

Ionophore Activity of Frangufoline

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Abstract □ The ionophore activity of frangufoline(**1**), a sedative cyclopeptide alkaloid isolated from *Zizyphus jujuba*, was investigated by UV and CD spectroscopic methods. Frangufoline(**1**) showed ion binding activity to calcium and magnesium ions.

Keywords □ Ionophore, frangufoline, cyclopeptide alkaloid

Interest on the ionophore-active compounds has rapidly expanded due to its potential biological activity through the modification of energy-linked transport of metal ions in microorganism¹. The authors' group reported frangufoline(**1**)^{2,3} as the sedative principle of "sanjoin", the seed of *Z. jujuba* which has been used for the treatment insomnia in the oriental medicine. Frangufoline(**1**) is a fourteen-membered cyclopeptide alkaloid consisted of three amino acids, (*N,N*-dimethylphenylalanine, leucine and β -hydroxy-leucine) and *p*-hydroxystyrylamine units, and the units are connected through amide and ether bonds. Recently, it was recognized that frangufoline has an affinity to calmodulin which is a calcium binding protein⁴.

This communication describing the ultraviolet and circular dichroism (CD) spectroscopic study on the ionophore² activity of (**1**) is a part of our investigation on the binding among calcium-binding protein, sedative cyclopeptide alkaloid (**1**) and calcium ion.

EXPERIMENTAL METHODS

Materials

Frangufoline(**1**) was isolated from *Z. jujuba* as previously reported⁵. Calcium perchlorate and potassium perchlorate were synthesized from calcium hydroxide and potassium hydroxide, respectively. All salts were dried under vacuum overnight prior to use. UV and CD spectra were recorded in spec-

troscopic grade acetonitrile (Uvasol, Merck) on Shimadzu UV-2100 UV spectrometer and Jasco J-20C CD spectrometer, respectively.

Examination by UV and CD

UV and CD spectra of (**1**) were taken in the presence of various added salts (perchlorate of Na⁺, K⁺, Ag⁺, Mg²⁺, Ca²⁺ or Ba²⁺). The concentration of (**1**) was fixed to 3.7×10^{-5} M, while that of metal ion was varied in the range of 3.0×10^{-5} M- 5.0×10^{-2} M.

RESULTS AND DISCUSSION

UV spectrum of (**1**) showed end absorption with shoulder at ca. 224 nm (Fig. 1). This shoulder was disappeared by the addition of Ca²⁺ or Mg²⁺ ion but not affected by Na⁺, K⁺, Ag⁺ or Ba²⁺ ion as shown in Fig. 1, which suggests that frangufoline (**1**) has ion binding activity to Ca²⁺ and Mg²⁺ ions.

The ion binding of ionophore leads to a change of its conformation which can sensitively be observed by CD spectroscopy⁶. The CD spectrum of (**1**) was markedly changed by the addition of calcium or magnesium ion, while it was not affected by sodium, potassium, silver or barium ion. This was consistent with the result of UV spectroscopic observation.

Fig. 2 shows the result of CD titration of (**1**) with calcium ion. The CD ellipticity ($\Delta\epsilon$) at 274 nm was varied from negative to positive and then negative

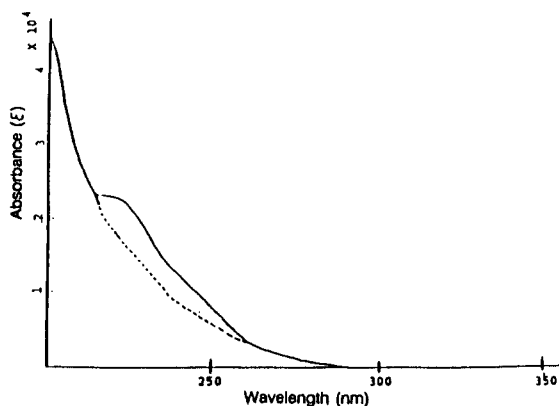


Fig. 1. UV spectrum of franguloline(1) in CH_3CN .
—: (1) alone, \cdots : (1) and 1.8×10^{-3} M of Ca^{2+} (or Mg^{2+}) Addition of Na^+ , K^+ , Ag^+ or Ba^{2+} ion leads no change in UV spectrum of (1).

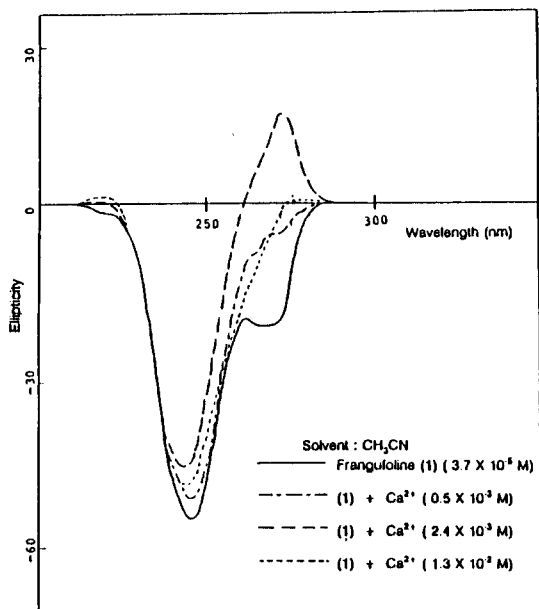


Fig. 2. CD spectrum of franguloline.

again to the concentration of calcium ion.

Fig. 3 shows the change of CD ellipticity ($\Delta\Delta\epsilon$) at 252 nm and at 274 nm to the change of log concentration of calcium ion ($\log [\text{Ca}^{2+}]$) added in

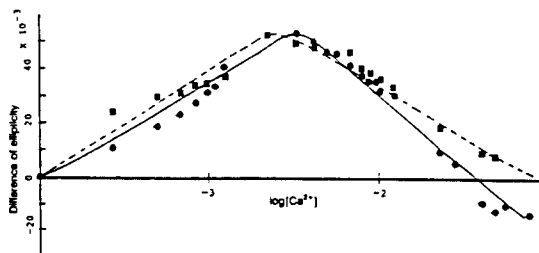


Fig. 3. CD titration curves of franguloline with $\text{Ca}(\text{ClO}_4)_2$.
— at 274 nm, \cdots at 252 nm

CH_3CN . In the curves obtained, two phases were observed indicating at least two types of complex may be formed as the concentration of calcium ion increased⁷⁾.

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