                              | 26.19            | 1.29 <i>s</i>   | 26.5     | 18'                | 12.94            | 1.68 <i>d</i> (6.8)                                | 12.3     |
| 19                              | 99.66            | -   | 98.6     | 19'                | 130.26           | 5.88 <i>q</i> (6.8)                                | 118.5    |
| 20                              | 36.96            | 2.42 <i>m</i> †                                       | 36.8     | 20'                | 126.41           | -  | 136.1    |
| 21                              | 31.07            | 2.07 <i>m</i> †<br>1.74 <i>br d</i> (17.5)            | 28.5     | 21'                | 59.26            | 5.23 <i>d</i> (14)<br>4.24 <i>d</i> (14)           | 52.9     |
| N <sub>1</sub> -CH <sub>3</sub> | 29.03            | 3.61 <i>s</i>   | 29.0     | 23'                | 67.21            | 5.93 <i>d</i> (10)<br>5.83 <i>d</i> (10)           | -        |
| N <sub>4</sub> -CH <sub>3</sub> | 41.75            | 2.32 <i>s</i>   | 41.8     | COOCH <sub>3</sub> | 52.45            | 3.72 <i>s</i>                                      | 51.7     |

\*in CDCl<sub>3</sub>; assignments based on DEPT, COSY, HETCOR and HMBC. † multiplet or overlapping with other peaks.

## Experimental

**Plant material.** The bark of *A. macrophylla* was collected from Sabah, Malaysia, and a voucher specimen (No. 138326) has been deposited at the Forest Research Centre, Sepilok.

**Extraction and isolation.** About 1.2 kg of the material was extracted with 95% EtOH. After most of the solvent had been removed, the residue was acidified with 5% HCl and the mixture filtered<sup>1</sup>. The mixture was neutralised with conc. NH<sub>4</sub>OH to pH 10; extraction of this solution with CHCl<sub>3</sub> gave 8.3 g of alkaloids. These were fractionated by silica gel CC using MeOH-CHCl<sub>3</sub> gradient and centrifugal chromatography (silica gel, 1 mm thickness) using hexane-Et<sub>2</sub>O-MeOH (7:2:1), and fractional recrystallization to give (+)-N<sub>6</sub>-methyl-N<sub>6</sub>,21-secotalpinine, alstophylline, 19,20-dehydro-10-methoxytalcarpine, pleiocarpamine, N<sub>6</sub>-demethylalstophyllal oxindole, alstonal, macralstonine hydroxyketone, villalstonine and alstoctazine.

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5. Villalstonine (2). 10 mg. EIMS 70 eV, m/z (rel. int.): 660 [M<sup>+</sup>] (85), 617(30), 601(28), 352(65), 338(92), 322(76), 293(25), 263(44), 197(100), 182(40), 170(46), 135(69), 121(58), 107(35). <sup>1</sup>HNMR (270 MHz, CDCl<sub>3</sub>): δ 7.54 (1H, *d*, 7.5 Hz, H-9), 7.33 (1H, *d*, 7.5 Hz, H-12), 7.22 (1H, *d*, 7.5 Hz, H-11), 7.15 (1H, *t*, 7.5 Hz, H-11), 7.15 (1H, *t*, 7.5 Hz, H-10), 6.98 (1H, *t*, 7.5 Hz, H-10'), 6.88 (1H, *d*, 7.5 Hz, H-9'), 6.69 (1H, *t*, 7.5 Hz, H-11'), 6.14 (1H, *d*, 7.5 Hz, H-12'), 5.36 (1H, *q*, 6.5 Hz, H-19'), 4.44 (1H, *d*, 3.5 Hz, H-16'), 4.19 (1H, *br d*, 12.5 Hz, H-21'), 3.99 (1H, *t*, 12 Hz, H-17), 3.86 (1H, *s*, H-3), 3.70-3.75 (2H, *m*, H-17, H-3'), 3.67 (3H, *s*, COOMe), 3.62 (3H, *s*, N-1 Me), 3.29 (1H, *dd*, 16.5, 6.5 Hz, H-6), 3.21 (1H, *d*, 3.5 Hz, H-15'), 3.13 (1H, *dd*, 14, 2.5 Hz, H-5'), 2.96 (1H, *d*, 12.5 Hz, H-21'), 2.91 (1H, *d*, 6.5 Hz, H-5), 2.63-2.73 (2H, *m*, H-5', H-14'), 2.47 (1H, *d*, 16.5 Hz, H-6), 2.36-2.46 (2H, *m*, H-14, H-21), 2.31 (3H, *s*, N-4 Me), 2.01-2.12 (2H, *m*, H-16, H-6'), 1.67-1.72 (1H, *m*, H-14'), 1.56-1.66 (2H, *m*, H-15, H-21), 1.55 (1H, *dd*, 7, 1.5 Hz, H-18'), 1.44 (1H, *d*, 12.5 Hz, H-14), 1.25 (1H, *s*, H-18), 1.16-1.19 (1H, *m*, H-20), 1.11 (1H, *d*, 13 Hz, H-6').
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