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PHYTOCHEMISTRY

Alkaloids from the stem-bark of *Alstonia macrophylla*

Toh-Seok Kam*, Ing-Ho Iek, Yeun-Mun Choo

Department of Chemistry, University of Malaya, 50603 Kuala Lumpur, Malaysia

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Abstract

Three new indole alkaloids, viz. 10-methoxyaffinisine, 10-methoxycathafoline and alstonerinal, in addition to alstonerine, alstonisine, alstonal, alstophylline, vincamajine, lochnerine and cathafoline were isolated from the stem-bark extract of *Alstonia macrophylla*.

Keywords: *Alstonia* species; Apocynaceae; Indole alkaloids

1. Introduction

Plants of the genus *Alstonia* numbering about 38 species are distributed in tropical areas including Africa, Indo-Malaya and Australia. Several species of *Alstonia* are used in traditional medicine throughout Southeast Asia for the treatment of malaria and dysentry (Burkhill, 1966; Perry & Metzger, 1980). *Alstonia macrophylla* are usually medium sized trees growing up to about 15 to 25 m. Representatives from Sri Lanka (Atta-ur-Rahman, Silva, Alvi, & De Silva, 1987; Ratnayake, Arambewela, De Silva, Atta-ur-Rahman, & Alvi, 1987; Atta-ur-Rahman, Qureshi, Muzaffar, & De Silva, 1988; Atta-ur-Rahman et al., 1991; Atta-ur-Rahman, Nighat, Sultana, & De Silva, 1994), the Phillipines (Abe, Yamauchi, & Padolina, 1994), Thailand (Abe, Yamauchi, & Santisuk, 1994; Keawpradub & Houghton, 1997), as well as one from Malaysian Borneo (Wong, Lim, & Chuah, 1996), have been previously investigated. As part of our study of Malaysian *Alstonia* (Kam, Jayashankar, Sim, & Yoganathan, 1997; Kam, Nyeoh, Sim, & Yoganathan, 1997), we would like to report the isolation of several new alkaloids from a Malayan *A. macrophylla*.

2. Results and discussion

The ethanol extract of the stem-bark of *Alstonia macrophylla* furnished the following alkaloids: alstonerine **1** (Ratnayake et al., 1987; Ghedira et al., 1988), alstonisine **2** (Ghedira et al., 1988), alstonal **3** (Wong et al., 1996), alstophylline **4** (Abe et al., 1994), vincamajine **5** (Abe et al., 1994), lochnerine **6** (Mors, Zaltzman, Beereboom, Pakrashi, & Djerassi, 1956), cathafoline **7** (Das, Cosson, & Lukacs, 1977), 10-methoxyaffinisine **8**, 10-methoxycathafoline **9** and alstonerinal **10**. Of these, the last three are new and 10-methoxyaffinisine **8** constitutes the predominant alkaloid present in the stem-bark. Compound **8** was obtained as white needles from MeOH and analysed for $C_{21}H_{26}N_2O_2$ in HREIMS. Besides the molecular ion (*m*/*z* 338), another major fragment was observed at *m*/*z* 307 due to the loss of CH_2OH. The UV spectrum showed absorption maxima characteristic of an indole chromophore (223, 283 and 300 nm) while the IR spectrum showed the presence of an OH function (3355 cm^{-1}). The ^1H NMR spectrum (Table 1) showed the presence of a substituted indole ring, *N*-methyl and aromatic methoxy groups, an ethylidene group and a hydroxymethyl function. These and the ^{13}C NMR spectrum (Table 2) suggested a sarpargine-type compound and comparison with affinisine **11** revealed **8** to be the 10-methoxy substituted derivative of the known alkaloid affinisine (Clivio et al., 1991). Compound **9**

* Corresponding author. Tel.: +60-3-759-4266; fax: +60-3-759-4193.

E-mail address: tskam@kimia.um.edu.my (T.S. Kam)

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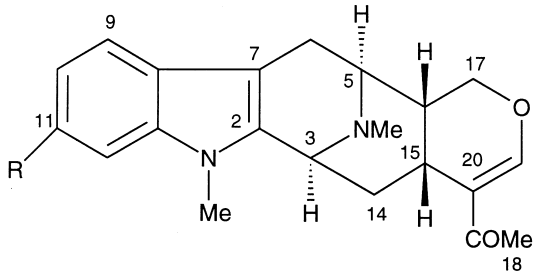
Table 1
^{1}H NMR spectral data for compounds (**1**, **8**–**10**) (400 MHz, $CDCl_3$)

H	8	9	10	1
2	–	2.44 s	–	–
3	4.20 dd (10, 2)	4.12 d (5)	3.86 t (3)	3.87 t (3)
5	2.79 m	2.64 m	3.06 brd (7)	3.07 brd (7)
5	–	3.88 td (14, 5)	–	–
6	2.59 dd (15, 1)	1.45 dd (15, 5)	2.48 d (16)	2.49 d (16)
6	3.04 dd (15, 5)	3.07 ddd (15, 14, 5)	3.31 dd (16, 7)	3.32 dd (16, 7)
9	6.92 d (1)	6.61 d (1)	7.44 brd (8)	7.44 brd (8)
10	–	–	7.08 td (8, 1)	7.08 td (8, 1)
11	6.83 dd (8, 1)	6.63 dd (8, 1)	7.19 td (8, 1)	7.19 td (8, 1)
12	7.17 d (8)	6.53 d (8)	7.31 brd (8)	7.31 brd (8)
14	1.67 ddd (12, 4, 2)	1.61 dd (14, 3)	1.79 td (12, 3)	1.81 td (12, 3)
14	2.01 ddd (12, 10, 2)	2.37 ddd (14, 5, 3)	2.12 ddd (12, 5, 3)	2.12 ddd (12, 5, 3)
15	1.79 m	3.60 brs	2.61 dt (12, 5)	2.61 dt (12, 5)
16	1.81 tdd (8, 6, 1)	2.94 d (4)	1.89 m	1.89 m
17	3.54 m	–	4.18 ddd (11, 4, 2)	4.16 ddd (11, 4, 2)
17	3.54 m	–	4.46 t (11)	4.40 t (11)
18	1.63 dt (7, 2)	1.50 dd (7, 3)	2.15 s	2.07 s
19	5.40 brq (7)	5.41 brq (7)	–	–
21	3.54 m	2.94 d (16)	9.65 s	7.52 s
21	3.54 m	3.95 brd (16)	–	–
N(1)–Me	3.59 s	2.65 s	3.63 s	3.64 s
N(4)–Me	–	–	2.31 s	2.31 s
10-OMe	3.85 s	3.73 s	–	–
CO_2Me	–	3.77 s	–	–

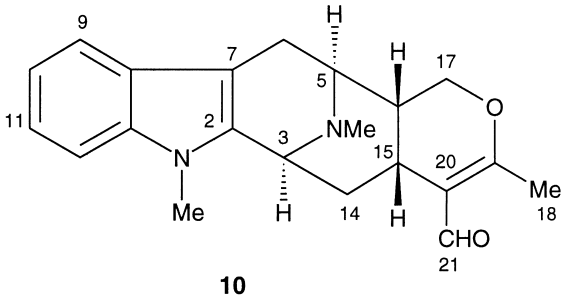
was obtained as light yellowish oil and analysed for $C_{22}H_{28}N_2O_3$. The UV spectrum indicated a dihydroindole chromophore (204, 249 and 315 nm) while the NMR spectral data showed the presence of a substituted aromatic ring, *N*-methyl and aromatic methoxy groups, an ester function and an ethylidene group. The NMR spectral data (Tables 1 and 2) indicated that **9** was an alkaloid of the akuammiline-type and corresponds to the 10-methoxy substituted derivative of cathafoline **7** which was also present in the stem-bark extract. Compound **10**, alstonerinal, coeluted with the type B macroline (Ghedira et al., 1988), alstonerine **1**, during chromatography and proved resistant to further resolution by chromatography as well as fractional crystallization. The ^{1}H NMR spectrum (Table 1) showed overlap involving most of the signals except for a few which were distinguishable. For example, in the ^{1}H NMR spectrum, only the H-18 (methyl) and the H-21 (aldehyde-H for **1**, vinylic-H for **10**) signals of both compounds are clearly distinguished, while the H-17 signals are partially overlapped. Other than these, the remaining signals are virtually coincident. In the case of the ^{13}C NMR spectrum (Table 2), the C-18, C-19 and C-21 signals are clearly distinguished, while the rest are either overlapped, or occur in pairs with very similar chemical shifts, which nevertheless can be distinguished by their relative intensities, since there is a 3.5-fold predominance of the type B macroline form. These compounds tend to coelute during isolation and are frequently obtained as a mixture with the type B macroline form predominating and since the majority of the peaks are overlapped in NMR, the presence of the minor type A macroline may some-

Table 2
^{13}C NMR spectral data for compounds (**1**, **7**, **8**–**11**) (100 MHz, $CDCl_3$)

C	11	8	7	9	1	10
2	139.4	140.2	79.3	79.5	133.1	133.1
3	49.2	49.4	47.4	47.5	54.6	54.6
5	54.5	54.4	50.8	50.3	53.7	53.6
6	27.2	27.4	31.2	30.1	22.8	22.3
7	103.4	103.1	43.1	43.0	105.8	105.8
8	127.2	127.6	140.5	141.5	126.5	126.5
9	118.0	100.6	121.0	109.3	117.7	117.3
10	118.6	153.7	119.4	153.7	120.7	121.0
11	120.6	110.5	127.2	110.9	118.6	118.6
12	108.5	109.3	109.4	109.8	108.9	108.9
13	137.1	132.6	153.2	147.2	137.1	137.1
14	32.5	32.8	33.9	33.6	38.5	38.5
15	27.0	27.1	34.4	34.9	32.3	31.8
16	44.0	44.2	52.9	52.7	41.7	41.7
17	64.6	64.9	–	–	67.7	68.0
18	12.6	12.7	13.0	13.0	22.7	16.5
19	116.2	116.5	119.2	120.0	195.7	170.6
20	135.6	135.8	139.3	138.0	126.5	126.5
21	55.9	56.2	54.9	54.7	157.4	188.6
N(1)–Me	29.1	29.6	34.0	34.2	29.0	29.0
N(4)–Me	–	–	–	–	24.9	24.9
10-OMe	–	56.0	–	55.8	–	–
CO_2Me	–	–	51.5	51.5	–	–
CO_2Me	–	–	172.9	172.6	–	–



1 R = H
4 R = OMe



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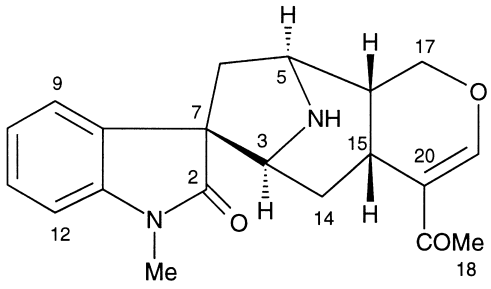

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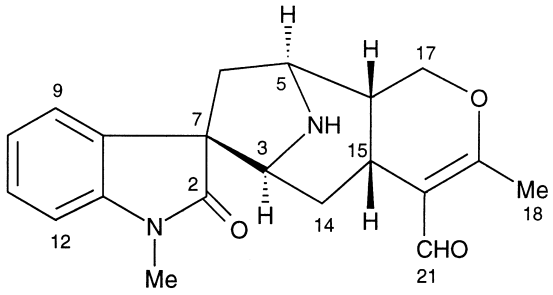

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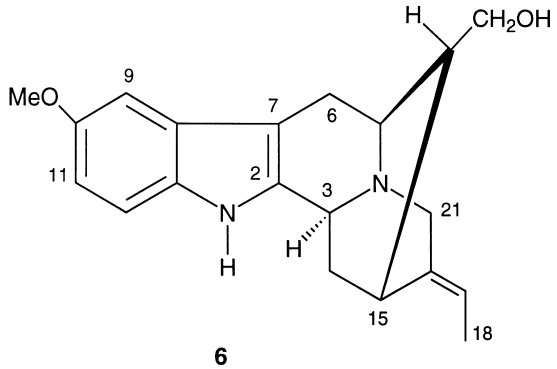

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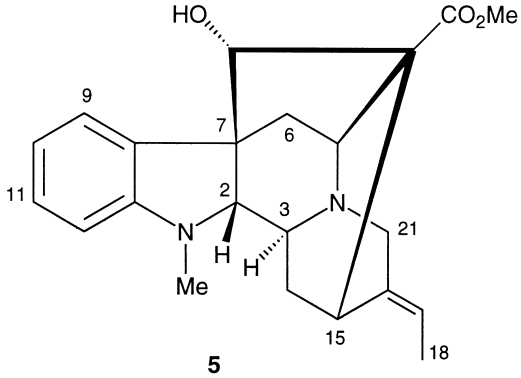

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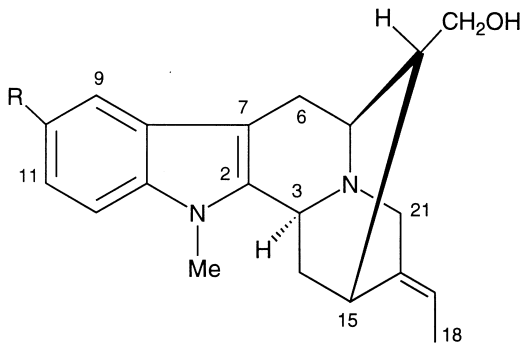
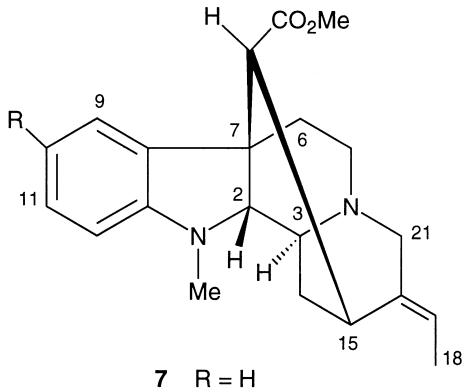
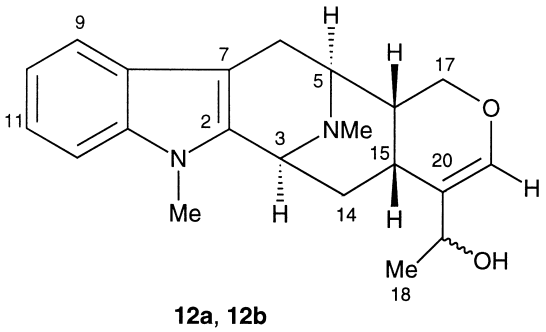
times escape detection. A mixture (ca. 3.5:1) of alstonerine **1** and alstonerinal **10**, when subjected to $NaBH_4$ reduction, yielded a mixture of three products, viz. the epimeric secondary alcohols **12a** and **12b**, from reduction of alstonerine, **1**, and the primary alcohol, **13**, from reduction of alstonerinal. All three products can be separated and characterized (see Section 3). The primary alcohol product **13**, from reduction of alstonerinal is distinguished by the presence of a characteristic AB doublet at δ 3.94 and 3.85 ($J = 12$ Hz), due to the hydroxymethyl protons (δ_C 66.6). Oxidation of **13** (PCC, CH_2Cl_2, 30°C, 3 h) regenerated the pure aldehyde, alstonerinal **10** (yield 46%). In common with many other *Alstonia*, the stem-bark alkaloids of the Malayan *A. macrophylla* showed a preponderance of the macroline unit, with the presence also of sarpagine- and akuammiline-type indole alkaloids.



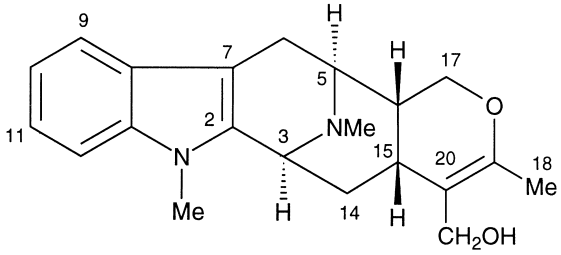
11 R = H
8 R = OMe



7 R = H
9 R = OMe



12a, 12b



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3. Experimental

3.1. Plant material

Stem-bark of *Alstonia macrophylla* Wall. was collected in the Genting Highlands area, Selangor, Malaysia and was identified by Dr. K.M. Wong of the Department of Botany, University of Malaya. Herbarium voucher specimens are deposited at the Herbarium of the Department of Chemistry, University of Malaya.

3.2. Extraction and isolation

The extraction of alkaloids was carried out in the usual manner which has been described in detail elsewhere (Kam & Tan, 1990). Essentially, the ground stem material was exhaustively extracted with 95% EtOH at ambient temperature. The EtOH extract was then concentrated under reduced pressure, partitioned into dilute HCl, basified with concentrated ammonia solution and liberated alkaloids were then taken into chloroform to give a basic fraction containing a mixture of alkaloids. The alkaloids were isolated by repeated fractionation using CC and centrifugal TLC on SiO_2. The solvent system used for CC was $CHCl_3$–MeOH. The solvent systems used for centrifugal TLC were hexane–Et_2O, hexane–$CHCl_3$, $CHCl_3$, $CHCl_3$–MeOH, EtOAc–Et_2O and EtOAc–MeOH. The yields (g kg^{-1}) of the alkaloids from the stem-bark extract were: **1** (0.088), **2** (0.035), **3** (0.012), **4** (0.024), **5** (0.035), **6** (0.024), **7** (0.008), **8** (2.88), **9** (0.005) and **10** (0.03).

3.3. 10-Methoxyaffinisine *8*

Needles from MeOH, m.p. 205–206°C, $[\alpha]_D = 75°$ ($CHCl_3$, *c* 0.62). UV (EtOH), λ_{max} nm ($\log \varepsilon$): 223 (4.25), 283 (4.02) and 300 (3.91). EIMS (probe) 70 eV, *m/z* (rel. int.): 338 [M^+] (69), 307 (24), 279 (13), 212 (30), 167 (14), 149 (28) and 83 (49). HREIMS, [M^+], found 338.1992, calcd for $C_{21}H_{26}N_2O_2$, 338.1994. ^1H and ^{13}C NMR: see Tables 1 and 2.

3.4. 10-Methoxycathafoline *9*

$[\alpha]_D = -57°$ ($CHCl_3$, *c* 0.08). UV (EtOH), λ_{max} nm ($\log \varepsilon$): 204 (4.23), 249 (3.74) and 315 (3.88). API-

LCMS, MH^+, m/z 369. EIMS (probe) 70 eV, m/z (rel. int.): 368 [M^+] (100), 337 (7), 295 (10), 232 (24), 194 (81), 174 (62), 154 (8) and 139 (24). HREIMS, [M^+], found 368.2105, calcd for $C_{22}H_{28}N_2O_3$, 368.2100. ^1H and ^{13}C NMR: see Tables 1 and 2.

3.5. Alstonerinal **10**

$[\alpha]_D = -32^\circ$ ($CHCl_3$, c 0.03). UV (EtOH), λ_{max} nm ($\log \varepsilon$): 228 (4.30), 267 (4.00) and 291 (3.80). API-LCMS, MH^+, m/z 337 ($C_{21}H_{24}N_2O_2 + H$). ^1H and ^{13}C NMR: see Tables 1 and 2.

3.6. Reduction of **1** and **10**

To a mixture (ca. 3.5:1) of compound **1** and **10** (60 mg) in MeOH was added $NaBH_4$ (73 mg). After 3 h at 30°C, excess solvent was removed under reduced pressure. Saturated NH_4Cl solution (10 ml) was added and the mixture extracted with chloroform. The extract was dried (Na_2SO_4) and then chromatographed (SiO_2, centrifugal TLC, Et_2O; $CHCl_3$–NH_3) to give **12a** (15 mg), **12b** (7 mg), **13** (6 mg) and unreacted **1** (16 mg).

3.7. Compound **12a** (major epimer)

$[\alpha]_D = -124^\circ$ ($CHCl_3$, c 0.15). UV (EtOH), λ_{max} nm ($\log \varepsilon$): 232 (4.14), 286 (3.73) and 293 (3.69). API-LCMS, MH^+, m/z 339 ($C_{21}H_{26}N_2O_2 + H$). ^1H NMR (400 MHz, $CDCl_3$): δ 1.07 (3H, d, $J = 6.5$ Hz, H-18), 1.93 (1H, m, H-16), 2.06 (2H, m, 2 × H-14), 2.23 (1H, dt, $J = 11$, 5.5 Hz, H-15), 2.32 (3H, s, N(4)–Me), 2.51 (1H, d, $J = 16.5$ Hz, H-6), 3.10 (1H, d, $J = 7$ Hz, H-5), 3.32 (1H, dd, $J = 16.5$, 7 Hz, H-6), 3.64 (3H, s, N(1)–Me), 3.90 (1H, t, $J = 3$ Hz, H-3), 4.00 (1H, ddd, $J = 11$, 4, 2 Hz, H-17), 4.11 (1H, q, $J = 6.5$ Hz, H-19), 4.27 (1H, t, $J = 11$ Hz, H-17), 6.46 (1H, s, H-21), 7.11 (1H, td, $J = 8$, 1 Hz, H-10), 7.21 (1H, td, $J = 8$, 1 Hz, H-11), 7.32 (1H, brd, $J = 8$ Hz, H-12), and 7.51 (1H, brd, $J = 8$ Hz, H-9). ^{13}C NMR (100 MHz, $CDCl_3$): δ 141.8 (C-21), 137.1 (C-13), 133.4 (C-2), 126.6 (C-8), 120.8 (C-11), 119.5 (C-20), 118.8 (C-10), 118.0 (C-9), 108.9 (C-12), 106.2 (C-7), 68.5 (C-19), 66.7 (C-17), 55.1 (C-5), 53.6 (C-3), 41.8 (N(4)–Me), 40.0 (C-16), 34.2 (C-14), 29.1 (N(1)–Me), 23.9 (C-15), 22.9 (C-6), and 21.3 (C-18).

3.8. Compound **12b**

$[\alpha]_D = -107^\circ$ ($CHCl_3$, c 0.07). UV (EtOH), λ_{max} nm ($\log \varepsilon$): 229 (3.95), 286 (3.29) and 294 (3.26). API-LCMS, MH^+, m/z 339 ($C_{21}H_{26}N_2O_2 + H$). ^1H NMR (400 MHz, $CDCl_3$): δ 1.15 (3H, d, $J = 6.5$ Hz, H-18), 1.95 (1H, m, H-16), 2.06 (3H, m, 2 × H-14, H-15), 2.33 (3H, s, N(4)–Me), 2.51 (1H, d, $J = 16.5$ Hz, H-6), 3.10 (1H, d, $J = 7$ Hz, H-5), 3.32 (1H, dd, $J = 16.5$, 7 Hz, H-6), 3.64 (3H, s, N(1)-Me), 3.91 (1H, brs, H-3), 3.99 (1H, ddd, $J = 11$, 4, 2 Hz, H-17), 4.02 (1H, q, $J = 6.5$ Hz, H-19), 4.31 (1H, t, $J = 11$ Hz, H-17), 6.49 (1H, s, H-21), 7.11 (1H, td, $J = 8$, 1 Hz, H-10), 7.21 (1H, td, $J = 8$, 1 Hz, H-11), 7.32 (1H, brd, $J = 8$ Hz, H-12) and 7.50 (1H, brd, $J = 8$ Hz, H-9). ^{13}C NMR (100 MHz, $CDCl_3$): δ 141.1 (C-21), 137.1 (C-13), 133.3 (C-2), 126.6 (C-8), 120.9 (C-11), 119.5 (C-20), 118.8 (C-10), 118.0 (C-9), 108.9 (C-12), 106.2 (C-7), 68.8 (C-19), 66.6 (C-17), 55.1 (C-5), 53.6 (C-3), 41.8 (N(4)–Me), 40.1 (C-16), 34.2 (C-14), 29.1 (N(1)–Me), 25.6 (C-15), 23.0 (C-6) and 22.8 (C-18).

3.9. Compound **13**

$[\alpha]_D = -155^\circ$ ($CHCl_3$, c 0.06). UV (EtOH), λ_{max} nm ($\log \varepsilon$): 235 (4.12), 285 (3.77) and 294 (3.74). API-LCMS, MH^+, m/z 339 ($C_{21}H_{26}N_2O_2 + H$). ^1H NMR (400 MHz, $CDCl_3$): δ 1.83 (3H, s, H-18), 1.96 (3H, m, 2 × H-14, H-16), 2.08 (1H, dt, $J = 11$. 5.5 Hz, H-15), 2.33 (3H, s, N(4)–Me), 2.49 (1H, d, $J = 16.5$ Hz, H-6), 3.09 (1H, d, $J = 7$ Hz, H-5), 3.30 (1H, dd, $J = 16.5$, 7 Hz, H-6), 3.64 (3H, s, N(1)–Me), 3.85 (1H, d, $J = 12$ Hz, H-21), 3.91 (1H, m, H-3), 3.94 (1H, d, $J = 12$ Hz, H-21), 3.99 (1H, ddd, $J = 11$, 4, 2 Hz, H-17), 4.24 (1H, t, $J = 11$ Hz, H-17), 7.11 (1H, td, $J = 8$, 1 Hz, H-10), 7.20 (1H, td, $J = 8$, 1 Hz, H-11), 7.31 (1H, brd, $J = 8$ Hz, H-12) and 7.49 (1H, brd, $J = 8$ Hz, H-9). ^{13}C NMR (100 MHz, $CDCl_3$): δ 150.5 (C-19), 137.5 (C-13), 133.4 (C-2), 127.2 (C-8), 120.9 (C-11), 118.9 (C-10), 118.0 (C-9), 109.9 (C-20), 108.9 (C-12), 106.2 (C-7), 66.6 (C-21), 62.0 (C-17), 55.1 (C-5), 53.7 (C-3), 41.8 (N(4)–Me), 40.6 (C-16), 33.5 (C-14), 29.1 (N(1)–Me), 27.0 (C-15), 22.9 (C-6) and 16.3 (C-18).

Acknowledgements

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References

Abe, F., Yamauchi, T., & Padolina, W. G. (1994). *Phytochemistry*, *35*, 253.

Abe, F., Yamauchi, T., & Santisuk, T. (1994). *Phytochemistry*, *35*, 249.

Atta-ur-Rahman, Nighat, F., Nelofer, A., Zaman, K., Choudhary, M. I., & De Silva, K. T. D. (1991). *Tetrahedron*, *47*, 3129.

Atta-ur-Rahman, Nighat, F., Sultana, A., & De Silva, K. T. D. (1994). *Natural Product Letters*, *5*, 201.

Atta-ur-Rahman, Qureshi, M. M., Muzaffar, A., & De Silva, K. T. D. (1988). *Heterocycles*, *27*, 725.

Atta-ur-Rahman, Silva, W. S. J., Alvi, K. A., & De Silva, K. T. D. (1987). *Phytochemistry*, *26*, 865.

Burkhill, I. M. (1966). *A dictionary of economic products of the Malay Peninsula*. Kuala Lumpur: Ministry of Agriculture and Co-operatives.

Clivio, P., Richard, B., Deverre, J. R., Sevenet, T., Zeches, M., & Le Men-Olivier, L. (1991). *Phytochemistry*, *30*, 3785.

Das, B. C., Cosson, J. P., & Lukacs, G. (1977). *Journal of Organic Chemistry*, *42*, 2785.

Ghedira, K., Zeches-Hanrot, M., Richard, B., Massiot, G., Le Men-Olivier, L., Sevenet, T., & Goh, S. H. (1988). *Phytochemistry*, *27*, 3955.

Kam, T. S., Jayashankar, R., Sim, K. M., & Yoganathan, K. (1997). *Tetrahedron Letters*, *38*, 477.

Kam, T. S., Nyeoh, K. T., Sim, K. M., & Yoganathan, K. (1997). *Phytochemistry*, *45*, 1303.

Kam, T. S., & Tan, P. S. (1990). *Phytochemistry*, *29*, 2321.

Keawpradub, N., & Houghton, P. J. (1997). *Phytochemistry*, *46*, 757.

Mors, W. B., Zaltzman, P., Beereboom, J. J., Pakrashi, S. C., & Djerassi, C. (1956). *Chemistry and Industry (London), 75*, 173.

Perry, L. M., & Metzger, J. (1980). *Medicinal plants of East and Southeast Asia*. Cambridge: MIT Press.

Ratnayake, C. K., Arambewela, L. S. R., De Silva, K. T. D., Atta-ur-Rahman, & Alvi, K. A. (1987). *Phytochemistry*, *26*, 868.

Wong, W. H., Lim, P. B., & Chuah, C. H. (1996). *Phytochemistry*, *41*, 313.