# Alasmontamine A, A First Tetrakis Monoterpene Indole Alkaloid from Tabernaemontana elegans

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# Alasmontamine A, A First Tetrakis Monoterpene Indole Alkaloid from *Tabernaemontana elegans*

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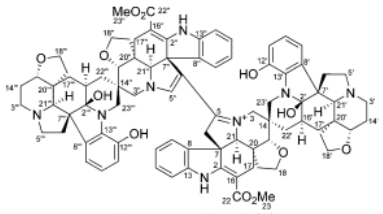
## ABSTRACT



alasmontamine A (**1**)

**A novel tetrakis monoterpene indole alkaloid, alasmontamine A (1) consisting of bis-vobtusine-type skeletons, was isolated from the leaves of *Tabernaemontana elegans*. The structure including the relative stereochemistry was elucidated on the basis of spectroscopic data. Alasmontamine A (1) exhibited moderate cell growth inhibitory activity against HL-60 cells.**

*Tabernaemontana elegans* Stapf is a member of the Apocynaceae family that occurs in tropical or subtropical regions including Indonesia, Malaysia, and Africa. Traditionally, the roots have been used as a remedy for pulmonary diseases in Africa.[1] *Tabernaemontana* species so far have been shown to produce various skeletal types of indole alkaloids, including iboga-type alkaloids such as ibogamine,[2] aspidosperma-type alkaloids such as taberhanine,[3] and vobasinyl-ibogan bisindole alkaloids such as conodiparine A.[4] Recently, we isolated a new type of bisindole alkaloids as biscarpamontamines A and B from *T. sphaerocarpa*.[5] In our search for structurally and biogenetically interesting alkaloids from tropical plants found in Indonesia, a first tetrakis monoterpene indole alkaloid, alasmontamine A (**1**) consisting of tetrakis aspidosperma-type skeletons, was isolated from the leaves of *T. elegans*, together with vobtusine[6] and vobtusine lactone.[7] In this paper, we describe the isolation and structure elucidation of the new alkaloid, alasmontamine A (**1**).

Alasmontamine A (**1**),[8,9] yellow amorphous solid, $[\alpha]^{27}_{D}$ −311 (*c* 1.0, MeOH), showed molecular formula, $C_{84}H_{91}N_8O_{12}$, which was determined by HRESITOFMS [*m*/*z*

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702.3415 $(M + H)^{2+}$, $\Delta$ +0.3 mmu]. IR absorptions (3440 and 1670 $cm^{-1}$) implied the presence of NH and/or OH, and conjugated ester carbonyl functionalities. $^1H$ and $^{13}C$ NMR, HMQC, and HMBC spectra revealed 84 carbon signals due to 18 $sp^2$ and 12 $sp^3$ quaternary carbons, 15 $sp^2$ and 10 $sp^3$ methines, 27 $sp^3$ methylenes, and 2 methyl groups (303 K by 600 MHz cryo probe). Among them, 8 $sp^3$ methylenes ($\delta_C$ 55.0; $\delta_H$ 4.11, and 4.26, $\delta_C$ 48.6; $\delta_H$ 2.37 and 2.88, $\delta_C$ 51.6; $\delta_H$ 2.31 and 3.09, $\delta_C$ 50.7; $\delta_H$ 3.71 and 3.83, $\delta_C$ 53.5; $\delta_H$ 3.82 and 3.92, $\delta_C$ 48.6; $\delta_H$ 2.37 and 2.88, $\delta_C$ 51.6; $\delta_H$ 2.31 and 3.09, and $\delta_C$ 45.2; $\delta_H$ 3.19, and 4.73), 1 $sp^2$ methine ($\delta_C$ 158.7; $\delta_H$ 7.68), 4 $sp^3$ methines ($\delta_C$ 76.2; $\delta_H$ 4.49, $\delta_C$ 64.7; $\delta_H$ 2.80, $\delta_C$ 72.1; $\delta_H$ 4.29, $\delta_C$ 64.7; $\delta_H$ 2.79), 7 $sp^2$ quaternary carbons ($\delta_C$ 161.4, 144.8, 168.2, 136.1, 158.7, 144.7, and 136.9), and 2 $sp^3$ quaternary carbons ($\delta_C$ 93.6 and 93.2) were ascribed to those bearing a nitrogen atom. Since 18 out of 44 elements of unsaturation were accounted for, **1** was inferred to possess 26 rings.

The gross structure of **1** was elucidated by analyses of 2D NMR data including $^1H-^1H$ COSY, HOHAHA, HSQC, and HMBC spectra in $CD_3OD$ at 313 K by using a 920 MHz NMR spectrometer (Figure 1). Each pair of the observed $^1H$

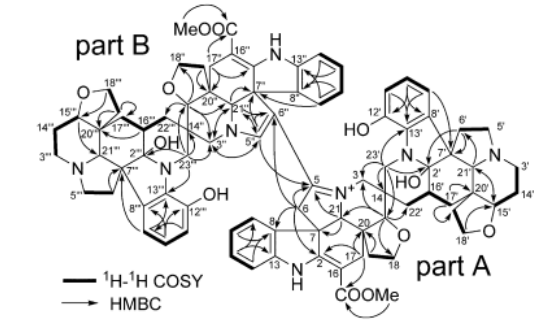



**Figure 1.** Selected 2D NMR correlations for alasmontamine A (**1**).

and $^{13}C$ NMR signals seemed to be due to each half moiety (parts A and B) of a dimeric compound. In part A, connectivities of C-9−C-12, C-18 to C-19, C-3′−C-15′, C-5′ to C-6′, C-9′−C-11′, C-18′−C-19′, and C-17′−C-22′ were deduced from $^1H-^1H$ COSY and HOHAHA correlations. In the HMBC spectrum, long-range $^1H-^{13}C$ correlations indicated that part A possessed a 12′-*O*-demethylvobtusine-type framework with an iminium functionality at C-5 ($\delta_C$ 168.2). The presence of an iminium carbon (C-5) was elucidated by HMBC correlations for $H_2$-6 and H-21 to C-5. In addition, the $^{13}C$ signals at 6- and 21-positions around the iminium functionality were observed at lower field due to deshielding effects (Table 1) compared with those of vobtusine. The relative configurations at C-7, 15, 20, 21, 7′, 15′, 16′, 20′, and 21′ in part A were based on NOESY correlations of H-9 and H-19a/H-21, H-15/$H_2$-17, H-9′ and H-18′b/H-21′, and H-16′/H-19′b, while the 3,3′-spirobipiperidine (C-3, 14, 15, 20, 21, N, 2′, 16′, 22′, 23′, and N) ring adopted a boat−chair conformation that was supported by NOESY correlations as shown in Figure 2. Furthermore the

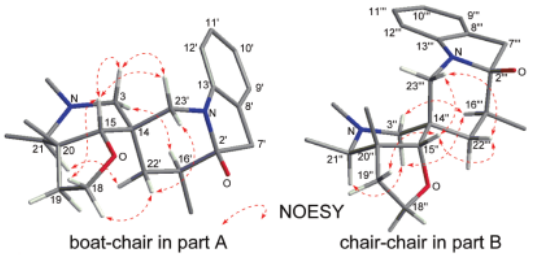



**Figure 2.** Selected NOESY correlations around two spiro carbons (C-14 and C-14′) in parts A and B of **1**.

$\beta$-configuration of the OH group at C-2′ was deduced from the upfield chemical shift of C-6′ ($\delta_C$ 30.6) by the $\gamma$-gauche effect.[6,7]

On the other hand, detailed analyses of the HMBC spectrum of **1** indicated that part B possessed a 12′-*O*-

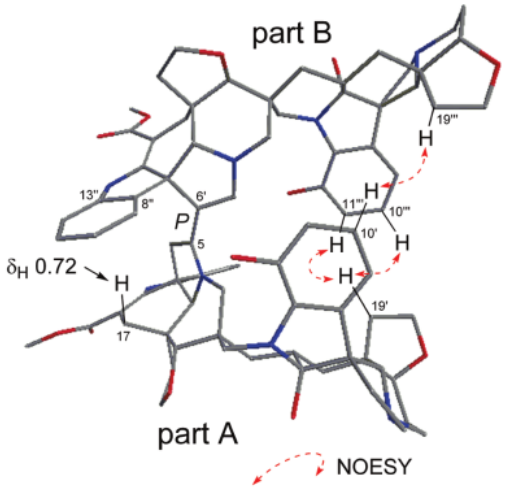



**Figure 3.** Selected NOESY correlations and the relative stereochemistry for alasmontamine A (**1**).

(8) The leaves of *T. elegans* collected at Alas Purwo, Indonesia in 2007 were extracted with MeOH, and the extract was partitioned between EtOAc and 3% tartaric acid. The aqueous layer was adjusted at pH 9 with saturated $Na_2CO_3$ aq and extracted with $CHCl_3$. $CHCl_3$-soluble alkaloidal materials were subjected to a silica gel column ($CHCl_3$/MeOH) twice followed by an LH-20 column ($CHCl_3$/MeOH) to afford alasmontamine A (**1**, 0.0005%) together with known alkaloids, vobtusine[6] and vobtusine lactone.[7]

(9) Alasmontamine A (**1**): yellow amorphous solid; $[\alpha]^{27}_D$ −311 (*c* 1.0, MeOH); IR (KBr) $\nu_{max}$ 3440, 2940, 1670, and 1630 $cm^{-1}$; UV (MeOH) $\lambda_{max}$ 365 ($\varepsilon$ 17 800), 329 (20 400), 295 (19 700), 259 (17 700), and 218 (55 400) nm; CD (MeOH) $\lambda_{max}$ 373 ($\theta$ −22 100), 338 (−13 100), 325 (−17 900), 295 (4 500), 280 (4 100), 265 (13 600), 250 (5 600), and 225 (33 100) nm; $^1H$ and $^{13}C$ NMR (Table 1); ESIMS (pos.) *m/z* 702 $(M + H)^{2+}$; HRESITOFMS *m/z* 702.3415 $(M + H)^{2+}$, calcd for $C_{84}H_{92}N_8O_{12}$ 1404.6824.

**Table 1.** $^{1}H$ and $^{13}C$ NMR Data of Alasmontamine A (**1**) in $CD_3OD$

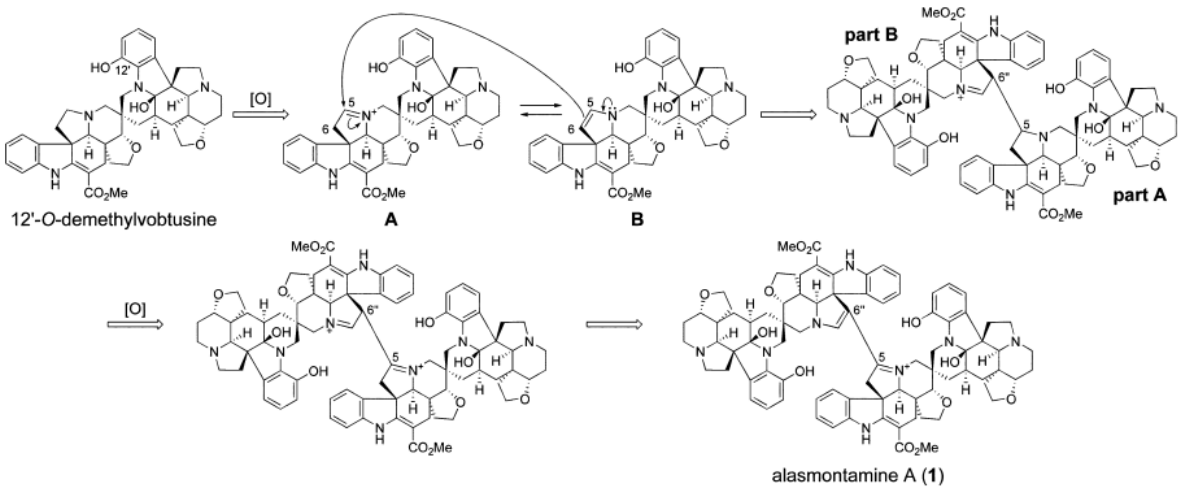
| unit A | | | | unit B | | | |
|---|---|---|---|---|---|---|---|
| position | $\delta_H$[a] | $\delta_C$[b] | HMBC[a] | position | $\delta_H$[a] | $\delta_C$[b] | HMBC[a] |
| 2 | | 161.4 | 6b, 17b | 2″ | | 158.7 | 17″ |
| 3a | 4.11 (1H, d, 13.6) | 55.0 | 22′b, 23′[b] | 3″a | 3.82 (1H, m) | 53.5 | 5″, 15″, 22‴,[b] 23‴ |
| 3b | 4.26 (1H, d, 13.6) | | | 3″b | 3.92 (1H, m) | | |
| 5 | | 168.2 | 6a,[b] 6b, 21, 5″[b] | 5″ | 7.68 (1H, s) | 158.7 | 3″,[b] 21″ |
| 6a | 2.76 (1H, m) | 48.7 | | 6″a | | 104.6 | 6a,[b] 5″, 21″[b] |
| 6b | 2.95 (1H, d, 18.6) | | | 6″b | | | |
| 7 | | 52.4[a,c] | 6, 21 | 7″ | | 60.9 | 5″, 9″, 21″[b] |
| 8 | | 134.9 | 6a,[b] 6b, 10, 12, 21 | 8″ | | 135.4 | 10″, 12″, 21″ |
| 9 | 7.56 (1H, m) | 122.6[a,c] | 11 | 9″ | 7.26 (1H, d, 7.3) | 124.4 | 11″ |
| 10 | 6.91 (1H, dd, 7.5, 7.2) | 122.8 | 12 | 10″ | 7.11 (1H, dd, 7.3, 7.3) | 123.4 | 12″ |
| 11 | 7.19 (1H, dd, 7.4, 7.2) | 130.0 | 9 | 11″ | 7.33 (1H, dd, 7.6, 7.3) | 131.2 | 9″ |
| 12 | 6.96 (1H, d, 7.4) | 111.1 | 10 | 12″ | 7.02 (1H, d, 7.6) | 111.6 | 10″ |
| 13 | | 144.8 | 9, 11 | 13″ | | 144.7 | 9″, 11″ |
| 14 | | 41.8[a,c] | 22′b, 23′b[b] | 14″ | | 39.8 | 3″,[b] 15″,[b] 22‴b,[b] 23‴b[b] |
| 15 | 2.79 (1H, s) | 87.3 | 17,[b] 18b, 19b,[b] 22′b[b] | 15″ | 3.46 (1H, s) | 87.1 | 3″b, 18″, 22‴b,[b] 23‴a |
| 16 | | 92.6 | 17 | 16″ | | 98.4 | 17″ |
| 17a | 0.72 (1H, d, 16.2) | 31.1 | | 17″a | 2.36 (1H, d, 14.5) | 28.2 | 15″,[b] 19″b,[b] 21″[b] |
| 17b | 2.51 (1H, d, 16.2) | | | 17″b | 2.66 (1H, dd, 14.5, 1.6) | | |
| 18a | 3.83 (1H, m) | 69.2 | 15[b] | 18″a | 3.75 (1H, m) | 64.3 | 19″b[b] |
| 18b | 4.05 (1H, dd, 8.4, 8.4) | | | 18″b | 3.75 (1H, m) | | |
| 19a | 1.56 (1H, 11.7, 11.7, 11.7) | 35.8 | 15,[b] 17,[b] 21[b] | 19″a | 1.43 (1H, ddd, 12.8, 8.5, 4.3) | 37.0 | 17″b,[b] 18″,[b] 21″[b] |
| 19b | 1.62 (1H, m) | | | 19‴b | 1.60 (1H, m) | | |
| 20 | | 50.8 | 15,[b] 17, 18b, 19b,[b] 21[b] | 20″ | | 49.1 | 17″, 19″[b] |
| 21 | 4.49 (1H, s) | 76.2 | 3b, 15,[b] 17 | 21″ | 4.29 (1H, s) | 72.1 | 3″b, 5″, 15″, 17″, 19″[b] |
| 22 | | 169.1 | 17b, 23 | 22″ | | 168.6 | 17″, 23″ |
| 23 | 3.78 (3H, s) | 51.7 | | 23″ | 3.69 (3H, s) | 51.9 | |
| 2′ | | 93.6[a,c] | 6′b, 22′b,[b] 23′b | 2‴ | | 93.2[a,c] | 17‴a, 22‴a, 22‴b,[b] 23‴b |
| 3′a | 2.37 (1H, m) | 48.6[a,c] | 15′ | 3‴a | 2.37 (1H, m) | 48.6[a,c] | 15‴ |
| 3′b | 2.88 (1H, m) | | | 3‴b | 2.88 (1H, m) | | |
| 5′a | 2.31 (1H, m) | 51.6[a,c] | 6′b | 5‴a | 2.31 (1H, m) | 51.6[a,c] | 6‴b |
| 5′b | 3.09 (1H, m) | | | 5‴b | 3.09 (1H, m) | | |
| 6′a | 1.20 (1H, m) | 30.6[a,c] | | 6‴a | 1.21 (1H, m) | 31.4[a,c] | |
| 6′b | 2.74 (1H, m) | | | 6‴b | 2.75 (1H, m) | | |
| 7′ | | 57.0 | 6′b, 9′ | 7‴ | | 56.0[a,c] | 6‴b, 9‴ |
| 8′ | | 136.6 | 10′ | 8‴ | | 135.9 | 10‴ |
| 9′ | 6.65 (1H, d, 7.7) | 116.3 | 11′ | 9‴ | 6.83 (1H, d, 7.5) | 115.9 | 11‴ |
| 10′ | 5.85 (1H, dd, 7.9, 7.7) | 121.6 | | 10‴ | 6.35 (1H, dd, 7.6, 7.5) | 120.9 | |
| 11′ | 6.22 (1H, d, 7.9) | 116.5 | 9′ | 11‴ | 6.41 (1H, d, 7.6) | 118.9 | 9‴ |
| 12′ | | 142.0 | 10′ | 12‴ | | 142.0 | 10‴ |
| 13′ | | 136.1 | 9′, 11′, 23′a, 23′b[b] | 13‴ | | 136.9 | 9‴, 11‴, 23‴a, 23‴b[b] |
| 14′a | 1.95 (1H, m) | 25.5[a,c] | | 14‴a | 1.95 (1H, m) | 25.5[a,c] | |
| 14′b | 1.99 (1H, m) | | | 14‴b | 1.99 (1H, m) | | |
| 15′ | 3.54 (1H, brt, 2.6) | 80.5[a,c] | 18′b | 15‴ | 3.51 (1H, brt, 2.7) | 80.5[a,c] | 18‴b |
| 16′ | 2.28 (1H, m) | 31.2[a,c] | | 16‴ | 1.99 (1H, m) | 32.3[a,c] | 22‴[b] |
| 17′a | 1.07 (1H, brd, 12.9) | 31.6[a,c] | | 17‴a | 0.97 (1H, d, 11.6) | 32.1[a,c] | |
| 17′b | 1.90 (1H, dd, 12.9, 12.9) | | | 17‴b | 2.00 (1H, m) | | |
| 18′a | 4.01 (1H, ddd, 10.1, 8.6, 3.6) | 65.1[a,c] | | 18‴a | 3.94 (1H, ddd, 9.9, 8.6, 3.2) | 64.8[a,c] | |
| 18′b | 4.23 (1H, ddd, 10.1, 8.1, 8.0) | | | 18‴b | 4.16 (1H, ddd, 9.9, 8.2, 7.9) | | |
| 19′a | 2.07 (1H, m) | 36.8[a,c] | 18′b[b] | 19‴a | 1.74 (1H, ddd, 10.5, 9.2, 9.4) | 36.2[a,c] | 18‴b[b] |
| 19′b | 2.59 (1H, ddd, 11.8, 8.0, 3.6) | | | 19‴b | 2.56 (1H, ddd, 10.5, 7.9, 3.2) | | |
| 20′ | | 46.3[a,c] | | 20‴ | | 46.1[a,c] | 16‴,[b] 19‴b[b] |
| 21′ | 2.80 (1H, s) | 64.7[a,c] | | 21‴ | 2.79 (1H, s) | 64.7[a,c] | |
| 22′a | 1.49 (1H, brd, 13.0) | 29.3[a,c] | | 22‴a | 1.67 (1H, brd, 14.4) | 36.0 | 3″,[b] 23‴b[b] |
| 22′b | 2.15 (1H, dd, 13.0, 12.8) | | | 22‴b | 2.20 (1H, dd, 14.8, 14.4) | | |
| 23′a | 3.71 (1H, d, 14.2) | 50.7 | 15, 22′a | 23‴a | 3.19 (1H, dd, 14.5, 1.6) | 45.2 | 15″, 22‴a[b] |
| 23′b | 3.83 (1H, m) | | | 23‴b | 4.73 (1H, m) | | |

[a] Recorded at 313 K by 920 MHz NMR. [b] Recorded at 303 K by 600 MHz cryo probe NMR. [c] Assigned by HSQC or HMBC.

demethylvobtusine type framework with an enamine on C-5″ and C-6″. HMBC correlations for H-3″b and H-5″ to C-21″ ($\delta_C$ 72.1), $H_2$-3″ and H-21″ to C-5″ ($\delta_C$ 158.7), and H-5″ and H-21″ to C-3″ ($\delta_C$ 53.5) supported connections among C-3″, 5″, and 21″ through a nitrogen atom. And H-5″, H-9″, and H-21″ to C-7″ ($\delta_C$ 60.9), and H-5″ and H-21″ to C-6″ ($\delta_C$ 104.6) revealed the presence of an enamine on C-5″ and C-6″. The relative configurations at C-7″, 14″, 15″, 20″, 21″, 7‴, 15‴, 16‴, 20‴, and 21‴ in part B were the same as corresponding ones of part A. However, the conformation of the 3,3′-spirobipiperidine (C-3″, 14″, 15″, 20″, 21″, N, 2‴, 16‴, 22‴, 23‴, and N) ring in part A was different from that in part B. NOESY correlations of $H_2$-3″/H-16‴, H-3″a/H-21″, H-15″/H-22‴b and H-23‴a, and H-22‴b/H-23‴a suggested the chair−chair conformation of a 3,3′-spirobipiperidine ring in part B. The $^{13}C$ NMR chemical shifts of part B except for an enamine moiety were in good agreement with those of vobtusine which also possessed a chair−chair conformation assigned by X-ray analysis.[5] The connection of C-5 and C-6″ between parts A and B was provided by HMBC correlations for H-5″ to C-5 and H-6a to C-6″.

Finally, the relative stereochemistry between parts A and B in **1** was elucidated by the combination of Monte Carlo (MC) search[10] in MacroModel program[11] and NOESY correlations. A total of 3000 MC steps were performed to confirm the reproducibility of calculation results. After the MC conformational search, each of the resulting conforma-

**Scheme 1.** Plausible Biogenetic Path for Alasmontamine A (**1**)




tions was subjected to the energy-minimization calculation by MMFF94s force field.[12] Low-energy conformers belonged to two separate clusters.

The lowest energy one (1234.59 kJ/mol) had an *M* rotation at the C-5−C-6″ axis, and the other one had a *P* rotation (1251.76 kJ/mol) that corresponded with the solution conformer as shown below. Since the latter only satisfied the NOESY correlations of H-10′/H-19‴, H-19′/H-10‴, and H-19′/H-11‴, the relative stereochemistry of **1** was assigned as Figure 3. The allylic proton signal for H-17a was shifted to higher field ($\delta_H$ 0.72) as compared with that of H-17″a ($\delta_H$ 2.36). This can be explained by the anisotropic effect of the benzene ring (C-8″ − C-13″) as shown in a computer generated 3D drawing (Figure 3).

Alasmontamine A (**1**) consisting of bis-vobtusine type skeletons is a novel tetrakis monoterpene indole alkaloid from nature. A plausible biogenetic pathway for alasmontamine A (**1**) is proposed as shown in Scheme 1. Tetrakis monoterpene indole skeleton might be formed through an iminium-enamine coupling (C-5−C-6) of two vobtusine-type skeletons **A** and **B**, which might be produced through Polonovski-type reaction[13] from the *N*-oxide of 12′-*O*-demethylvobtusine. Formation of alasmontamine A (**1**) might occur through further oxidation, which was accompanied by enamine formation as shown in Scheme 1.

Alasmontamine A (**1**) showed moderate cell growth inhibitory activity against HL-60 cells ($IC_{50}$ 31.7 $\mu$M).

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**Supporting Information Available:** 1D and 2D NMR spectra for compound **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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# Alasmontamine A, A First Tetrakis Monoterpene Indole Alkaloid from Tabernaemontana elegans

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GENERAL COMMENTS

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