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Biologically active vallesamine, strychnan, and rhazinilam alkaloids from *Alstonia*: Pneumatophorine, a *nor-seco*vallesamine with unusual incorporation of a 3-ethylpyridine moiety

Jun-Lee Lim [a], Kae-Shin Sim [b], Kien-Thai Yong [b], Bi-Juin Loong [c], Kang-Nee Ting [c], Siew-Huah Lim [a], Yun-Yee Low [a], Toh-Seok Kam [a,*]

[a] Department of Chemistry, University of Malaya, 50603 Kuala Lumpur, Malaysia
[b] Institute of Biological Sciences, University of Malaya, 50603 Kuala Lumpur, Malaysia
[c] Department of Biomedical Sciences, University of Nottingham Malaysia Campus, Jalan Broga, 43500 Semenyih, Selangor, Malaysia

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ABSTRACT

Four alkaloids comprising two vallesamine, one strychnan, and one pyranopyridine alkaloid, in addition to 32 other known alkaloids were isolated from two Malayan *Alstonia* species, *Alstonia pneumatophora* and *Alstonia rostrata*. The structures of these alkaloids were determined using NMR and MS analyses, and in one instance, confirmed by X-ray diffraction analysis. The *nor*-6,7-*seco*vallesamine alkaloid, pneumatophorine, is notable for an unusual incorporation of a 3-ethylpyridine moiety in a monoterpenoid indole. The rhazinilam-type alkaloids (rhazinicine, *nor*-rhazinicine, rhazinal, and rhazinilam) showed strong cytotoxicity toward human KB, HCT-116, MDA-MB-231, and MRC-5 cells, while pneumatophorine, the uleine alkaloid undulifoline, and the strychnan alkaloids, N4-demethylalstogustine and echitamidine, induced concentration dependent relaxation in phenylephrine-precontracted rat aortic rings.

1. Introduction

Plants of the genus *Alstonia* are distributed over the tropical regions of Central America, Africa, and Asia (Markgraf, 1974; Sidiyasa, 1998). These plants are typically shrubs or trees and are usually rich in alkaloids (Kam, 1999; Kam and Choo, 2006). About seven species (local name *Pulai*) occur in Peninsular Malaysia (Middleton, 2011), some of which are used in traditional medicine (Burkill, 1966; Perry and Metzger, 1980). A number of the *Alstonia* bisindole alkaloids have been shown to possess antiproliferative and antimalarial properties (Kam et al., 2008; Keawpradub et al., 1999; Wright et al., 1993). As part of our systematic study of the Malaysian members (Ku et al., 2011; Lim et al., 2011, 2012, 2013; Tan et al., 2010a, 2014), we investigated the alkaloid content of two species, viz., *Alstonia pneumatophora* Backer *ex* Den Berger and *Alstonia rostrata* C.E.C. Fisch, and herein report the results. Plants belonging to the former species are usually encountered in swampy areas (Middleton, 2011).

2. Results and discussion

The basic fraction from the EtOH extract of *A. pneumatophora* yielded a total of 22 alkaloids, of which three (**1**, **4**, **5**) are new, while a total of 21 alkaloids were isolated from *A. rostrata*, of which one, the monoterpene alkaloid **6** is new (Fig. 1).

Compound **1** (pneumatophorine) was isolated as a light yellowish oil with $[\alpha]_D^{25}$ −41 ($CHCl_3$, *c* 0.57). The IR spectrum showed bands due to NH/OH (3380 cm^{-1}) and ester carbonyl functions (1725 cm^{-1}), while the UV spectrum showed characteristic indole absorptions at 231, 282, and 290 nm. The ESIMS showed an $[M+H]^+$ peak at *m*/*z* 434, the odd mass (M^+ *m*/*z* 433) indicating the presence of a third nitrogen. This was confirmed by ^{13}C NMR and HRESIMS data, which established the molecular formula as $C_{26}H_{31}N_3O_3$.

The ^1H (Table 1) and ^{13}C NMR (Table 2) spectra of **1** appeared complex and indicated the presence of two sets of signals with very similar or coincident chemical shifts, corresponding to the presence of two unresolvable components of nearly identical structure (**1a**:**1b** = 0.55:0.45 in $CDCl_3$). This was especially true for the ^{13}C NMR spectrum where the majority of the signals appear to occur in pairs with very similar chemical shifts. Thus of a total of

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

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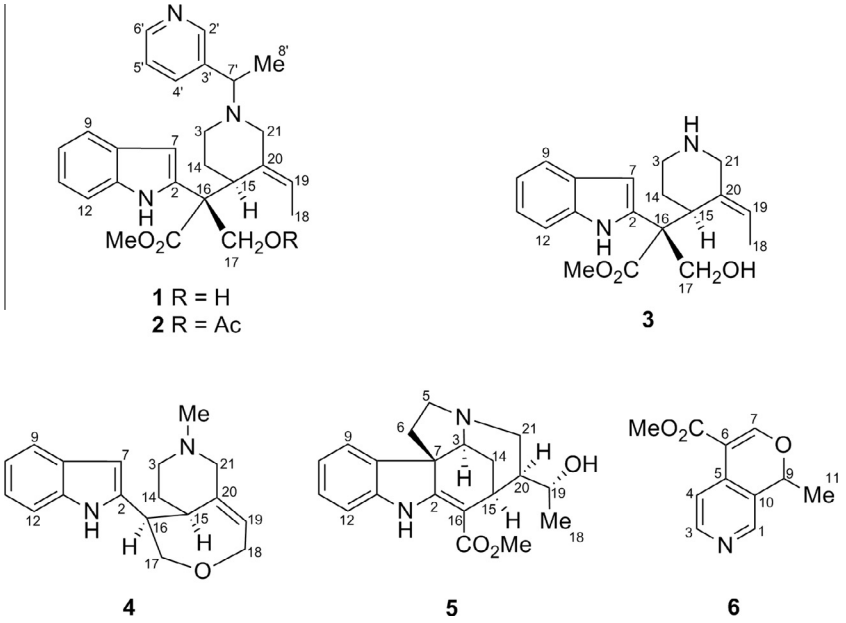

Fig. 1. Structures of compounds **1–6**.

26 carbons as required by the molecular formula, 24 of the resonances appeared as pairs with very similar chemical shifts (average $\Delta\nu$ for all paired ^{13}C signals is 0.11 ppm, Table 2) and were therefore indistinguishable, while three resonances were overlapped or coincident. In the ^{1}H NMR spectrum, 12 resonances were overlapped or coincident, while 12 resonances appeared as pairs with very similar chemical shifts (average $\Delta\nu$ for all paired ^{1}H signals is 0.05 ppm, Table 1). Assignment of the ^{1}H resonances into the two respective sets required spectra obtained at 600 MHz, combined with the application of DEPT and 2-D HSQC experiments.

The possibility that these components correspond to a pair of equilibrating conformers was ruled out based on the following observations. First, variable temperature NMR experiments showed that the spectrum remained unchanged when the temperature was raised (no evidence of any signal broadening up to 80 °C in toluene-d_8). In addition, the ratio of the two components as determined by ^{1}H NMR spectroscopy remained constant as the temperature was increased or lowered. Furthermore, the ratio of the two components was also essentially invariant when the spectra were obtained in different solvents (**1a**:**1b** = 0.55:0.45 in $CDCl_3$, CD_2Cl_2, toluene-d_8, benzene-d_6) (Garrido et al., 2003). Treatment of the mixture with Ac_2O/pyridine yielded a mixture of the corresponding *O*-acetyl derivatives (**2a**, **2b**), which also proved resistant to chromatographic resolution and which also occurred in essentially the same proportions (**2a**:**2b** ~ 6:4) as determined by ^{1}H NMR. These observations constituted strong evidence that the two components shown in the NMR spectrum do not correspond to a pair of equilibrating conformers, but represents a mixture of two diastereomers which could not be further resolved. Attempts to separate the two components by conventional column chromatography, TLC, radial chromatography, Sephadex LH20, and HPLC, including chiral-phase HPLC, were in every instance singularly unsuccessful. Similar behavior has also been previously documented with other alkaloids (Lim and Kam, 2009; Lim et al., 2009; Tan et al., 2012).

The ^{1}H NMR data of pneumatophorine (Table 1, **1a**) showed the presence of eight aromatic resonances (δ 7.08–8.39), an indolic NH (δ 9.88), a methyl ester group (δ 3.74), a hydroxymethyl group (δ 4.20, 4.40; d, J = 12 Hz), a CHMe (δ 1.26, 3.28), and an ethylidene side-chain (δ 1.64, 5.54). The ^{13}C NMR data (Table 2, **1a**) showed a total of 26 carbon resonances, comprising three methyl, four methylene, twelve methine, four quaternary, one ester carbonyl, and two tertiary carbons linked to the indolic nitrogen (corresponding to C-2 and C-13). The presence of a hydroxymethyl group was supported by the ready conversion of **1** to its *O*-acetyl derivative **2**. The NMR data of **2** also showed the same doubling of signals corresponding to the presence of two diastereomers (See Section 4).

Of the nine aromatic hydrogen resonances, four are contiguous as shown by the COSY spectrum and can be readily assigned to the aromatic hydrogens (H-9, H-10, H-11, H-12) of the indole moiety. The NOE observed between the aromatic doublet at δ 7.36 and the indolic NH at δ 9.88, facilitated the assignment of this doublet to H-12, and the doublet at δ 7.52 to H-9. Furthermore an NOE was observed between H-9 and the aromatic-H singlet at δ 6.48 (δ_C 101.94, Fig. 2), indicating that these hydrogens are proximate and suggesting that this relatively shielded aromatic H is due to H-7 of a 6,7-*seco* monoterpenoid indole (Tan et al., 2010b; Yamauchi et al., 1990; Zeches et al., 1987). This was further supported by the COSY spectrum, which showed long-range coupling between this hydrogen to the indolic NH. Of the remaining four aromatic resonances, three correspond to a CH–CH–CH fragment as shown by the COSY data (Fig. 3), while one corresponds to an isolated aromatic hydrogen. Since five additional aromatic carbon resonances were observed and the MS data required the presence of a third nitrogen, a 3-substituted pyridine moiety is suggested. The relatively deshielded aromatic resonance at δ 8.34 (δ_C 149.2) is characteristic of the α-hydrogen (α-carbon) of a pyridine moiety, likewise, the aromatic resonance at δ 8.39 (δ_C 148.6). These assignments were supported by the HMBC data (Fig. 3). The alkyl substituent at C-3′ of the pyridine moiety is readily deduced to be a $CHCH_3$ from the ^{1}H NMR spectrum. This group is attached to N-4 of the monoterpenoid indole as shown by the HMBC data (Fig. 3).

The identity of the monoterpenoid indole half of the alkaloid was deduced by linking the various substructures shown by the COSY spectrum with the help of the HMBC data (Fig. 3). The presence of an aromatic hydrogen at C-7 alluded to earlier suggested a *nor*-6,7-*seco*-vallesamine type alkaloid, of which a number occur in *Alstonia* (Tan et al., 2010b; Yamauchi et al., 1990; Zeches et al., 1987). The quaternary carbon at *ca.* δ_C 58 is characteristic of C-