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Structures of stemoxazolidinones A–F, alkaloids from *Stemona sessilifolia*

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ABSTRACT

Six new alkaloids, stemoxazolidinones A–F, were isolated from the roots of *Stemona sessilifolia* (Miq.) Miq. (Stemonaceae). The structures of stemoxazolidinones A–C, and E were determined by interpretation of their spectroscopic data and those of stemoxazolidinones D and F by X-ray crystallography. These alkaloids possess a novel structural unit in which an oxazolidin-2-one unit fuses with a pyrrolo[1,2-*a*]azepine nucleus of the rearranged or normal tuberostemonine-type skeleton.

1. Introduction

Plants of the genus *Stemona*, belonging to the Stemonaceae family, are distributed in Southeast Asia through Malaysia to North Australia,[1] and are known to produce alkaloids of unique structures, which are characterized by the presence of a pyrrolo[1,2-*a*]azepine nucleus.[2–4] In continuation of our phytochemical investigation of the roots of *Stemona sessilifolia* (Miq.) Miq.,[5–7] we isolated six novel *Stemona* alkaloids, stemoxazolidinones A–F (**1**–**6**). These alkaloids were shown to be characterized by having a unique structural unit in which an oxazolidin-2-one unit fused with a pyrrolo[1,2-*a*]azepine nucleus of the rearranged or normal tuberostemonine-type skeleton (Fig. 1). This paper describes their isolation and structure determination.

2. Results and discussion

A mixture of neutral and acidic components (300 g), obtained from a MeOH extract of the roots of *Stemona sessilifolia*,[5] was subjected to silica gel column chromatography. The fraction obtained by eluting the column with EtOAc–MeOH (10/1) was subjected to a series of chromatographic separations to give six new alkaloids, **1** (4.3 mg), **2** (2.5 mg), **3** (1.3 mg), **4** (7.3 mg), **5** (11.6 mg), and **6** (97.0 mg) (Fig. 1).

Stemoxazolidinone A (**1**) was obtained as an amorphous solid. Its molecular formula, $C_{24}H_{30}N_2O_7$, determined from the HRESIMS indicated that it was an alkaloid possessing two nitrogens in the molecule with eleven degrees of unsaturation. The IR spectrum indicated the presence of carbonyl groups (1760 cm^{-1}). Its ^1H NMR spectrum showed characteristic signals of one terminal methyl (δ_H

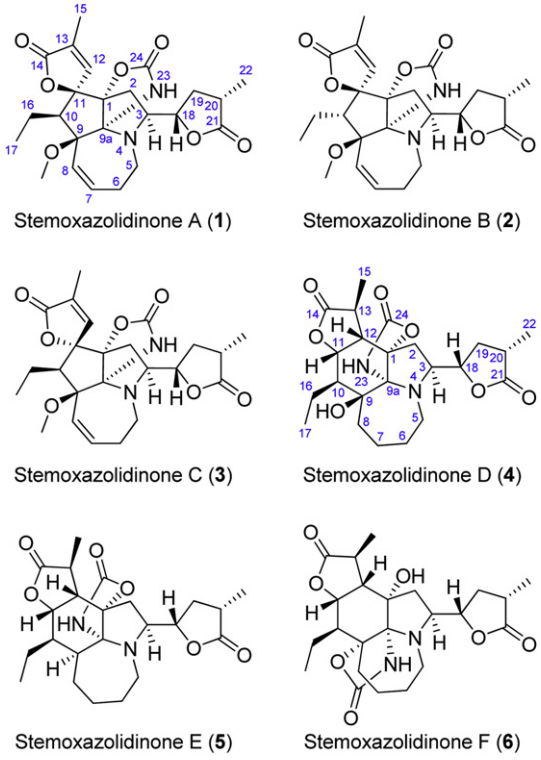

Fig. 1. Structures of stemoxazolidinones A–F (**1**–**6**).

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0.91), one secondary methyl (δ_H 1.25), one allylic methyl (δ_H 1.94) and one methoxy group (δ_H 3.38) and one oxymethine (δ_H 4.47) and three olefinic methine protons (δ_H 5.87, 6.21, and 7.08), and an amide-like NH proton (δ_H 5.80) (Table 1). Its ^{13}C NMR spectrum showed 24 signals caused by four methyls, five methylenes, seven methines, and eight quaternary carbons including three carbonyl carbons. Analysis of the $^1H-^1H$ COSY and HMQC spectra revealed the presence of three molecular fragments, i.e., a three-carbon chain fragment (C-10–C-16–C-17) in which C-17 was a terminal methyl, a *cis*-substituted homoallyl fragment (C-5–C-6–C-7–C-8, $J_{H\text{-}7,H\text{-}8}$=10.7 Hz) in which C-5 ($\delta_C$ 43.1) was a nitrogen-substituted methylene and C-7 (δ_C 136.3) and C-8 (δ_C 129.4) were olefinic methines, and a six-carbon chain fragment (C-2–C-3–C-18–C-19–C-20–C-22) in which C-3 (δ_C 67.9) was a nitrogen-substituted methine, C-18 (δ_C 81.3) was an oxymethine, and C-22 (δ_C 14.8) was a secondary methyl (Fig. 2).

The linkages between those carbon chain fragments and the quaternary carbons including three carbonyl carbons were deduced from the HMBC experiment. The correlations from H-3 (δ_H 3.35) to C-5 and from H-5a (δ_H 3.42) to C-3 suggested that C-5 of the homoallyl fragment and C-3 of the six-carbon chain fragment were connected via a nitrogen atom. Also, the correlations from H-2a (δ_H 2.11) and H-2b (δ_H 1.77) to C-1 (δ_C 98.7), from H-2b to C-9a (δ_C 93.3), from H-5a and H-5b (δ_H 2.73) to C-9a, from H-7 (δ_H 6.21) to C-9 (δ_C 84.0) and from H-8 (δ_H 5.87) to C-9 and C-9a revealed the presence of a carbon sequence, C-2–C-1–C-9a–C-9–C-8 with C-9a being connected to the nitrogen atom. Accordingly, alkaloid **1** was proved to incorporate a pyrrolo[1,2-*a*]azepine nucleus in the structure as characteristically observed in *Stemona* alkaloids. Correlations from H-2a to C-11 (δ_C 91.9), from H-10 (δ_H 2.59) to C-8, C-9, and C-11, and from H_2-16 (δ_H 1.68 and 1.37) to C-9 and C-11 suggested that C-1, C-9a, C-9, C-10, and C-11 constituted a cyclopentane ring and

Table 1
NMR data of stemoxazolidinones A–C (**1**–**3**) in $CDCl_3$[a]

Position	**1**		**2**		**3**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		98.7		100.3		93.5
2	a 2.11 (dd, 13.1, 10.4)	31.9	a 2.10 (dd, 13.0, 10.2)	32.0	a 1.92 (dd, 12.8, 4.2)	35.0
	b 1.77 (dd, 13.1, 4.7)		b 1.71 (dd, 13.0, 4.9)		b 1.56 (dd, 12.8, 10.6)	
3	3.35 (ddd, 10.4, 7.7, 4.7)	67.9	3.38 (ddd, 10.2, 7.8, 4.9)	68.4	3.28 (ddd, 10.6, 7.9, 4.2)	68.1
5	a 3.42 (dt, 12.2, 4.5)	43.1	a 3.43 (m)	43.5	a 3.51 (dt, 12.8, 4.2)	43.4
	b 2.73 (ddd, 12.9, 12.2, 1.8)		b 2.70 (m)		b 2.70 (td, 12.8, 2.2)	
6	a 2.52 (m)	29.1	a 2.66 (m)	28.6	a 2.45 (m)	29.0
	b 2.18 (m)		b 2.14 (m)		b 2.22 (m)	
7	6.21 (ddd, 10.7, 8.5, 4.0)	136.3	6.17 (m)	136.4	6.25 (ddd, 10.8, 9.0, 3.7)	136.8
8	5.87 (dd, 10.7, 2.8)	129.4	5.92 (dd, 10.9, 2.4)	128.7	5.85 (dd, 10.8, 3.1)	128.3
9		84.0		85.3		82.6
9a		93.3		94.1		92.2
10	2.59 (t, 6.7)	57.8	2.37 (dd, 9.3, 2.4)	58.8	2.90 (t, 7.1)	57.7
11		91.9		92.5		92.6
12	7.08 (q, 1.5)	149.1	7.32 (q, 1.5)	147.7	7.32 (d-like, 1.5)	148.6
13		130.3		131.1		130.3
14		173.4		172.6		172.1
15	1.94 (d, 1.5, 3H)	10.6	1.98 (d, 1.5, 3H)	10.7	1.96 (d, 1.5, 3H)	10.8
16	a 1.68 (m)	16.5	a 1.65 (m)	20.6	a 1.50 (m)	18.4
	b 1.37 (m)		b 1.55 (m)		b 1.28 (m)	
17	0.91 (t, 7.6, 3H)	14.0	0.94 (t, 7.4, 3H)	16.1	0.92 (t, 7.6, 3H)	12.8
18	4.47 (ddd, 10.9, 7.7, 5.4)	81.3	4.54 (ddd, 11.0, 7.8, 5.4)	81.0	4.39 (ddd, 11.0, 7.9, 5.5)	81.1
19	a 2.40 (ddd, 12.6, 8.4, 5.4)	34.8	a 2.39 (m)	34.8	a 2.38 (m)	34.9
	b 1.47 (ddd, 12.6, 12.3, 10.9)		b 1.45 (td, 12.5, 11.0)		b 1.51 (m)	
20	2.60 (m)	34.6	2.60 (m)	34.6	2.59 (m)	34.6
21		179.1		179.1		178.7
22	1.25 (d, 7.0, 3H)	14.8	1.24 (d, 7.0, 3H)	14.8	1.26 (d, 7.2, 3H)	14.8
24		155.8		155.6		156.1
NH	5.80 (s)		5.66 (s)		5.82 (s)	
OMe	3.38 (s, 3H)	53.7	3.16 (s, 3H)	50.3	3.39 (s, 3H)	53.9

[a] Recorded at 500 MHz for ^1H and 125 MHz for ^{13}C; *J*-values given in Hz in parentheses.

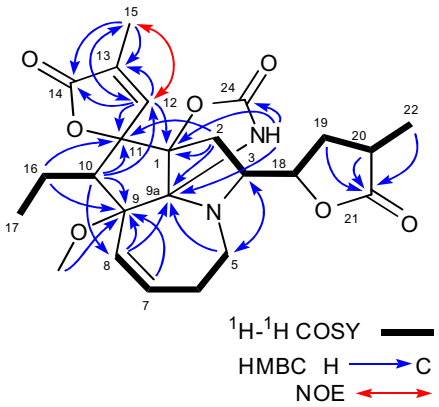

Fig. 2. Key $^1H-^1H$ COSY, HMBC, and NOE correlations of **1**.

a correlation from the methoxy protons to C-9 that the methoxy group was at C-9. The HMBC correlations from H-12 (δ_H 7.08) to C-1 and C-11 and from H-10 to C-12 indicated that C-12 was connected to C-11. The correlations from H-12 to C-13 (δ_C 130.3), C-14 (δ_C 173.4), and C-15 (δ_C 10.6) and from H_3-15 (δ_H 1.94) to C-12 (δ_C 149.1), C-13, and C-14 showed that C-12, C-13, C-14, and C-15 constituted an α-methylpropenoyl group. Since C-12/C-13 double bond had a *Z* configuration as implied by the NOE correlation between H-12 and H_3-15, **1** was concluded to have a lactone of a spiro structure with C-12 and O linking to C-11.

The carbonyl carbon resonated at δ_C 179.1 was assigned to C-21 by the correlations from H-19a (δ_H 2.40), H-20 (δ_H 2.60), and H_3-22 (δ_H 1.25) to C-21, forming a part of the γ-lactone often observed in the *Stemona* alkaloids.

The HMBC correlation from the NH proton to C-24 and the chemical shift value of C-24, δ_C 155.8, a characteristic value of a carbamoyl carbon, indicated the presence of a carbamoyl group in

1. The quaternary carbon C-9a, connecting to C-1, C-9, and N-4, resonated in very low-field, at δ_C 93.3, suggesting that the fourth atom to which C-9a linked was a nitrogen atom, i.e., that of the carbamoyl group. This partial structure was supported by the HMBC correlations from the NH proton to C-1 and C-9a. On the basis of these observations and its eleven degrees of unsaturation, alkaloid **1** was considered to possess a six-ring structure in which an oxazolidin-2-one ring and the core skeleton fused together at C-1 and C-9a (Fig. 2).

The NOESY spectrum gave information about the stereochemistry of this alkaloid. Correlations between the proton signals, H-3/NH, H-3/H-5b, H-5b/NH, H-6a/OCH_3-9, H-10/H-12, H-10/NH, H_2-16/OCH_3-9, and H-18/H-20, indicated that H-3, H-5b, H-10, C-12, and the oxazolidinone nitrogen were on the same side of the molecule, whereas H-6a and OCH_3-9 were on the opposite side, and H-18 and H-20 were of a *cis* orientation (Fig. 3). The stereochemical relations between the main core unit and the γ-lactone moiety linked to C-3 were also established by the NMR technique. The coupling constant between H-3 and H-18 was 7.7 Hz, and NOE correlations were observed between H-2a/H-18, H-2a/H-19a, H-2b/H-19a, H-2b/H-19b, and H-3/H-19b. These data suggested that H-3 and H-18 were in an *anti* relationship and that C-2 and C-19 were in a *gauche* relationship, indicating that **1** had (3*R**,18*R**) relative configuration. Accordingly, alkaloid **1** was concluded to have the (1*R**,3*S**,9*S**,9a*S**,10*R**,11*R**,18*S**,20*S**) relative configuration as shown in Fig. 1.

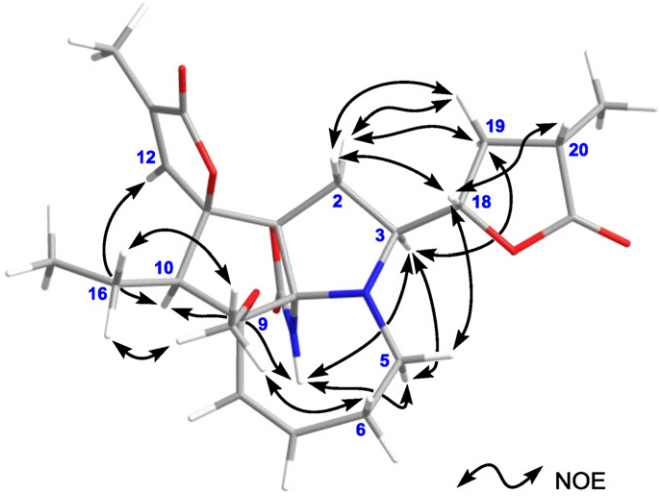

Fig. 3. Selected NOE correlations of **1**.

Stemoxazolidinone B (**2**) was isolated as an amorphous solid. The HRESIMS indicated its molecular formula to be $C_{24}H_{30}N_2O_7$, which is the same as that of **1**. Its ^1H and ^{13}C NMR spectra were very similar to those of **1**, and analyses of its ^1H–^1H COSY, HMQC, and HMBC spectra revealed that **2** had the same planar structure as **1** (Table 1). Thus, alkaloid **2** was considered to be a stereoisomer of **1**. Its NOESY spectrum showed correlations between H-2a/H-18, H-2a/H-19a, H-2b/H-19b, H-3/H-5b, H-3/H-19b, H-3/NH, H-5b/NH, H-6a/OCH_3-9, and H-18/H-20, and the *J*-value for H-3/H-18 was 7.8 Hz, indicating that **2** had the same relative configuration as **1** at positions 1, 3, 9, 9a, 18, and 20 (Fig. 4). This compound further showed NOE correlations between OCH_3-9/H-10, H_2-16/H-12, and H_2-16/NH, indicating that H-10 and OCH_3-9 were on the same side of the molecule, whereas C-12 was on the opposite side, i.e., on the same side as the oxazolidinone nitrogen. Accordingly, alkaloid **2** was determined to have the (1*R**,3*S**,9*S**,9a*S**,10*S**,11*R**,18*S**,20*S**) relative configuration as shown in Fig. 1.

Stemoxazolidinone C (**3**) was isolated as an amorphous solid. Its molecular formula, $C_{24}H_{30}N_2O_7$, determined from the HRESIMS, was

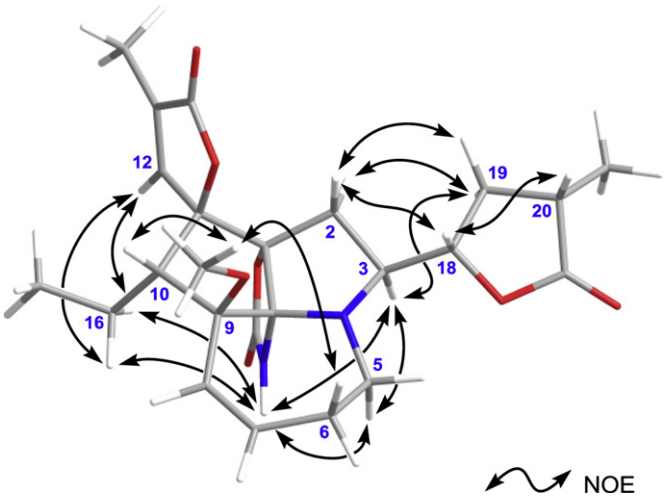

Fig. 4. Selected NOE correlations of **2**.

the same as those of **1** and **2**. Analyses of its ^1H–^1H COSY, HMQC, and HMBC spectra indicated that **3** was also a stereoisomer of **1**. Its NOESY spectrum showing correlations between H-2a/H-19b, H-2b/H-18, H-2b/H-19a, H-3/H-5b, H-3/H-19b, H-5b/NH, H-6a/OCH_3-9, H-10/NH, H_2-16/OCH_3-9, and H-18/H-20, and the *J*-value between H-3 and H-18 of 7.9 Hz, indicated that **3** had the same relative configuration as **1** at positions 1, 3, 9, 9a, 10, 18, and 20 (Fig. 5). However, since H-12 showed NOE correlations with H-2b, H_2-16, and OCH_3-9, C-12 was determined to be on the same side of the molecule as C-16 and OCH_3-9. Thus, alkaloid **3** was concluded to be an epimer of **1** at C-11, having the (1*R**,3*S**,9*S**,9a*S**,10*R**,11*S**,18*S**,20*S**) relative configuration as shown in Fig. 1.[8]

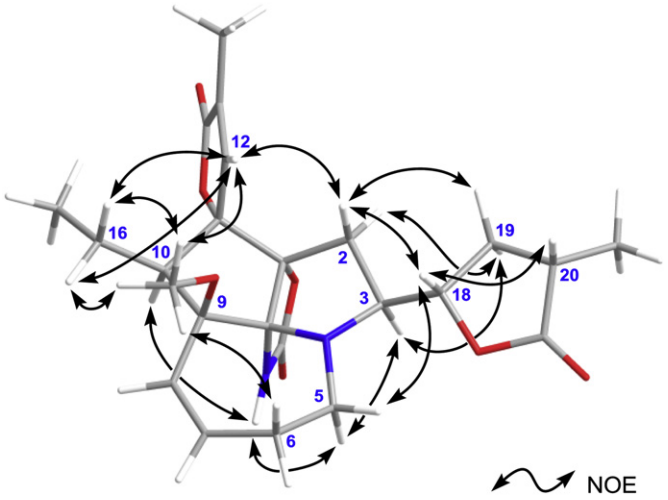

Fig. 5. Selected NOE correlations of **3**.

Stemoxazolidinone D (**4**) was obtained as colorless prisms. Its molecular formula, $C_{23}H_{32}N_2O_7$, determined from the HRESIMS indicated that it was also an alkaloid of the same type as **1**–**3** possessing two nitrogens in the molecule, with one carbon less and with nine degrees of unsaturation. The ^1H NMR spectrum of **4** showed signals of one terminal methyl (δ_H 1.04) and two secondary methyl (δ_H 1.28 and 1.40) groups, two oxymethines (δ_H 4.26 and 4.62), a hydroxyl group (δ_H 3.44), and an amide-like NH group (δ_H 6.88) (Table 2). The ^{13}C NMR spectrum indicated the presence of

Table 2
NMR data of stemoxazolidinones D–F (**4**–**6**) in $CDCl_3$[a]

Position	**4**		**5**		**6**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		90.4		87.0		79.3
2	a 2.39 (dd, 13.7, 6.4)	40.0	a 2.23 (dd, 13.8, 6.8)	40.5	a 2.07 (dd, 14.6, 10.6)	36.7
	b 1.63 (dd, 13.7, 10.3)		b 1.44 (dd, 13.8, 9.4)		b 1.72 (dd, 14.6, 4.2)	
3	3.21 (ddd, 10.3, 7.5, 6.4)	65.3	3.37 (m)	62.1	3.07 (m)	62.8
5	a 3.41 (ddd, 12.5, 10.1, 8.1)	48.0	a 3.45 (brd, 15)	45.1	a 2.99 (brd, 14)	48.4
	b 2.68 ([b])		b 2.56 (brt, 13)		b 2.69 (m)	
6	a 1.96 (m)	26.0	a 1.69 (m)	30.3	a 1.64 (m)	30.1
	b 1.51 (m)		b 1.37 (m)		b 1.47 (m)	
7	a 1.68 ([b])	20.0	a 1.98 (m)	30.9	a 1.69 (m)	22.3
	b 1.61 ([b])		b 1.21 (m)		b 1.64 (m)	
8	a 2.09 (m)	35.6	a 1.61 (m)	23.2	a 2.14 (dd, 14.2, 5.8)	28.7
	b 1.37 (m)		b 1.20 (m)		b 1.46 (m)	
9		74.5	1.92 (m)	46.9		90.0
9a		87.9		87.3		88.1
10	1.74 (m)	43.2	1.68 (m)	40.1	2.52 (m)	45.9
11	4.62 (dd, 7.9, 7.3)	78.1	4.33 (dd, 9.5, 7.8)	80.6	4.37 (dd, 9.4, 7.2)	81.9
12	2.67 (dd, 7.3, 5.0)	49.4	2.16 (dd, 7.8, 1.8)	48.5	2.19 (dd, 7.2, 1.8)	48.2
13	2.73 (qd, 7.4, 5.0)	37.5	2.82 (qd, 7.7, 1.8)	36.7	2.77 (q, 7.7)	36.7
14		177.9		178.2		180.0
15	1.40 (d, 7.4, 3H)	16.7	1.40 (d, 7.7, 3H)	18.2	1.37 (d, 7.7, 3H)	17.7
16	a 1.89 (m)	21.0	a 1.89 (m)	24.4	a 1.84 (m)	23.6
	b 1.64 ([b])		b 1.48 (m)		b 1.55 (m)	
17	1.04 (t, 7.5, 3H)	11.2	0.96 (t, 7.4, 3H)	11.7	1.06 (t, 7.5, 3H)	13.8
18	4.26 (ddd, 11.0, 7.5, 5.4)	81.2	4.22 (ddd, 10.6, 7.7, 5.5)	80.9	4.13 (ddd, 10.7, 7.6, 5.6)	81.9
19	a 2.40 (ddd, 12.3, 8.4, 5.4)	34.5	a 2.42 (m)	34.8	a 2.41 (m)	34.4
	b 1.55 (td, 12.3, 11.0)		b 1.57 (m)		b 1.49 (m)	
20	2.63 (ddq, 12.3, 8.4, 7.0)	34.7	2.61 (m)	34.6	2.64 (m)	35.1
21		178.4		178.8		178.8
22	1.28 (d, 7.0, 3H)	14.8	1.27 (d, 7.2, 3H)	14.8	1.26 (d, 7.0, 3H)	14.9
24		156.9		157.7		158.8
NH	6.88 (s)		7.06 (s)		7.36 (s)	
OH-1					3.79 (d, 1.8)	
OH-9	3.44 (s)					

[a] Recorded at 500 MHz for ^1H and 125 MHz for ^{13}C; *J*-values given in Hz in parentheses.
[b] Multiplicity patterns were unclear due to signal overlapping.

three methyls, seven methylenes, seven methines, and six quaternary carbons, of which three are carbonyl carbons. One of the carbonyl carbons resonated at δ_C 156.9, suggesting the presence of a carbamoyl group as in **1**. Analogous studies on its ^1H–^1H COSY, HMQC, and HMBC data as for **1** revealed that alkaloid **4** possessed a tuberostemonine-type skeleton with one hydroxyl group and one oxazolidin-2-one moiety (Fig. 6). The HMBC data showed that the hydroxyl proton was correlated with C-9 (δ_C 74.5), C-9a (δ_C 87.9), and C-10 (δ_C 43.2), and the NH proton with C-1 (δ_C 90.4), C-9a, and C-24 (δ_C 156.9), which indicated that the hydroxyl group was at C-9, and that the oxazolidin-2-one fused to the core skeleton at C-1 and C-9a (Fig. 6). Its (1*R**,3*R**,9*R**,9a*R**,10*R**,11*R**,12*R**,13*R**,18*R**,20*R**) relative stereochemistry was established unequivocally by the X-ray crystallographic analysis (Fig. 7) as shown in Fig. 1.

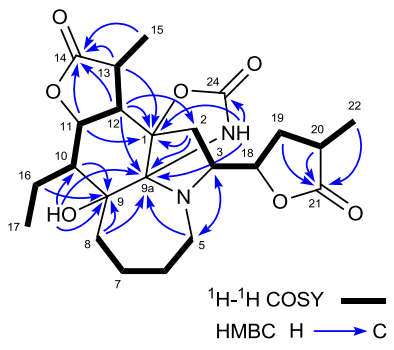

Fig. 6. ^1H–^1H COSY and key HMBC correlations of **4**.

Stemoxazolidinone E (**5**) was isolated as an amorphous solid. The molecular formula, $C_{23}H_{32}N_2O_6$, obtained from its HRESIMS was one oxygen less than that of **4**. Its NMR spectra were generally similar to those of **4**, and its NMR data including 2D NMR spectra indicated that alkaloid **5** possessed the same tuberostemonine-type skeleton with an oxazolidin-2-one ring as **4**. The major difference in the NMR spectra between **4** and **5** was that in **5**, the C-9 was observed as a methine carbon (δ_H 1.92/δ_C 46.9), whereas in **4**, it was an oxyquaternary carbon (δ_C 74.5) (Table 2). The location of the oxazolidin-2-one ring was determined to be at C-1 and C-9a of the core unit as in **4** with the nitrogen of the oxazolidin-2-one connected to C-9a and the oxygen of that to C-1, because the HMBC correlations from NH to C-1 and C-9a and the ROE correlations between NH/H-9 and NH/H-10 were observed (Fig. 8). Its stereochemistry was established by the ROESY experiment. ROE correlations between H-3/H-5b, H-3/NH, H-5b/NH, H-9/NH, and H-10/NH indicated that H-3, H-5b, H-9, H-10, and the oxazolidinone nitrogen were on the same side of the molecule, whereas correlations between H-2b/H-12, H-8b/H-12, H-11/H-12, H-11/H_3-15, and H-12/H_3-15 indicated that H-2b, H-8b, H-11, H-12, and Me-15 were on the opposite side (Fig. 9). Also, ROE correlations between H-2a/H-19b, H-2b/H-18, H-2b/H-19a, H-3/H-19b, and H-18/H-20 and a *J*-value of 7.7 Hz for H-3/H-18 showed that H-18 and H-20 were in a *cis* relation, and H-3 and H-18, and C-2 and C-19 were in *anti* and *gauche* relations, respectively, as in alkaloids **1**–**4**. Thus, alkaloid **5** was determined to have the (1*R**,3*R**,9*R**,9a*R**,10*S**,11*R**,12*R**,13*R**,18*R**,20*R**) relative configuration as shown in Fig. 1.

Stemoxazolidinone F (**6**) was isolated as colorless prisms. Its molecular formula, $C_{23}H_{32}N_2O_7$, determined from the HRESIMS, was

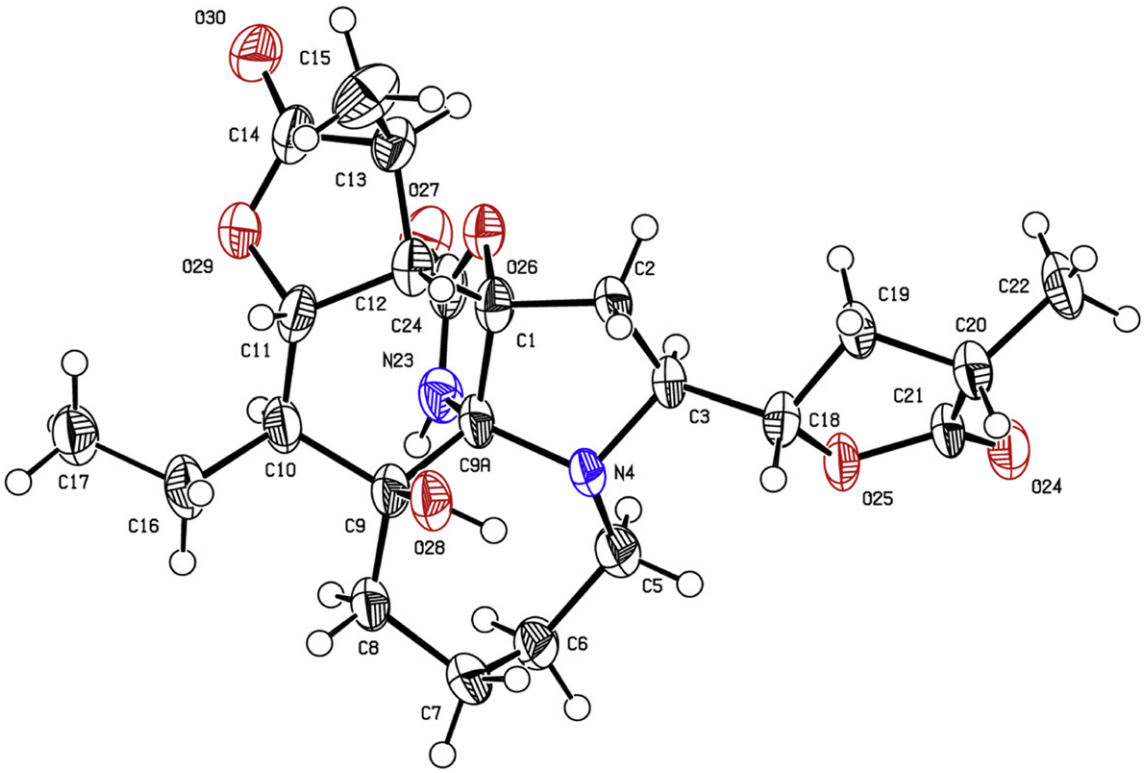

Fig. 7. ORTEP representation of stemoxazolidinone D (**4**).

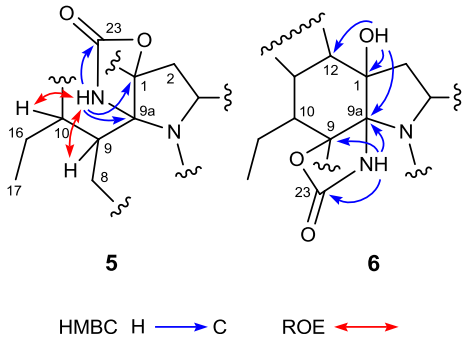

Fig. 8. Key HMBC and ROE correlations of **5** and **6** establishing the locations of the oxazolidin-2-one unit.

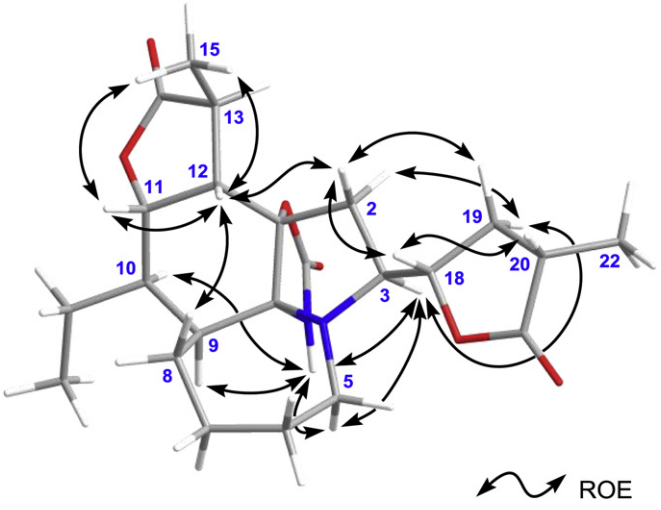

Fig. 9. Selected ROE correlations of **5**.

the same as that of stemoxazolidinone D (**4**). Analysis of its NMR data including 2D NMR spectra indicated that alkaloid **6** possessed the same carbon framework as that of **4** with one hydroxyl group and one oxazolidin-2-one ring as in **4**. However, the HMBC correlations from the hydroxyl proton (δ_H 3.79) to C-1 (δ_C 79.3), C-9a (δ_C 88.1), and C-12 (δ_C 48.2), and the presence of a long-range coupling (J=1.8 Hz) between the hydroxyl proton and H-12 (δ_H 2.19) indicated that the hydroxyl group was at C-1 and not at C-9 (Table 2, Fig. 8). The HMBC correlations from NH (δ_H 7.36) to C-9 (δ_C 90.0) and C-9a implied that the oxazolidin-2-one ring fused to the main unit at C-9 and C-9a with the C-9 oxygen forming part of the oxazolidin-2-one. The structure of **6** having the (1*R**,3*R**,9*S**,9a*S**,10*R**,11*R**,12*R**,13*R**,18*R**,20*R**) relative stereochemistry was established by the X-ray crystallographic analysis (Fig. 10) to be as shown in Fig. 1.

The present studies revealed that stemoxazolidinones A–F (**1**–**6**) from *Stemona sessilifolia* roots are alkaloids possessing quite unique structural features: They all contain an oxazolidin-2-one ring in the molecule, which is rarely seen in the natural products[9,10] and a pyrrolo[1,2-*a*]azepine ring, which is commonly and characteristically seen in the *Stemona* alkaloids. In the present alkaloids, those two ring units fuse together at positions 1 and 9a (**1**–**5**) or at 9 and 9a (**6**) of the pyrrolo[1,2-*a*]azepine ring with the nitrogen of the oxazolidin-2-one linking to C-9a to construct a six ring system in the molecule. Such structural features have not been reported in the alkaloid skeleton before. Further, stemoxazolidinones A–C (**1**–**3**) possess a novel carbon framework in their mother skeleton in which the C-1 of a tuberostemonine-type skeleton was rearranged from C-12 to C-11 to form a spiro structure.

3. Experimental

3.1. General

Melting points were determined on a Yanaco MP-3 apparatus and recorded uncorrected. Optical rotations were measured on a JASCO P-1030 digital polarimeter, UV spectra on a JASCO V-530

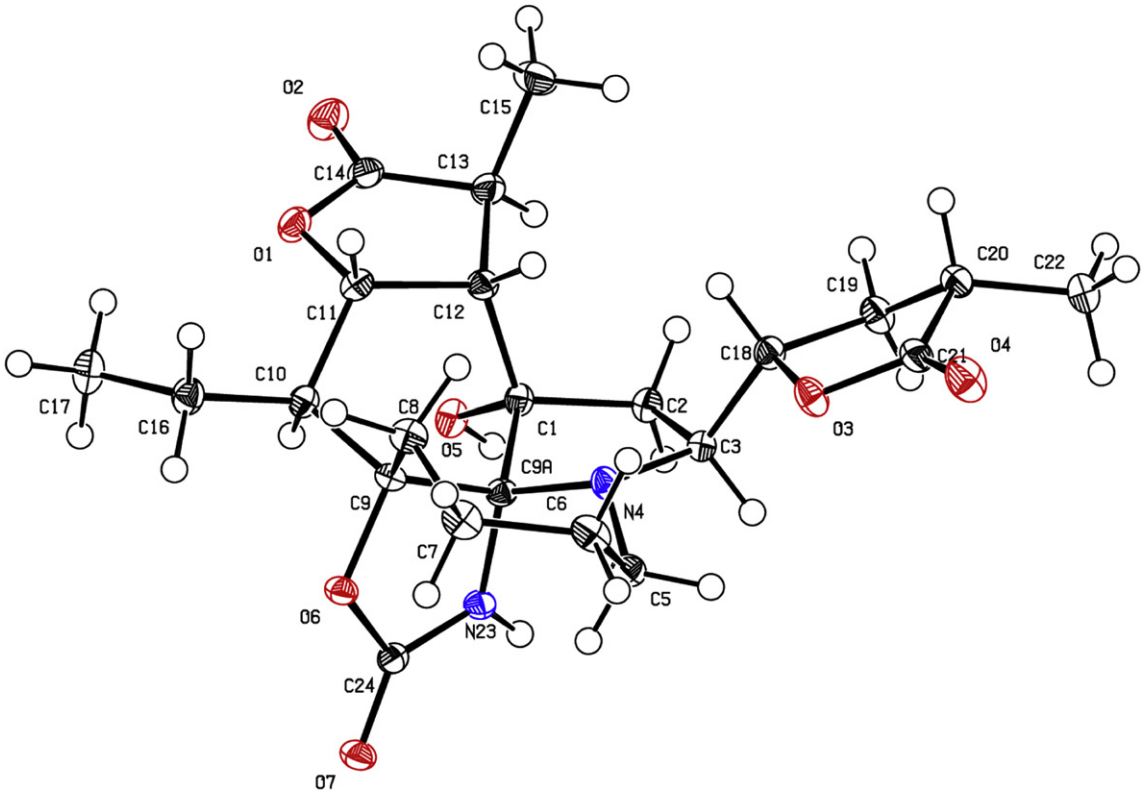

Fig. 10. ORTEP representation of stemoxazolidinone F (**6**).

spectrophotometer, and IR spectra on a JASCO FT/IR 620 spectrophotometer. Mass spectra were obtained with a Micromass LCT spectrometer. NMR spectra were obtained on a Bruker DRX-500 spectrometer at 300 K. In ^{1}H NMR spectra, the chemical shifts (δ) are given in parts per million relative to the resonance of residual $CHCl_3$ at 7.26 ppm, and in ^{13}C NMR spectra, the chemical shifts are given in parts per million relative to the resonance at 77.03 ppm for $CDCl_3$. Preparative HPLC was carried out on a Shimadzu LC-6AD system equipped with an SPD-10A UV detector (220 nm) and a reversed-phase column, Wakosil-II 5C18HG prep (5 μm, 20×250 mm), using a MeOH–H_2O or a MeCN–H_2O mixture as mobile phase, at a flow rate of 10 mL/min. Single-crystal X-ray analysis was carried out on a Mac Science DIP diffractometer with Mo Kα radiation (λ=0.71073 Å).

3.2. Plant material

The procurement and identification of plant material were made as described in the previous paper.[5]

3.3. Isolation

The mixture of neutral and acidic components (300 g), obtained from a MeOH extract (8 kg) of *S. sessilifolia* roots (15 kg),[5] was subjected to silica gel (1700 g) column chromatography eluting sequentially with hexane–EtOAc (3/1, 5 L), hexane–EtOAc (1/1, 5 L), EtOAc (5 L), EtOAc–MeOH (10/1, 8 L), and MeOH (8 L) to afford six fractions. The fourth fraction (43.6 g), a part of the EtOAc–MeOH (10/1) eluate, was subjected to aminopropyl-bonded silica gel (570 g) column chromatography eluting sequentially with hexane–EtOAc (1/0, 3/1, 1/1, 1/3, and 0/1, 4 L each), EtOAc–MeOH (10/1, 8 L), and MeOH (8 L) to give seven fractions (fractions 1–7). After removal of the solvent to dryness, fraction 4 (hexane–EtOAc 1/3 eluate, 0.72 g) was subjected to HPLC using MeOH–H_2O (35/65, 65/35, and 100/0) to afford 13 fractions (fractions 4A–4M). Fractions 4G (8.4 mg) and 4J (11.7 mg) were each subsequently purified by HPLC using MeCN–H_2O (23/77 and 25/75, respectively) to give **2** (2.5 mg) and **1** (4.3 mg), respectively.

Fraction 5 (EtOAc eluate, 0.33 g) was subjected to HPLC using MeOH–H_2O (40/60, and 100/0) to afford nine fractions (fractions 5A–5I). Fractions 5C (29.9 mg) and 5G (15.2 mg) were each subsequently purified by HPLC using MeCN–H_2O (32/68). Fraction 5C gave **3** (1.3 mg) and **4** (7.3 mg), and fraction 5G gave **5** (11.6 mg).

Fraction 6 (EtOAc–MeOH 10/1 eluate, 5.59 g) was subjected to silica gel (170 g) column chromatography eluting sequentially with $CHCl_3$–MeOH (30/1, 20/1, 10/1, 5/1, and 0/1) to afford 11 fractions. The third fraction (1.26 g), a part of the $CHCl_3$–MeOH (30/1) eluate, was then subjected to HPLC using MeOH–H_2O (40/60) to give seven fractions, the seventh fraction (0.65 g) of which was crystallized from dioxane to afford **6** (97.0 mg).

3.4. Characteristics of each alkaloid

*3.4.1. Stemoxazolidinone A (**1**).* Amorphous solid, $[\alpha]_D^{25}$ −124 (*c* 0.22, $CHCl_3$); UV λ_{max} (MeOH) 212 nm (log ε 4.21), 256sh (log ε 3.35); IR ν_{max} (film) 3304, 2972, 2934, 1760, 1171, 1019, 753 cm^{-1}; HRESIMS *m*/*z* 459.2098 $[M+H]^+$ (calcd for $C_{24}H_{31}N_2O_7$, 459.2131).

*3.4.2. Stemoxazolidinone B (**2**).* Amorphous solid, $[\alpha]_D^{25}$ −169 (*c* 0.12, $CHCl_3$); UV λ_{max} (MeOH) 212 nm (log ε 4.22); IR ν_{max} (film) 3325, 2969, 2932, 1759, 1169, 1098, 1020, 756 cm^{-1}; HRESIMS *m*/*z* 459.2099 $[M+H]^+$ (calcd for $C_{24}H_{31}N_2O_7$, 459.2131).

*3.4.3. Stemoxazolidinone C (**3**).* Amorphous solid, $[\alpha]_D^{25}$ −114 (*c* 0.065, MeOH); UV λ_{max} (MeOH) 212 nm (log ε 4.07), 320 nm (log ε

2.89); IR ν_{max} (film) 3290, 2971, 2935, 1766, 1171, 1022, 758 cm^{-1}; HRESIMS *m*/*z* 459.2148 $[M+H]^+$ (calcd for $C_{24}H_{31}N_2O_7$, 459.2131).

*3.4.4. Stemoxazolidinone D (**4**).* Colorless prisms, mp 286–289 °C (MeOH), $[\alpha]_D^{25}$ −87 (*c* 0.083, MeOH); IR ν_{max} (film) 3467, 3292, 2974, 2935, 2873, 1772, 1335, 1193, 755 cm^{-1}; HRESIMS *m*/*z* 449.2294 $[M+H]^+$ (calcd for $C_{23}H_{33}N_2O_7$, 449.2288).

*3.4.5. Stemoxazolidinone E (**5**).* Amorphous solid, $[\alpha]_D^{25}$ −110 (*c* 0.11, MeOH); IR ν_{max} (film) 3291, 2934, 1766, 1201, 1005, 756 cm^{-1}; HRESIMS *m*/*z* 433.2323 $[M+H]^+$ (calcd for $C_{23}H_{33}N_2O_6$, 433.2339).

*3.4.6. Stemoxazolidinone F (**6**).* Colorless prisms, mp 232–236 °C ($MeOH-H_2O$), $[\alpha]_D^{25}$ +9.6 (*c* 0.11, MeOH); IR ν_{max} (film) 3409, 2934, 2875, 1760, 1204, 994, 754 cm^{-1}; HRESIMS *m*/*z* 449.2282 $[M+H]^+$ (calcd for $C_{23}H_{33}N_2O_7$, 449.2288).

3.5. X-ray crystallographic study

*3.5.1. Stemoxazolidinone D (**4**).* $C_{23}H_{32}N_2O_7$, *M*=448.51, 0.30×0.25×0.20 mm, orthorhombic, $P2_12_12_1$, *a*=11.3940(15) Å, *b*=12.9130(9) Å, *c*=14.9960(17) Å, *V*=2206.4(4) Å,[3] *Z*=4, D_x=1.350 Mg m^{-3}, μ(Mo Kα)=0.100 mm^{-1}, 2603 measured reflections, 2603 unique reflections, 1448 observed reflections [$I>2\sigma(I)$], *R*1=0.0553, *wR*2=0.1229 (observed data), GOF=0.870; *R*1=0.0905, *wR*2=0.1318 (all data).

*3.5.2. Stemoxazolidinone F (**6**).* $C_{23}H_{32}N_2O_7 \cdot H_2O$, *M*=466.52, 0.50×0.38×0.38 mm, orthorhombic, $P2_12_12_1$, *a*=8.72500(10) Å, *b*=9.77100(10) Å, *c*=26.8070(3) Å, *V*=2285.35(4) Å,[3] *Z*=4, D_x=1.356 Mg m^{-3}, μ(Mo Kα)=0.102 mm^{-1}, 2894 measured reflections, 2894 unique reflections, 2772 observed reflections [$I>2\sigma(I)$], *R*1=0.0291, *wR*2=0.0774 (observed data), GOF=1.066; *R*1=0.0305, *wR*2=0.0780 (all data).

The structures were solved by direct methods using the maXus crystallographic software package,[11] and refined by full-matrix least-squares on F^2 using the program SHELXL-97.[12] The absolute structures could not be determined crystallographically.

CCDC 790097 and 790098 contain the supplementary crystallographic data for compounds **4** and **6**, respectively, from this paper. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/data_request/cif, by e-mailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.tet.2010.11.013.

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8. As regards the methoxy group at C-9 of stemoxazolidinones A–C (**1**–**3**), we cannot completely eliminate the possibility that it is an artifact introduced during the isolation and purification process. However, since many *Stemona* alkaloids are known to have a methoxy group in their structures, and since no C-9-hydroxy congener was isolated in the present studies, it is more likely that **1**–**3** are genuine natural products bearing biogenetically formed methoxy groups.
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