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Pratiwi Pudjiastuti
Airlangga University

Stephen G. Pyne
University of Wollongong, spyne@uow.edu.au

S Sugiyanto
Bureau of Cultivation and Plantation Protections, Ambon, Molucas

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

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Keywords
tuberosa, lour, croomine, novel, stemona, tuberospironine, derivative, isolation, CMMB

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Isolation of tuberospironine A, a novel croomine derivative from *Stemona tuberosa* Lour.

Pratiwi Pudjiastuti[a,*], Stephen G. Pyne[b,*] Sugiyanto[c], Wilford Lie[b]

[a]*Department of Chemistry, Airlangga University, Surabaya, Indonesia*

[b]*School of Chemistry, University of Wollongong, Wollongong, NSW, 2522, Australia*

[c]*Institute of Cultivation and Plantation Protections, Ambon, Moluccas, Indonesia*

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

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

Keywords:

Stemona alkaloid

Croomine

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ABSTRACT

A novel croomine derivative, tuberospironine A (3−*epi*−tuberospironine) was isolated from the root extracts of *Stemona tuberosa* Lour. found growing on Seram Island, Moluccas Province, Indonesia. The structure of this novel alkaloid, with unprecedented configuration at C-3 for a croomine derivative, was determined from interpretation of its NMR spectroscopic data.

1. Introduction

The *Stemona* family of alkaloids includes more than one hundred different natural products (Pilli et al., 2005; Greger, 2006) which have been structurally classified by Pilli into eight different groups. The pyrrolo[1,2-*a*]azepine nucleus is common to six of these groups,

while a pyrido[1,2-*a*]azepine ring system is found in the more recently discovered Stemocurtisine group of *Stemona* alkaloids (Pyne et al., 2007). A miscellaneous group comprising five *Stemona* alkaloids has also been identified (Pilli et al. 2005). Greger has recently classified the *Stemona* alkaloids into three skeletal types based on their proposed biosynthetic origins (Greger, 2006). The root extract of *Stemona tuberosa* Lour. has been extensively studied and has been shown to contain croomine and/or stichoneurine-type alkaloids (Greger, 2006) including, croomine **1** (Lin et al. 2008; Schinnerl et al., 2005, 2007), 6-hydroxycroomine **2** (Jiang et al., 2006a, Lin et al., 2008; Schinnerl et al., 2005, 2007), 10–hydroxycroomine **3** (Lin et al., 2008) and tuberospironine **4** (Jiang et al., 2006b) and tuberostemonine **5** (Götz et al. 1961; Hu, et al., 2009; Jiang et al., 2006; Lin et al., 2008; Schinnerl et al., 2005, 2007; Xu; Y. T. et al., 2010; Xu; R-S. et al. 1982) and tuberostemonine A **6** (Schinnerl et al.,2005, 2007), respectively (Figure 1). We report here the isolation of a novel croomine-like alkaloid, tuberospironine A **7** (3–*epi*–tuberospironine), from the root extracts of *Stemona tuberosa* Lour., which has the opposite configuration at C-3 and C-9 to that of croomine **1** itself (Figure 1) .

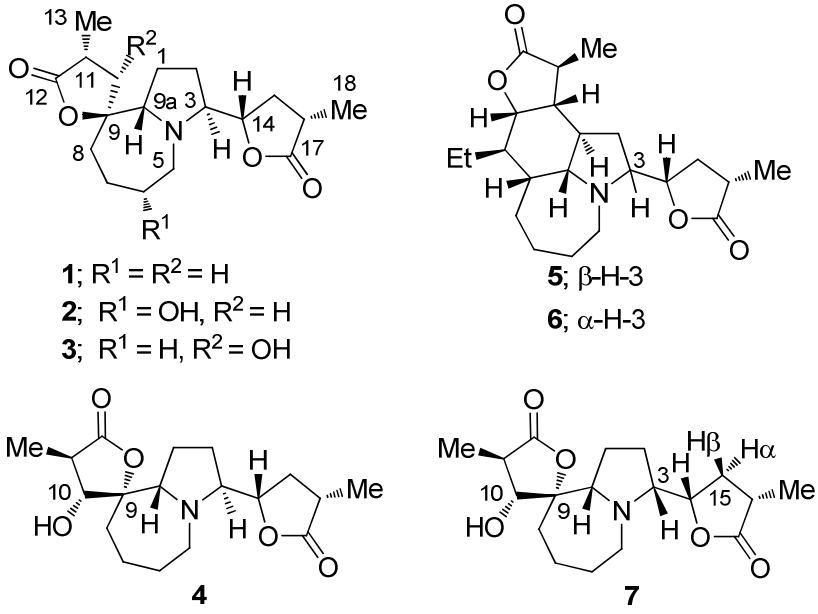

Figure 1. The structures of representative croomine and tuberostemonine alkaloids.

2. Results and Discussion

The roots of *Stemona tuberosa* Lour. were collected from Seram Island, Moluccas Province, Indonesia. The ethanol extract of the dried and powered roots was concentrated *in vacuo* and the resulting residue was acidified to pH 1-2 and then partitioned between water and CH_2Cl_2. The aqueous layer was made basic with ammonia and then extracted with CH_2Cl_2. The crude alkaloid mixture was subjected to successive separations by column chromatography and finally preparative TLC to give a pure sample of tuberospironine A **7**.

Compound **7** was obtained as a pale brown, amorphous solid. Its molecular formula was determined to be $C_{18}H_{27}NO_5$ from its HRESIMS, (*m/z* 338.1953 $[M+H]^+$), the same molecular formula as 10-hydroxycroomine **3** and tuberospironine **4** (Figure 1). The IR spectrum of **7** showed bands for the presence of a γ-lactone (1730 cm^{-1}) and a hydroxyl group (3420 cm^{-1}). The ^1H NMR spectrum of **7** (Table 1) showed a downfield resonance at δ 4.37 (m, 1H, H–14) and two methyl doublet signals (δ 1.27 (d, 3H, *J* = 7.0 Hz, H–18 and δ 1.29 (d, 3H, *J* = 7.5 Hz, H–13) typical resonances for the spirocyclic (at C–9) and the appended (at C–3) γ-lactone moieties of croomine-derived alkaloids. The ^1H NMR spectrum of **7** (Table 1) showed a doublet resonance at δ 3.95 (d, *J* = 10.5 Hz, H–10) diagnostic of a C-10 hydroxyl group. The chemical shift and coupling constant of H–10 were different to that of 10-hydroxycroomine **3** (δ 4.32 (d, *J* = 7.8 Hz, H–10) (Lin et al.) and tuberospironine **4** (δ 4.09 (d, *J* = 10.3 Hz, H–10) (Jiang et al.) but were closer in magnitude to those of tuberospironine **4** which has the opposite configuration at C-9 to the alkaloids **1**–**3** and at C-10 to 10-hydroxycroomine **3** (Figure 1). The ^1H, COSY, ^{13}C NMR and HSQC and HMBC spectra of **7** (Table 1) indicated that this compound was a diastereomer of **3** and **4**.

The relative configuration of **7** was determined from NOESY NMR experiments (Figure 2), with the absolute configuration at C-9a assumed to be the same as that of croomine **1**. The NOESY correlations between H–10 and H–9a and H–1α indicated that the C-9 configuration

of **7** was opposite to that of the alkaloids **1**-**3** and was the same as that of tuberospironine **4** (Figure 1). The NOESY correlation between H-10 and the C-11 methyl group (H-13) indicated their relative *syn*-relationship, as observed in tuberospironine **4**, which was further supported by the aforementioned similarity in magnitude of $J_{10,11}$ in **7** (10.5 Hz) and **4** (10.3 Hz). The NOESY correlations between H–3 and H–15α and H–14 and the two H-5 protons, and H–15β (Figure 2, see Figure 1 for the definition of 15α and 15β) indicated the β-configuration of H–3, opposite that found in all previous naturally occurring croomine-like alkaloids (Pilli et al., 2005; Greger, 2006). This C-3 configuration is rare and has been only found in two tuberostemonines, tuberostemonine A **6** (Schinnerl et al., 2005, 2007) and tuberostemonine M (Sastraruji, 2006).

In conclusion, we have isolated a novel 10-hydroxycroomine derivative from the root extracts of *Stemona tuberosa* Lour. which we have named tuberospironine A **7** based on its same C–9/C–9a relative configuration to tuberospironine **4**. The structure of this novel alkaloid, with unprecedented configuration at C–3 for a croomine derivative, was determined from interpretation of its NMR spectroscopic data.

Table 1 ^{13}C NMR (125 Hz) and ^{1}H NMR (500 MHz) spectroscopic data for compound **7** in $CDCl_3$.

Position	δ_C (DEPT)	δ_H (mult., *J* (Hz))	HMBC
1α	26.5 (CH_2)	1.97 (m)	H–2, H–3, H–9a
1β		1.73 (m)	
2α	26.6 (CH_2)	1.89 (m)	H–1, H–9a, H–14
2β		1.54 (m)	
3	66.2 (CH)	3.42 (dd, 7.5, 14.5)	H–5 (weak), H–9a (weak), H–14
5α	47.8 (CH_2)	3.09 (m)	H–3, H–6, H–9a
5β		3.30 (br d, 13.5)	
6a	26.9(CH_2)	1.72 (m)	H–5
6b		1.64 (m)	
7a	22.1 (CH_2)	1.77 (m)	H–8a
7b		1.69 (m)	
8a	30.3 (CH_2)	2.06 (m)	
8b		1.80 (m)-	
9	87.8 (C)	-	H–8, H–9a, H–10
9a	67.8 (CH)	3.58 (dd, 7.5, 8.0)	H–1, H–2, H–5, H–6, H–9, H–10
10	78.0 (CH)	3.95 (d, 10.5)	H–8, H–9, H–9a, H–11
11	40.2 (CH)	2.65 (m)	
12	175.5 (C)	-	H–13
13	12.7 (CH_3)	1.31 (d, 7.5)	H–12
14	79.4 (CH)	4.37 (m)	H–3
15α	35.0 (CH_2)	1.52 (m)	H–17
15β		2.39 (m)	
16	35.5 (CH)	2.61 (m)	
17	179.3 (C)	-	15β
18	14.9 (CH_3)	1.27 (d, 7.0)	,
OH	-	1.93 (br s)	

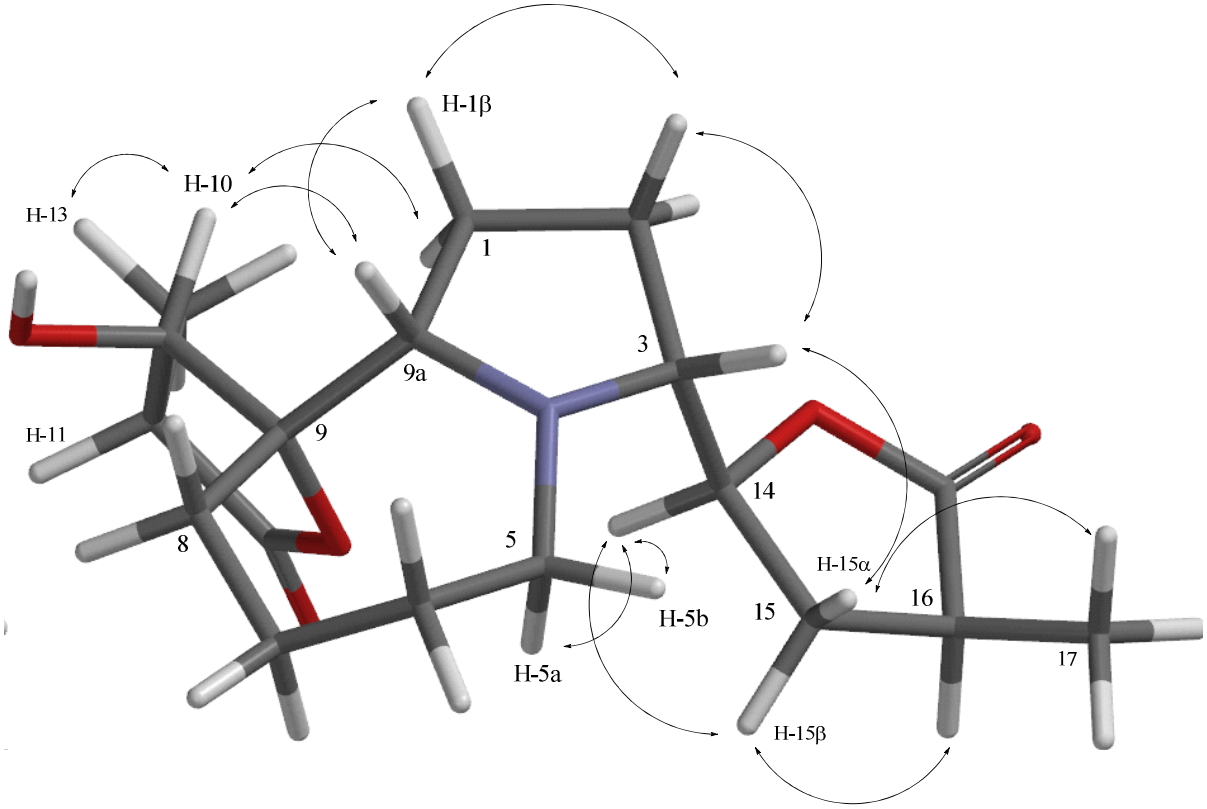

Figure 2. Selected NOESY correlations (shown as double headed arrows) for compound **7** based on a SPARTAN (AM3) generated structure.

3. Experimental

3.1 General Experimental Procedure

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 Micromass Platform LCZ and factory modified Waters QToF *Ultima* Mass Spectrometer (Wyntheshawe, UK). NMR spectra were recorded on Variant-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 viewed at 254 nm and visualized by Dragendorff's reagent.

3.2 Plant material

The roots of *S. tuberosa* Lour were collected from Seram Island, Moluccas Province, Indonesia in July 2011. 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.14) was deposited.

3.3 Extraction and isolation

The roots of *S. tuberosa* (3.0 kg) were dried and ground into a powder (840 g) and extracted with 95% ethanol three times for 12 days. The filtrate was evaporated under vacuum at 50^{o} C temperature, to give 148.0 g of crude extract. The crude extract was acidified with 5% of HCl until pH 1-2 and partitioned between H_2O and CH_2Cl_2. The aqueous fraction was basified with NH_4OH until pH 10-11 and extracted using CH_2Cl_2 to give 4.13 g of crude alkaloid extract. The crude alkaloid extract (4.0 g) was subjected to column chromatography over silica gel with gradient elution from CH_2Cl_2/MeOH (100:0) to CH_2Cl_2/MeOH (70:30) to give 27 fractions. Fractions 6-8 (1.864 g) were combined and separated by column chromatography and then preparative TLC to afford tuberospironine A **7** (13.8 mg).

3.4 Tuberospironine A **7**

Pale brown, amorphous, $[\alpha]_D^{24}$ -6.32 (*c* 0.005, $CHCl_3$); IR υ_{max} (cm^{-1}); 3420, 2924, 2861, 1730, 1453, and 1156; ^{1}H and ^{13}C NMR data are shown in Table 1; HRESIMS *m/z* 338.1953 $[M+H]^+$, calcd for $C_{18}H_{28}NO_5$ 338.1962.

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