Absolute Structures of Stemona-Lactam S and Tuberostemospiroline, Alkaloids from *Stemona tuberosa*

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A new alkaloid, stemona-lactam S, and a known alkaloid, tuberostemospiroline, were isolated from the roots of *Stemona tuberosa* Lour. (Stemonaceae). Their structures and absolute stereochemistry were established by X-ray crystallography and vibrational circular dichroism.

**Key words** stemona-lactam S; tuberostemospiroline; *Stemona tuberosa*; absolute configuration; X-ray crystallography; vibrational circular dichroism

The roots of *Stemona japonica* (BLUME) MIQ., *S. tuberosa* LOUR., and *S. sessilifolia* (MIQ.) MIQ. are used as an antitussive and an insecticide in traditional medicine in China and Japan, and the alkaloidal components are responsible for such biological activities. In the course of our work on the Japan, and the alkaloidal components are responsible for such activities and an insecticide in traditional medicine in China and

The absolute configuration of compound 1 was established by VCD spectroscopy, which is a useful method for the determination of the absolute configuration in chiral molecules and has been applied to several natural products. This method is based on the comparison of experimental IR and VCD spectra with those obtained by density functional theory (DFT) calculations. Conformational analysis of 1 with 8R, 9S, 10S, and 11R configuration by the Monte Carlo conformational search with MMFF94S force field gave five conformers within 5kcal/mol from the global minimum energy conformation. In order to estimate their conformational population, single-point energy calculations were performed for those conformers at the DFT/B3LYP/6-31G(d,p) level. The results indicated that among the five conformers, the three lowest energy conformers, 1a, 1b, and 1c, were in the relative energy range of 0.6kcal/mol and were estimated to contribute to 94.8% of the total population. The geometries of the three conformers were further optimized at the B3PW91/DGDZVP2 level and the calculated relative energies and the Boltzmann populations are summarized in Table 1. The three optimized conformers of 1 possessed essentially the same conformation except for the

(C–H 0.98 Å) and treated as riding on their parent atoms. The final R indices were \( R_1 = 0.0250, wR_2 = 0.0649 \) for reflections with \( I > 2\sigma(I) \) and \( R_1 = 0.0255, wR_2 = 0.0655 \) for all data. The Flack absolute structure parameter \( 2013 The Pharmaceutical Society of Japan

![Fig. 1. Structures of Stemona-Lactam S (1) and Tuberostemospiroline (2)](image)

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side chain torsions (Fig. 3). The IR and VCD frequencies and intensities for each optimized conformer were calculated and the theoretical VCD spectrum of 1 was obtained by combining the spectra of the conformers weighted according to the Boltzmann population (Fig. 4). As can be seen from Fig. 4, the calculated VCD spectrum of $(8R,9S,10S,11R)$-1 was in good agreement with the experimental VCD spectrum of natural 1. Thus, the absolute structure of stemona-lactam S (1) was determined to be that shown in Fig. 1 with $8R$, $9S$, $10S$, and $11R$ configuration.

Tuberostemospiroline (2) was previously isolated from this species by Hua et al. Although its relative structure was determined by interpretation of NMR data, the structure depicted in the literature was incorrect. We confirmed its relative structure by X-ray crystallography and established its
absolute structure by VCD spectroscopy. A crystal of optimal size (0.30×0.21×0.17 mm), which was obtained by recrystallization from MeOH–H$_2$O by slow evaporation at room temperature, was submitted for X-ray crystallographic analysis. The experimental X-ray data consisting of 5643 reflections were collected at the scan width of 0.5° and the exposure time of 3 s/frame in the range of 2.5 to 25.0° with the index ranges of −10≤h≤8, −11≤k≤11, and −16≤l≤15. The number of independent reflections was 2069 (R$_{int}$=0.019) and that of observed reflections with I>2σ(I) was 2018. The crystal belongs to the orthorhombic system and the space group was P2$_1$2$_1$2$_1$ with cell dimensions of $a$=8.46659(9)Å, $b$=10.0481(11)Å, $c$=13.7603(15)Å, and $V$=1170.62(2)Å$^3$, $D$$_{calc}$=1.346 g/cm$^3$, $Z$=4, and $F$(000)=512. Non-hydrogen atoms were refined anisotropically and all hydrogen atoms were placed in geometrically calculated positions (C–H 0.98 Å) and treated as riding on their parent atoms. The final R indices were $R_1$=0.0291, $wR_2$=0.0729 for reflections with I>2σ(I) and $R_1$=0.0301, $wR_2$=0.0738 for all data. The Flack absolute structure parameter was −0.1(10). The S value was 1.06 and the largest peak and hole in the final difference map were 0.17e/Å$^3$ and −0.23e/Å$^3$, respectively. The ORTEP representation of 2 is shown in Fig. 5. Monte Carlo conformational search of 2 with 9S, 9aS, and 11R configuration gave four conformers and single-point energy calculations were carried out for those four conformers at the B3LYP/6-31G(d,p) level. The results showed that the energy difference between the lowest energy conformer and the second lowest energy conformer was 3.34 kcal/mol, and that between the lowest energy conformer and the third lowest energy conformer was 11.84 kcal/mol. Thus, the conformational distribution of this lowest energy conformer was estimated to be more than 99.6% in 2. The theoretical VCD spectrum of 2 was calculated using this lowest energy conformer structure as a 1:1 complex with a CHCl$_3$ molecule at the B3PW91/DGDZVP2 level. As shown in Fig. 6, the experimental spectrum of 2 and the calculated VCD spectrum of the CHCl$_3$ complex of 2 were very similar. Thus, tuberostemospiroline (2) was determined to have 9S, 9aS, and 11R configuration, as shown in Fig. 1. Stemono-lactam S (1) and tuberostemospiroline (2) are structurally related to stemoninoamide$^7$ and croinine,$^8$ respectively. As the absolute configuration of the chiral centers of alkaloids 1 and 2 is the same as those of the corresponding carbon atoms of stemoninoamide and croinine, respectively, alkaloids 1 and 2 are considered to be biogenetically derived from those alkaloids, respectively.

**Experimental**

**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 and IR spectra, on a JASCO FT/IR 620 spectrophotometer. NMR spectra were recorded on a Bruker AV-600 or DRX-500 spectrometer at 300 K. The $^1$H chemical shifts in CDCl$_3$ and CD$_2$OD were measured relative to the residual chlorine peak (δ 7.26 ppm) or CD$_3$OD (δ 3.31 ppm) resonance, and the $^{13}$C chemical shifts, to the solvent resonance (δ 77.03 or 49.0 ppm). Mass spectra were obtained with a Micromass LCT spectrometer. Single crystal X-ray diffraction data were collected using a Bruker SMART APEX II CCD diffractometer equipped with a multilayer confocal mirror and a fine-focus rotating anode (MoKα, λ=0.71073 Å) in the phi and omega scan modes at 90 K. Preparative HPLC was carried out using a Shimadzu LC-6AD system equipped with an SPD-10A UV detector (220nm) and a reversed phase column, Wakosil-II 5C18H prep (5 μm, 20×250mm), with a MeOH–H$_2$O mixture as the mobile phase, at the flow rate of 10mL/min.

**Table 1. Calculated Relative Energies (kcal/mol) and Populations (%) of the Three Lowest Energy Conformers of 1**

<table>
<thead>
<tr>
<th>Conformer</th>
<th>$\Delta G$ (a)</th>
<th>P (%) (b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>0.00</td>
<td>65.4</td>
</tr>
<tr>
<td>1b</td>
<td>0.54</td>
<td>26.2</td>
</tr>
<tr>
<td>1c</td>
<td>1.21</td>
<td>8.4</td>
</tr>
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(a) Calculated relative energies to 1a with $\Delta G=$−5650.57 kcal/mol at the DFT/B3PW91/DGDZVP2 level. (b) Boltzmann population at T=298 K and 1 atm.

**Plant Material** The procurement and identification of plant material were made as described in the previous paper.$^3$

**Extraction and Isolation** The air-dried roots (15 kg) were extracted with hot MeOH (3×35 L). The solvent was evaporated to give a crude MeOH extract (8 kg), which was, after acidification with 3% aqueous tartaric acid (8L), treated with EtOAc (3×8L). The combined EtOAc layers were evaporated in vacuo to give a residue (mixture of neutral and acidic components, 300 g). The aqueous layer was adjusted to pH 9 with solid Na$_2$CO$_3$ and extracted with CHCl$_3$ (3×8L). The combined CHCl$_3$ extracts were evaporated in vacuo to give a residue (basic fraction, 250 g), which was subjected to HP-20 (DIAION, 1250 g) column chromatography eluting with MeOH (10L), and then with acetone (3L). The residue from the MeOH fraction (206 g) was placed on an alumina column (Merck Aluminiumoxid 90, 2 kg) and eluted sequentially with CHCl$_3$ (4L), CHCl$_3$–MeOH (5:1, 2L), and MeOH (2L). The residue from the CHCl$_3$ fraction (150 g) was placed on a silica gel column (Merck Kieselgel 60, 70–230 mesh, 900 g) and eluted with petroleum ether containing an increasing amount of EtOAc (4:1 to 0:1, 22 L), and then with CHCl$_3$–MeOH (10:1, 4.5L). The CHCl$_3$–MeOH (10:1) fraction (13.5 g) was further subjected to silica gel column chromatography eluting sequentially with petroleum ether–acetone (1:1, 4L), acetone (1L), and MeOH (1L) to give four fractions. The third fraction (4.42g, acetone eluate) gave, by ODS HPLC eluting with MeOH–0.1 M aqueous NH$_2$OAc (35:65), stemoninoamide (2.0 g). This stemoninoamide was not pure and recrystallization from Et$_2$O–acetone (1:1) gave stemoninoamide (1.5 g) and the mother liquor. The mother liquor was concentrated and applied to an ODS HPLC column eluting with MeOH–0.1 M aqueous NH$_2$OAc (35:65) to give tuberostemospiroline (2) (30 mg).

The mixture of neutral and acidic components (300 g) was subjected to silica gel (1700 g) column chromatography eluting sequentially with hexane–EtOAc (3:1, 5L), hexane–EtOAc (1:1, 5L), EtOAc (5L), EtOAc–MeOH (10:1, 8L), and MeOH (8L) to afford six fractions. The fourth fraction (43.6 g), which was 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, 4L each), EtOAc–MeOH (10:1, 8L), and MeOH (8L) to give seven fractions (fractions 1–7). After evaporating the solvent to dryness, fraction 4 (hexane–EtOAc 1:3 eluate, 0.72 g) was subjected to HPLC using MeOH–H$_2$O (35:65, 0.1%)](https://example.com)
65: 35, and 100: 0) to afford stemona-lactam S (1) (13.2 mg). Fraction 5 (EtOAc eluate, 0.33 g) was subjected to HPLC using MeOH–H2O (40: 60, and 100: 0) to afford nine fractions. The fifth fraction (3.72 mg) was subsequently purified by HPLC using MeOH–H2O (32: 68) to give stemona-lactam S (1) (5.3 mg).


**IR and VCD Measurements** IR and VCD spectra were measured with a Dual-PEM Chiral IR-2X FT-VCD spectrometer (BioTools, Inc., Jupiter, FL, U.S.A.) using a resolution of 4cm–1. 3.6 mg of 1 and 3.1 mg of 2 were each dissolved in 150µL of CDCl3 and placed in a BaF2 cell with a path length of 75µm. Six data blocks for 2h were collected and averaged, and the baseline was corrected by subtracting the spectrum of CDCl3 acquired under the same conditions.

**Molecular Modeling and VCD Calculations** The conformational analysis of 1 and 2 was performed by a Monte Carlo search with the MMFF94S force field using MacroModel ver. 7.0 software (Schrödinger Inc., Portland, OR, U.S.A.). Calculations consisted of 50000 steps. Five conformers for 1 and four conformers for 2 were found within 5 kcal/mol from the global minimum conformations, respectively. All the obtained structures were subjected to single point calculations at the DFT/B3LYP/6-31G(d,p) level. The geometric optimization and the calculations of the free energies and the frequencies were performed at the DFT/B3PW91/DGDZVP2 level using Gaussian 09W software (Gaussian, Inc., Wallingford, CT, U.S.A.). The calculated IR and VCD spectra were obtained with Lorentzian band shapes of 4cm–1 half-width at half-height. The frequencies of the calculated spectra were scaled by a factor of 0.98 using CompareVOA software (BioTools, Inc., Jupiter, FL, U.S.A.).

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**References**