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Alkaloids from the roots of Stemona javanica (Kunth) Engl. (Stemonaceae) and their anti-malarial, acetylcholinesterase inhibitory and cytotoxic activities

Rosdayati Alino Ramli
University of Wollongong, rar968@uowmail.edu.au

Pratiwi Pudjiastuti
Airlangga University

Tjitjik S. Tjahjandaric
Airlangga University

Wilford Lie
University of Wollongong, wilford@uow.edu.au

Roonglawan Rattanajak
BIOTEC, Thailand

See next page for additional authors

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Abstract
Two new protostemonine-type alkaloids, javastemonine A and B (3 and 4) have been isolated from the root extracts of Stemona javanica together with four known Stemona alkaloids, 13-demethoxy-11(S*),12(R*)-dihydroprotostemonine (1), isoprotostemonine (2), protostemonine and isomaistemonine. The structures and relative configurations of the new alkaloids were determined by spectroscopic analysis. The alkaloids 1 and 2 and protostemonine showed moderated antiplasmodial activities against the Plasmodium falciparum strains, TM4 (IC50 values of 17.7 ± 3.7, 16.8 ± 5.4, 16.0 ± 4.2 μg/mL, respectively) and K1 (IC50 values of 16.8 ± 3.1, 14.1 ± 3.7, 11.9 ± 3.3 μg/mL, respectively). These compounds showed no significant cytotoxicities against KB or Vero cells or acetylcholinesterase inhibitory activities.

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Authors
Rosdayati Alino Ramli, Pratiwi Pudjiastuti, Tjitjik S. Tjahjandaric, Wilford Lie, Roonglawan Rattanajak, Sumalee Kamchonwongapaisan, and Stephen G. Pyne

Alkaloids from the roots of *Stemona javanica* (Kunth) Engl. (Stemonaceae) and their anti-malarial, acetylcholinesterase inhibitory and cytotoxic activities

Rosdayati Alino Ramli[a,b], Pratiwi Pudjiastuti[c], Tjitjik S. Tjahjandaric[c], Wilford Lie[a], Roonglawan Rattanajak[d], Sumalee Kamchonwongapaisan[d] and Stephen G. Pyne[a,*]

[a]*School of Chemistry, University of Wollongong, Wollongong, New South Wales 2522, Australia*

[b]*Faculty of Science and Techonology, Islamic Science University of Malaysia 71800 Nilai, Malaysia*

[c]*Department of Chemistry, Faculty of Science and Technology, Airlangga University, Surabaya, Indonesia*

[d]*Medical Molecular Biology Research Unit, National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, Thailand Science Park, Pathumthani, 12120, Thailand.*

**Corresponding author: Tel: +61-02-4221 3511; fax: +61-02-4221 4287*

E-mail address:spyne@uow.edu.au

ABSTRACT

Two new protostemonine-type alkaloids, javastemonine A and B (**3** and **4**) have been isolated from the root extracts of *Stemona javanica* together with four known *Stemona* alkaloids, 13-demethoxy-11(*S**),12(*R**)-dihydroprotostemonine (**1**), isoprotostemonine (**2**), protostemonine and isomaistemonine. The structures and relative configurations of the new alkaloids were determined by spectroscopic analysis. The alkaloids **1** and **2** and protostemonine showed moderated antiplasmodial activities against the *Plasmodium falciparum* strains, TM4 (IC_{50} values of 17.7 ± 3.7, 16.8 ± 5.4, 16.0 ± 4.2 μg/mL, respectively) and K1 (IC_{50} values of 16.8 ± 3.1, 14.1 ± 3.7, 11.9 ± 3.3 μg/mL, respectively). These compounds showed no significant cytotoxicities against KB or Vero cells or acetylcholinesterase inhibitory activities.

Keywords: *Stemona javanica*, javastemonine A and B, anti-malarial, human acetylcholinesterase, cytotoxicity.

1. Introduction

The Stemonaceae family comprises three genera, *Stemona*, *Croomia* and *Stichoneuron* (Inthachub et al., 2009). Extracts of the roots of the *Stemona* species have been used in traditional medicine to treat the symptoms of bronchitis, pertussis and tuberculosis and as anti-parasitics on humans and animals (Greger, 2006; Kongkiatpaiboon, 2001, Pilli et al., 2010, 2005). More than 190 unique *Stemona* alkaloids have been isolated, some of these have significant antitussive activity (Chung et al., 2003; Lin et al., 2008a, 2008b, 2006; Xu et al., 2010, 2006; Yang et al., 2009; Zhou et al., 2009;), as well as insect toxicity, antifeedant, and repellent activities (Brem et al., 2002; Jiwajinda et al., 2001; Kaltenegger et al., 2003; Kongkiatpaiboon et al., 2013, 2012; Mungkornasawakul et al., 2009, 2004a; Phattharaphan et al., 2010; Sanguanpong and Hummel, 2008; Sakata et al., 1978; Tang et al., 2008). These latter properties are most likely associated with the ability of these alkaloids to inhibit insect acetylcholinesterase (AChE) (Brem et al., 2002; Jiwajinda et al., 2001; Kaltenegger et al., 2003; Kongkiatpaiboon et al, 2013, 2012; Mungkornasawakul et al., 2009, 2004; Phattharaphan et al., 2010; Sanguanpong and Hummel 2008; Sakata et al., 1978; Sastraruji et al., 2012; Tang et al., 2008). While other alkaloids have shown oxytocin antagonism (Phuwapraisirisan et al., 2006), nitric oxide inhibition (Hosoya et al., 2011) and the ability to inhibit P-glycoprotein in multi-drug-resistant cancer cell lines (Chanmahasathien et al., 2011a, 2011b; Limtrakul et al., 2007; Umsumarng et al., 2013). In the present paper, we describe the results of our phytochemical investigation of the roots of *S. javanica* collected from Alas Purwo in Indonesia.

2. Results and discussion

The roots of *S. javanica* were collected from Alas Purwo, East of Java, Indonesia in June and December 2012. Successive purification of the crude MeOH extract (35.2 g) by column chromatography gave pure samples of 13-demethoxy-11(*S**),12(*R**)-dihydroprotostemonine (**1**) (Tang et al., 2008), protostemonine (Kaltenegger et al., 2003), isoprotostemonine (**2**) (Ye et al., 1994), isomaistemonine (Guo et al., 2008), and two new *Stemona* alkaloids, javastemonine A (**3**) and javastemonine B (**4**) (Figure 1).

Compounds **3** and **4** are new compounds; we have named these alkaloids based on their botanical origins. Compound **3** was obtained as a brown gum. Its molecular formula was determined as $C_{22}H_{32}NO_5$ from its HRESIMS (*m/z* 390.2280 $[M+H]^+$, calc. for 390.2291). The IR spectrum of **3** showed characteristic bands at 1771 cm^{-1} and at 1761 and 1657 cm^{-1} for a saturated and an unsaturated γ-lactone, respectively. The ^{13}C/DEPT NMR spectrum displayed resonances for three methyl [δ 15.9 (C-17), 15.1 (C-22) and 11.0 (C-16)], six methylene [δ 48.0 (C-5), 35.3 (C-7), 34.9 (C-19), 26.9 (C-1), 26.8 (C-2), and 19.8 (C-6)], five methine [δ 65.1 (C-3), 59.7 (C-9a), 54.6 (C-9), 39.8 (C-10), and 35.1 (C-20)], four oxymethine [δ 83.5 (C-11), 82.6 (C-18), 80.7 (C-12), and 80.6 (C-8)], two olefinic [δ 146.4 (C-13) and 131.2 (C-14)] and two quaternary [δ 179.5 (C-21) and 174.5 (C-15)] carbons (Table 1). The H/H COSY correlations of **3** indicated the spin system H-1−H-2−H-3−H-18−H-19−H-20−H-22, typical of the pyrrolidine ring of the *Stemona* alkaloids, with a γ-lactone substituent at C-3 (Figure 2). Also observed were the COSY correlations between the vicinal pairs of contiguous protons along the C-5−C-13 and H-9−H-1 backbones and between H-10 and H-17 (Figure 2). The ^1H NMR spectrum indicated resonances for two different methyl groups, which were attached to methine carbons, with ^1H NMR resonances at δ 1.26 (d, J = 7.0 Hz, 3H, H-22) and δ 1.10 (d, J = 6.5 Hz, 3H, H-17), and a singlet methyl

resonance at δ 1.95 (s, 3H, H-16). Key HMBC correlations for **3** are shown in Figure 2, while full details are provided in Table 1. HSQC and HMBC experiments identified the protons and carbons (C-18−C-22) of the γ-butyrolactone moiety (Figure 2), which was clearly attached to C-3 from the HMBC correlation between H-3 (δ 3.23, ddd, 6.6, 7.0, 10.0) and C-18 (δ 82.6). HSQC and HMBC experiments further helped to identified the protons and carbons (C-11−C16) associated with the unsaturated γ-butyrolactone moiety of **3** (Figure 2). The ^{1}H NMR spectra of **1**, **3**, and the known alkaloid stemocochinine **5** (Kaltenegger, 2003; Tang et al., 2008) were nearly superimposable, except for small, but noticeable differences in the chemical shifts of some ^{1}H and ^{13}C NMR resonances. Notably, the ^{1}H NMR chemical shifts of H-9a and H-18 for these three compounds were significantly different [{**1**: H-9a (δ 3.63, ddd, J = 5.0, 5.6, 10.5) and H-18 (δ 4.14, ddd, J = 5.3, 7.5, 11.1)}; {**3**: H-9a (δ 3.77-3.75, overlap) and H-18 (δ 4.31, ddd, J = 5.5, 7.4, 12.8)}and {**5**: H-9a (δ 3.64, ddd, J = 9.8, 4.9, 4.9) and H-18 (δ 4.14, ddd, J = 11.2, 7.9, 5.3)}] (Kaltenegger, 2003; Tang et al., 2008). These differences suggested that **3** was a diastereomer of **1** and **5**. This assumption was also supported by their different chemical shifts for some ^{13}C NMR resonances [{**1**: C-11 (δ 85.0), C-12 (δ 83.1) and C-18 (δ 83.1)}; {**3**: C-11 (δ 83.5), C-12 (δ 80.7) and C-18 (δ 82.6)}and {**5**: C-11 (δ 83.4), C-12 (δ 80.5) and C-18 (δ 84.0)}] (Kaltenegger, 2003; Tang et al., 2008). The relative configuration of **3** was established from analysis of the ROESY NMR correlations of **3** and was further supported by molecular modelling experiments. TOCSY-type artifacts, which have the same phase as the diagonal peaks, were essentially eliminated (not observed) in these ROESY experiments (ROESY cross peaks have the opposite phase) (Bax, 1985). Compound **3** showed ROESY correlations between H-9a and H-1α, H-2α, H-3 and H-10 (Figure 3 and Table 1), indicating the mutal *syn*-stereochemical relationships between these five hydrogens. However, no ROESY correlations were observed between H-9a and H-9, or H-9a and H-17. These data suggested the α-configuration of the

proton at C-9a, which is opposite to that found in compounds **1** and **5**. ROESY correlations were also observed between H-1α and H-2α, H-3, H-5α, H-8, and H-10; and H-2α and H-3. These observations indicated the α-configuration of these protons. The ROESY correlations between H-18 and H-19β and H–20; H-19β and H-20; and H-19α and H-22 indicated the relative *syn*-configuration between H-18 and H-20 of the γ-lactone moiety (Figure 3). Because of the relatively free rotation around the C-3−C-18 bond in both possible diastereomeric structures for **3** ((3*S*, 18*S*, 20*S*)-**3** or (3*S*, 18*R*, 20*R*)-**3**), it was not possible to confidently assign the relative configurations of these two vicinal (C-3 and C-18) stereocentres from molecular modelling and ROESY NMR studies. However, we have tentatively assign the 3*S*, 18*S*, 20*S* configuration to **3** since this is the most commonly found absolute configuration of the *Stemona* alkaloids (Greger 2006; Kongkiatpaiboon, 2001; Pilli et al., 2010, 2005). The configurations assigned to C-11 and C-12 in **3** were based on ROESY correlations and the magnitude of $J_{11,12}$ when compared to the known *Stemona* alkaloids 11*S*,12*S*-saxorumamide (ROESY correlation between H-12 and H-17, and $J_{11,12}$ = 2.0 Hz) and 11*S*,12*R*-isosaxorumamide (ROESY correlation between H-10 and H-13, and $J_{11,12}$ = 6.9 Hz) (Wang et al., 2007) and related dihydrostemofoline alkaloids (Mungkornasawakul et al., 2004b). The relative small $J_{11,12}$ value for **3** (1.3 Hz) and the ROESY correlations between H-12 and H-17, as well as the correlation between H-11 and H-17, are consistent with the relative 11*S*,12*S* configurations assigned to compound **3**. Detailed analysis of the ^{1}H and ^{13}C-NMR spectra of **3**, as well as 2D-NMR analyses (COSY, HMBC and ROESY, Table 1) established the complete structure and relative configuration of **3**.

Compound **4** was obtained as a pale yellow gum. The molecular formula of **4** as $C_{23}H_{32}NO_6$ was determined from its HRESIMS (*m*/*z* 418.2230 $[M+H]^+$, calcd for $C_{23}H_{32}NO_6$ 418.2226). The IR spectrum of **4** showed characteristic signals at 1780 cm^{-1} and at 1745 and 1670 cm^{-1} for a saturated and an unsaturated γ-lactone ring, respectively. The ^{13}C/DEPT

NMR spectrum of **4** displayed resonances for three methyl [δ 8.7 (C-17), 14.6 (C-22), and 16.6 (C-16)], six methylene [δ 49.5 (C-5), 33.1 (C-7), 35.0 (C-19), 25.9 (C-2), 25.9 (C-1), and 17.8 (C-6)], five methine [δ 67.5 (C-3), 61.8 (C-9a), 50.9 (C-9), 41.7 (C-10), and 34.6 (C-20)], two oxymethine [δ 77.2 (C-18) and 82.7 (C-8)], four olefinic [δ 163.6 (C-13), 98.1 (C-14), 126.1 (C-12), and 149.2 (C-11)] and two quaternary [δ 178.3 (C-21) and 170.6 (C-15) carbons and a methoxy group [δ 59.5 (C-23)] (Table 1). The chemical shifts of these ^{13}C NMR signals were similar to these of the known *Stemona* alkaloids, protostemonine (the 11(*Z*) isomer of **2**) and isoprotostemonine (**2**) which were isolated previously from *S. japonica* (Ye et al., 1994). The COSY and HMBC spectroscopic analysis of **4** showed analogous correlations to those observed for **3**. The correlations in the COSY spectrum of **4** indicated the spin system H-1−H-2−H-3−H-18−H-19−H-20−H-22, typical of the pyrrolidine ring of the *Stemona* alkaloids with a γ-lactone substituent at C-3 (Figure 4). COSY correlations were also observed between the vicinal pairs of contiguous protons along the C-5−C-6−C-7−C-8−C-9−C-9a−C-1 backbone and between H-10 and H-17 (Figure 4). The ^{1}H NMR/COSY spectra indicated resonances for the presence of two methyl groups (δ 1.27 (d, J = 7.0 Hz, 3H, H-22), and 1.48 (d, J = 7.0 Hz, 3H, H-17), which were attached to methine groups with ^{1}H NMR resonances at δ 2.70 (ddq, J = 7.0, 7.5, 11.5 Hz, 1H, H-20), and δ 3.04 (ddd, J = 7.1, 7.1, 13.4, 1H, H-10), respectively. The HMBC experiment identified another methyl group at δ 2.02 (s, 3H) which was clearly attached to C-14 from the correlation between Me-16 and C-15. Key HMBC correlations are indicated in Figure 4, while full details are provided in Table 2. While compound **4** had the same molecular formula, and related structure features, to isoprotostemonine **2** and protostemonine there were notable differences in their NMR spectroscopic data (Wang et al., 2007). The aforementioned two known alkaloids had very similar ^{13}C NMR chemical shifts, while those of **4** were significantly different, especially those for C-3 (δ 64.4/64.3 vs δ 67.5 for **4**), C-5 (δ 46.6/46.5

vs δ 49.5 for **4**), C-9a (δ 58.4/58.8 vs δ 61.8 for **4**) and C-18 (δ 83.3/83.9 vs δ 77.2 for **4**). These chemical shift differences indicated that **4** was diastereomer of these known alkaloids. Compound **4** showed NOESY correlations between H-18 and H-19β; and H-19β and Me-22 (Figure 5 and Table 2), indicating the *syn*-relationship between H-18, H-19β and Me-22. ROESY correlations were also observed between H-9a and H-1β and H-9; H-1β and H-2β; H-2β and H-3; and H-9 and H-17, indicating the *syn*-relationship between these pairs of protons and the relative β-configuration of H-3. Because of the relatively free rotation around the C-3−C-18 bond in both possible diastereomeric structures for **4** [(3*R*, 18*S*, 20*R*)-**4** or (3*R*, 18*R*, 20*S*)-**4**], it was not possible to confidently assign the relative configurations of these two vicinal (C-3 and C-18) stereocentres from molecular modelling and NOESY NMR studies. However, we have tentatively assign the 3*R*, 18*S*, 20*R* configuration to **4** since the 18*S* configuration is the most commonly found absolute configuration of the *Stemona* alkaloids (Greger 2006; Kongkiatpaiboon, 2001; Pilli et al., 2010, 2005). The lack of a NOESY correlations between the OMe group and the protons on the C-ring was consistent the *E*-configuration of the C-11−C-12 alkene. These NOESY studies indicated that compound **4** was an epimer of **2** at C-3 and C-20 (see Table 2). Detailed analysis of the ^{1}H and ^{13}C-NMR spectra of **4**, as well as 2D-NMR analyses (COSY, HMBC and NOESY, Table 2) established the complete structure and relative configuration of **4**.

Compounds **1**–**4** and protostemonine were examined for their antiplasmodial, cytotoxicity and human acetylcholinesterase (hAChE) inhibitory activities (Table 3). Compounds **1**, **2** and protostemonine demonstrated moderate *in vitro* antiplasmodial activity against the *P. falciparum* strains, TM4 (a wild type chloroquine and antifolate sensitive strain) with IC_{50} values of 17.7μg/mL, 16.8 μg/mL and 16.0 μg/mL, respectively, and K1 (multidrug resistant strain) with IC_{50} values of 16.8 μg/mL, 14.1 μg/mL and 11.9 μg/mL, respectively. Compounds **3** and **4** did not show antiplasmodial activity even at the highest

tested concentration of 38.9-41.7 μg/mL. However, none of these isolated alkaloids showed mammalian cell line (KB and Vero cells) toxicity or human acetylcholinesterase inhibitory activity (Table 3).

3. Experimental

3.1 General experimental procedures

The IR spectra were recorded on a MIRacle 10 Shimadzu Spectrometer and optical rotations on a Jasco P-2000 polarimeter. The ESIMS and HRESIMS were recorded on a Micromass Platform LCZ and factory modified Waters QToF Ultima Mass Spectrometer (Wyntheshawe, UK). NMR spectra were recorded on a Varian-500 MHz NMR spectrometer. Silica gel was used for column chromatography and TLC was carried out on silica gel 60 GF254 plates Merck HX1 15287. The TLC spots were visualized by Dragendorff's reagent.

3.2 Plant material

The material was identified at the Conservation Institute of Purwodadi Botanical Garden, Pasuruan, East of Java, Indonesia, where a voucher specimen (No. IV.D.IV.7) was deposited.

3.3 Extraction and isolation

The dry, ground roots of *S. javanica* (1.3 kg) were extracted with 95% MeOH (4 × 1000 mL) over 3 days at room temperature. The MeOH solution was evaporated to give a dark residue (35.2 g). The residue was chromatographed on silica gel (200 mL) using gradient elution from CH_2Cl_2/MeOH (0:10) to CH_2Cl_2/MeOH (8:2). A total of 4 L of eluent was collected in test tubes of 200 mL. These fractions were pooled on the basis of TLC analysis to give three alkaloid fractions; A (2.2 g), B (5.2 g) and C (1.2 g). A portion of fraction A (100 mg) was further separated by preparative thin layer chromatography (PTLC) using *n*-hexane/EtOAc (1:9) as the eluent to give isomaistemonine (2.5 mg) and protostemonine (55.3 mg). Fraction B (2.5 g) was then separated by column chromatography using isocratic eluent (CH_2Cl_2/MeOH; 8:2) to give fraction B1 (507 g) and B2 (700 mg). A portion of fraction B1

(240 mg) was further purified by PTLC (*n*-hexane/EtOAc (9:1)) and gave 8.3 mg of pure 13-demethoxy-(11*S**,12*R**)-dihydroprotostemonine (**1**) and javastemonine A (**3**, 11.7 mg). Fraction B2 (700 mg) was chromatographed on silica gel (*n*-hexane/EtOAc (9:1)) to give isoprotostemonine (**2**, 30 mg). A portion of fraction C (130 mg) was further chromatograped by PTLC (*n*-hexane/EtOAc (9:1)) to give javastemonine B (**4**, 49.7 mg).

*3.3.1 Javastemonine A (**3**)*

Brown gum; $[\alpha]^{23}_{D}$ +35.5 (*c* 0.09, $CHCl_3$); IR film *v*max 3436, 2963, 2874, 1771, 1761, 1657, 1455, 1159, 1044, 726 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) see Table 1; HRESIMS *m*/*z* 390. 2280 $[M + H]^+$ (calcd for $C_{22}H_{32}NO_5$ 390.2291).

*3.3.2 Javastemonine B (**4**)*

Brown gum; $[\alpha]^{23}_{D}$ -45.2 (*c* 0.01, $CHCl_3$); IR film *v*max 2940, 1780, 1745, 1670, 1610, 1455, 1399, 1195, 1165, 1000 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) see Table 2; HRESIMS *m*/*z* 418.2230 $[M + H]^+$ (calcd for $C_{23}H_{32}NO_6$ 418.2226).

3.4 Bioassay methodology

3.4.1 Antiplasmodial assay

Compounds **1**−**4** and protostemonine were tested in vitro against a multidrug resistant K1CB1 strain and a wild type chloroquine and antifolate sensitive TM4/8.2 strain of *Plasmodium falciparum* using a previously described method (Trager and Jensen, 1976; Wangchuk et al., 2011; Desjardins et al., 1979; Kamchonwongpaisan et al., 2004).

3.4.2 Cytotoxicity assay

Cytotoxicity assays against normal Vero cells from kidney of African green monkey, *Cecopithecus aethiops* and human oral carcinoma KB cells were performed as previously described (Wangchuk et al., 2012).

3.4.3 hAChE inhibitory assays

hAChE inhibitory assays were performed as described by us previously (Ramli et al., 2013).

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Supplementary data

Copies of the ^{1}H and ^{13}C NMR spectra of compounds **3** and **4**, plus tables comparing the NMR spectroscopic data of **1**, **3** and **5**, and those of **4**, isoprotostemonine **2** and protostemonine.

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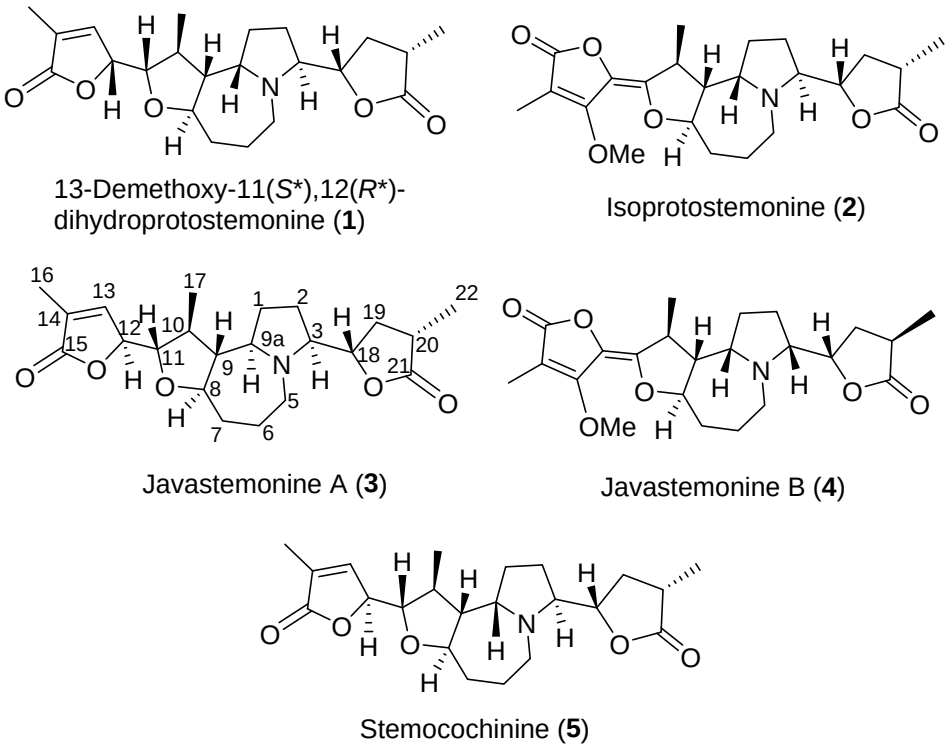


Fig. 1. Alkaloids (**1**−**4**) from *S. javanica* and stemocochinine (**5**)

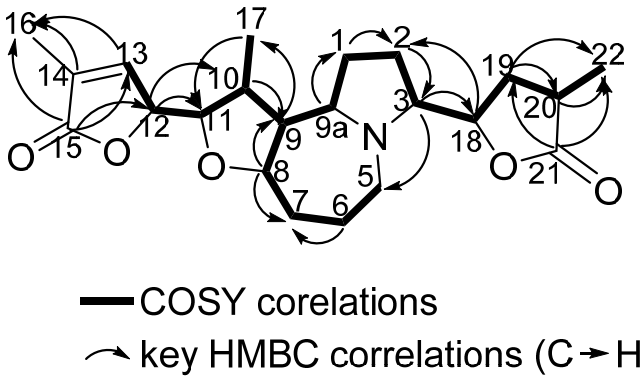


Fig. 2. Key COSY and HMBC correlations for compound **3**.

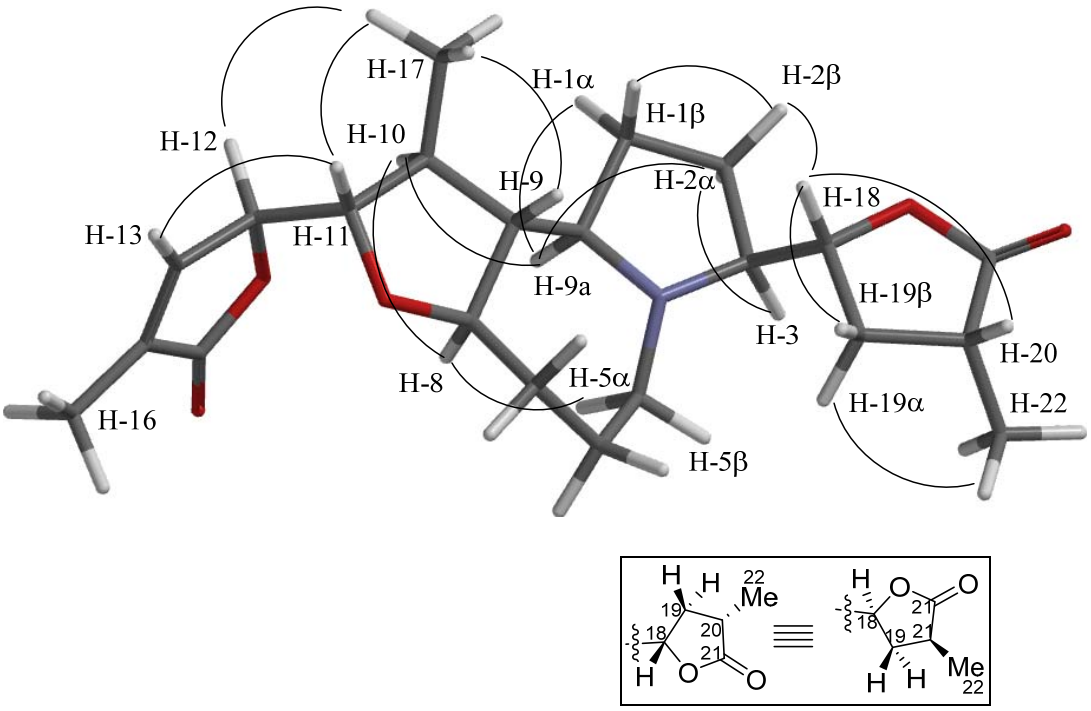


Fig. 3. Spartan '10 generated lowest energy conformation of **3** showing key ROESY cross-peaks. The structures shown here and in Figure 5 were generated using Spartan '10 and conformational searching (MMFF).

Table 1

^{13}C NMR (125 Hz), ^{1}H NMR (500 MHz) and 2D spectroscopic data for compound **3** in $CDCl_3$.

Position	δ_C (DEPT)	δ_H (mult., J (Hz)	HMBC	ROESY	COSY
1α	26.9	1.69-1.61 (overlap)	2, 3	2α, 3, 5α, 8, 9a, 10	2, 9a
1β		1.94-1.90 (overlap)		2β	
2α	26.8	1.91-185 (overlap)	1, 3	1α, 3, 9a, 10	1, 3
2β		1.50-1.42 (overlap)		1β, 18	
3	65.1	3.23 (ddd, 6.6, 7.0, 10.0)	1, 2, 5, 18	1α, 2α, 5β, 9a, 18, 19α	2, 18
5α	48.0	3.47 (dd, 15.3, 5.0)	3	1α, 5β, 8	6
5β		2.96 (dd, 15.3, 11.5)		3, 5α, 9	
6α	19.8	1.56-1.51 (overlap)	7	-	5, 7
6β		1.50-1.42 (overlap)		-	
7α	35.3	2.08 (dd, 3.0, 8.7)	5, 9	8	6, 8
7β		1.38-1.29 (m)		-	
8	80.6	3.83-3.79 (m, overlap)	7, 9a	1α, 5α ,7α, 10	7, 9
9	54.6	2.04-1.99 (m)	1, 17	5β, 11, 17	8, 9a, 10
9a	59.7	3.77-3.75 (overlap)	1, 2, 5	1α, 2α, 3, 10	1, 9
10	39.8	2.25-2.17 (m)	9, 17	1α, 2α, 8, 9a, 12, 17	9, 11, 17
11	83.5	3.82-3.78 (overlap)	7	9, 12, 13, 17	10, 12
12	80.7	4.90 (d, 1.3)	10, 11, 13	10, 11, 13, 17	11, 13
13	146.4	7.00 (br s)	16	11, 12, 16	12
14	131.2	-	16	-	-
15	174.5	-	13, 16	-	-
16	11.0	1.95 (s)		13	-
17	15.9	1.10 (d, 6.5)	11	9, 10, 11, 12	10
18	82.6	4.31 (ddd, 5.5, 7.4, 12.8)	2, 3, 19	3, 2β, 19β, 20	3, 19
19α	34.9	1.55-1.49 (m)	20, 22	3, 22	18, 20
19β		2.40 (ddd, 5.4, 8.3, 12.0)		18, 20	
20	35.1	2.66-2.58 (m)	19, 22	18, 19β, 22	19, 22
21	179.5	-	19, 22	-	-
22	15.1	1.26 (d, 7.0)	19, 20	19α, 20	20

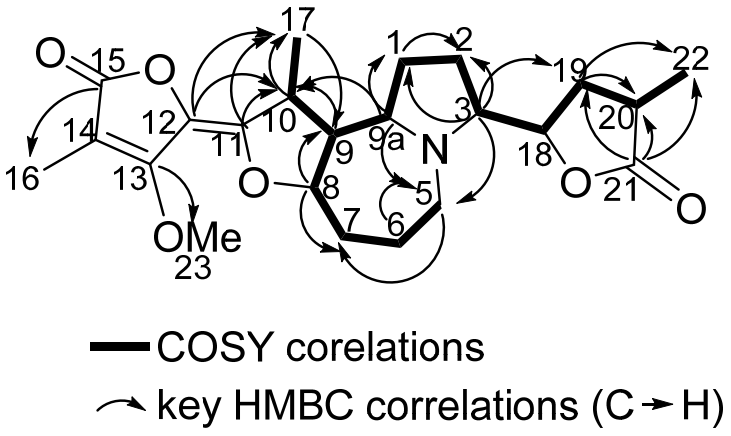


Fig. 4. Key COSY and HMBC correlations for compound **4**.

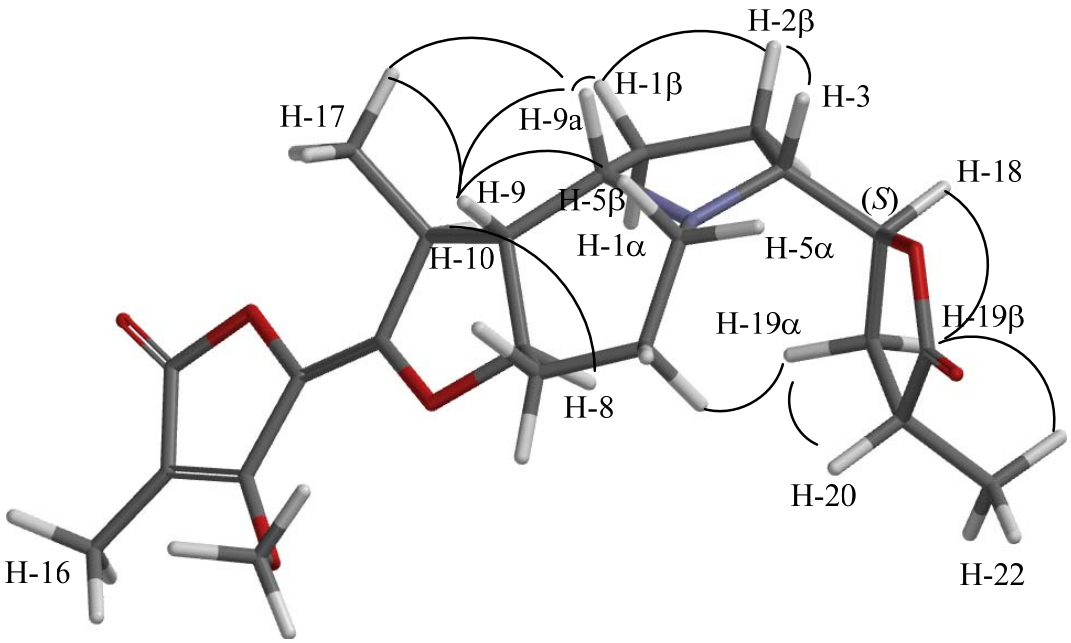


Fig. 5. Spartan '10 generated lowest energy conformation of **4** showing key ROESY cross-peaks.

Table 2

^{13}C NMR (125 Hz), ^{1}H NMR (500 MHz) and 2D spectroscopic data for compound **4** in $CDCl_3$.

Position	δ_C (DEPT)	δ_H (mult., J (Hz)	HMBC	ROESY	COSY
1α	25.9	1.92-1.86 (overlap)	2	1β, 2α, 8, 19α	2, 9a
1β		2.24-2.16 (overlap)		1α, 2β, 9a, 10	
2α	25.9	2.18-2.10 (overlap)	1	1α, 2β, 19α,10	1, 3
2β		1.86-1.80 (overlap)		1β, 2α, 3, 9a, 18,	
3	67.5	3.63 (m)	1, 2, 5, 19	2β, 19β	2, 18
5α	49.5	3.79 (dd, 6.0, 14.7)	7	5β, 6α, 18, 19β	6
5β		3.35 (dd, 12.5, 14.7)		5α, 5β, 6α, 6β, 9	
6α	17.8	1.86-1.82 (m)	5	5α, 6β, 7α, 8	5, 7
6β		1.98-1.92 (m)		5β, 6α, 7β	
7α	33.1	2.44 (dd, 3.7, 12.6)	5	6α, 7β, 8	6, 8
7β		1.66-1.58 (overlap)		6β, 7α, 9	
8	82.7	4.30 (ddd, 3.9, 11.0, 15.0)	7, 9	1α, 6α, 7α, 10	7, 9
9	50.9	2.29 (ddd, 4.0, 10.7, 14.6)	10	5β, 7β, 9a, 17	8, 9a
9a	61.8	4.36 (ddd, 6.2, 10.9, 11.1)	1, 2, 5, 9, 10	1β, 2β, 9, 17	1, 9
10	41.7	3.04 (ddd, 7.1, 7.1, 13.4)	9, 17	1β, 2α, 8, 17	9, 17
11	149.2	-	10, 17	-	-
12	126.1	-	10, 17	-	-
13	163.6	-	23	-	-
14	98.1	-	16	-	-
15	170.6	-	16	-	-
16	16.6	2.02 (s)	-	23	-
17	8.7	1.48 (d, 7.0)	9, 10	9, 9a, 10	10
18	77.2	5.03 (ddd, 6.4, 6.4, 9.7)	19	2β, 5α, 19β	3, 19
19α	35.0	2.54 (ddd, 5.6, 8.3, 12.6)	20, 22	1α, 2α, 19β, 20	18, 20
19β		1.64-1.54 (overlap)		3, 5α, 18, 19α, 22	
20	34.6	2.70 (ddq, 7.0, 7.5, 11.5)	19, 22	19α, 22	19, 22
21	178.3	-	19, 20, 22	-	-
22	14.6	1.27 (d, 7.0)	19, 20	19β, 20	20
23	59.5	4.11 (s)	-	16	-

Table 3

Antiplasmodial (IC_{50} in µg/mL), cytotoxicity and human acetylcholinesterase inhibitory activities of the alkaloids **1**-**4** and protostemonine.

Compounds	**Antiplasmodial (µg/mL)**		**Cytotoxicity (µg/mL)**		**hAChE (µM)**
	TM4	K1	VERO	KB	
13-Demethoxy-11(*S**),12(*R**)-dihydroprotostemonine (**1**)	17.7 ± 3.7	16.8 ± 3.1	> 38.9	> 38.9	>100
Protostemonine	16.8 ± 5.4	14.1 ± 3.7	> 41.7	> 41.7	>100
Isoprotostemonine (**2**)	16.0 ± 4.2	11.9 ± 3.3	> 41.7	>41.7	>100
Javastemonine A (**3**)	> 38.9	> 38.9	> 38.9	> 38.9	>100
Javastemonine B (**4**)	> 41.7	> 41.7	> 41.7	> 41.7	>100
Chloroquine[a]	0.010	0.089	-	-	-
Cycloguanil[a]	0.009	0.810	-	-	-
Pyrimethamine[a]	0.020	7.700	-	-	-
Ellipticine[b]	-	-	0.093	-	-
Doxorubicin[b]	-	-	-	0.56	-
Galanthamine[b]	-	-	-	-	0.55

[a]reference drugs for antiplasmodial activity.
[b]reference drugs for cytotoxicity activity.
[c]reference drugs for acetylcholinesterase activity.

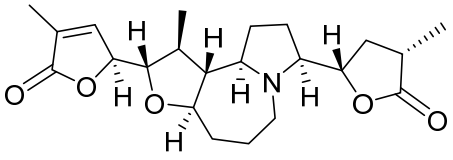
Graphical abstract

Alkaloids from the roots of *Stemona javanica* (Kunth) Engl. (Stemonaceae) and their anti-malarial, acetylcholinesterase inhibitory and cytotoxic activities

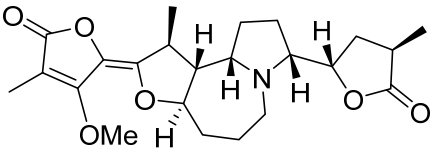
Rosdayati Alino Ramli, Pratiwi Pudjiastuti, Tjitjik S. Tjahjandaric, Wilford Lie, Roonglawan Rattanajak, Sumalee Kamchonwongapaisan and Stephen G. Pyne*

**Corresponding author: Tel: +61-02-4221 3511; fax: +61-02-4221 4287*

E-mail address:spyne@uow.edu.au



Javastemonine A (**3**)



Javastemonine B (**4**)

Highlights

- Two new alkaloids, javastemonine A and B, isolated from *Stemona javanica*.
- Structural elucidations were made using spectroscopic analysis.
- Javastemonine A was a C-9a epimer of stemocichinine.
- Javastemonine B was a C-3 epimer of isoprotostemonine.
- Three known alkaloids isolated were active against *Plasmodiyum falciparum*.