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ABSOLUTE CONFIGURATION OF A CYCLOPEPTIDE ALKALOID, SANJOININE-G1, FROM *ZIZYPHUS VULGARIS* VAR. *SPINOSUS*

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Key Word Index—*Zizyphus vulgaris* var. *spinosus*; Rhamnaceae; seeds; cyclopeptide alkaloid; sanjoinine-G1; absolute configuration.

Abstract—A new cyclopeptide alkaloid, sanjoinine-G1, has been isolated from the seeds of *Zizyphus vulgaris* var. *spinosus*. The structure and absolute configuration has been established by spectroscopic analysis and the circular dichroism exiton-coupling method, as *N*-[(*S*)-(*N'*,*N'*)-dimethylphenylalanyl]-cyclo[*O*-(2*S*,3*S*)-β-oxyleucyl-(*S*)-leucyl-(2-mino-(1*R*)-hydroxyethyl)-*p*-phenyl]. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

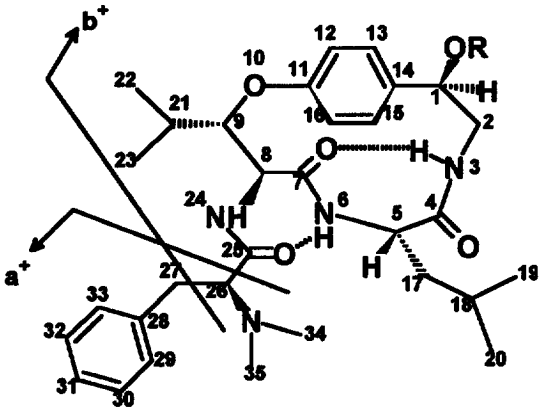
In the course of studies on the sedative and/or tranquilizing principles of the seeds of *Zizyphus vulgaris* var. *spinosus*, the most important tranquilizing agent in Oriental medicine, eight cyclopeptide alkaloids and six aporphinoids have been isolated [1, 2]. Herein, we describe the determination of the absolute configuration of a cyclopeptide alkaloid, sanjoinine-G1 (**1**).

RESULTS AND DISCUSSION

Sanjoinine-G1 (**1**), mp 236–238°, was isolated as a crystalline powder in a 3.5×10^{-5} % yield from the ether-soluble alkaloid fraction of a methanolic extract of the seeds by a combination of column chromatography and preparative TLC. Its molecular formula was determined to be $C_{31}H_{44}N_4O_5$, *m/z* 552.3273 $[M]^+$ by HR mass spectrometry. The IR spectrum exhibited bands corresponding to hydroxyl (3300 cm^{-1}), *N*-methyl (2270 cm^{-1}), amide (1670–1625 cm^{-1}) and phenol ether (1230 cm^{-1}) functions. Acid hydrolysis yielded leucine and *N*,*N*-dimethylphenylalanine.

The mass spectrum followed the typical fragmentation pattern of a frangulanine-type 14-membered peptide alkaloid [3]; the $[M]^+$ (*m/z* 552) and b^+ (*m/z* 461) ions were 18 mu greater than those of frangufoline [4]. However, the two olefinic proton peaks at δ 6.36 and δ 6.50 of frangufoline [2] were absent in 1 (Table 1). Instead, three proton peaks at δ 5.17 (1H, *d*, *J* = 3.8 Hz), 4.27 (1H, *ddd*, *J* = 3.8, 11, 14.4 Hz) and 3.06 (1H, *d*, *J* = 14.4 Hz) were observed, indicating that the double bond in the *p*-hydroxystyrylamine unit in frangufoline was hydrated in compound **1**. The presence of a hydroxyl group was also confirmed by acetylation and benzoylation (monoacetate of **1**, mp 282–285°, $[M]^+$ (*m/z* 594), ^1H NMR δ 2.18, 3H, *s*). ^1H–^1H COSY spectra and decoupling experiments supported the structure assigned to compound **1**, in which the hydroxyl group was placed at C-1.

In order to determine the absolute configuration at C-1, the circular dichroic exciton chirality method [5, 6] was applied to *p*-substituted benzoate derivatives of compound **1**. Sanjoinine-G1 benzoate (**2**) and *p*-bromobenzoate (**3**) were prepared by reaction of compound **1** with benzoic anhydride and *p*-bromobenzoyl chloride in pyridine, respectively. The UV spectra measured in



1 : **Sanjoinine-G1, R = H**

2 : **R = C_6H_5CO**

3 : **R = *p*-BrC_6H_4CO**

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Table 1. ^{1}H NMR spectra data for compounds **1** and **2** (360 MHz, $CDCl_3$, TMS as internal standard)

	1	**2**
H	δ_H (*J*, Hz)	δ_H (*J*, Hz)
1α	5.17 *d* (3.8)	6.35 *d* (4.5)
2α	4.27 *ddd* (3.8, 11, 14.4)	4.48 *ddd* (4.5, 11, 14.5)
2β	3.06 *d* (14.4)	3.25 *d* (14.5)
3NH	5.66 *d* (11)	5.34 *d* (11)
5β	3.95 *dt* (6.7, 9.2)	3.98 *dt* (6.0, 9.4)
6NH	6.05 *d* (9.2)	5.78 *d* (9.4)
8α	4.32 *dd* (8.6, 9.8)	4.28 *dd* (8.6, 9.8)
9β	4.79 *dd* (1.8, 8.6)	4.79 *dd* (2.0, 8.6)
12α	6.81 *dd* (2.5, 8.0)	6.89 *dd* (2.5, 8.4)
13α	6.97 *dd* (2.0, 8.0)	7.12 *dd* (2.0, 8.4)
15β	7.32 *dd* (2.0, 8.2)	7.19 *dd* (2.0, 9.0)
16β	6.94 *dd* (2.5, 8.2)	6.95 *dd* (2.5, 9.0)
17	1.27 2H, *dd* (5.8, 6.7)	1.27 2H, *dd* (6.0, 6.5)
18	1.31 *m*	1.31 *m*
19, 20	0.76 6H, *d* (5.4)	0.76 6H, *d* (5.4)
21	1.92 *m*	1.92 *m*
22	0.95 3H, *d* (6.5)	0.95 3H, *d* (6.6)
23	1.18 3H, *d* 6.5)	1.12 3H, *d* (6.6)
24NH	7.40 *d* (9.8)	7.33 *d* (9.8)
26	3.22 *t* (6.2)	3.12 *t* (6.2)
27	2.90 *dd* (6.2, 14)	2.90 *dd* (6.2, 14)
	3.12 *dd* (6.2, 14)	3.19 *dd* (6.2, 14)
29–33	7.19–7.22 5H, *m*	7.22–7.28 5H, *m*
34–35	2.23 6H, *s*	2.23 6H, *s*
–OH	6.43 *s*	
Benzoate		7.53 2H, *t* (7.5)
		7.64 *tt* (1, 7.5)
		8.15 2H, *m*

MeCN, and other spectral data, indicated that the secondary alcohol was converted into benzoates. As shown in Fig. 1, completely split CD spectra were observed, which originated from the interaction between the *p*-hydroxyphenyl and (*p*-bromo)benzoyl chromophores. From the negative A values (−30.4 and −13.3), it was concluded that the benzoates exhibit negative exciton chirality, indicating the *R* configuration at C-1. The stereochemistry at other chiral centres was assigned as 5*S*, 8*S*, 9*S*, and 26*S* by means of GC analyses of diastereomeric derivatives of individual amino acid units in the acid hydrolysates and NOE experiments [7].

The stereochemistry and solution of the conformation of compound **1**, elucidated on the basis of the analysis of ^{1}H–^{1}H vicinal coupling constants (Table 1) and ^{1}H NOE measurement (Table 2), were in good agreement with those of a 14-membered ring with a *p*-hydroxystyrylamine unit studied by X-ray, and high-resolution NMR analysis of frangulanine [8–10], dihydromauritine A [11] and synthetic model compounds [12, 13]. With respect to the geometry of the two amides in the macrocyclic ring, the *trans*-conformation of the N3-C4 amide was demonstrated by the significant NOE (12.1%) from the N3 amide proton to the C5-proton. Furthermore, a pronounced NOE (3.0%) to the resonance at δ 3.25, attributable to the H-2β, also confirmed the *trans* conformation of the N3-C4 amide, where the N3 amide proton points toward the β-face of the macrocyclic ring. The assignment of the *trans* geometry for the N6–C7 amide, where the N6 amide proton points toward the α-face of the ring, was based on the observed NOE from the 6N-H to H-8 (13.5%) and the aromatic proton H-12 (1.4%). The fact that 3N-H and 6N-H protons could not be exchanged with D_2O indicated they participated in hydrogen bonding (γ-turn) with the C7-carbonyl and C25-carbonyl groups, respectively, rendering the macrocyclic ring structure more rigid.

The unusually highfield chemical shift of the 3N-H resonance at δ 5.34 for the hydrogen-bonded amide proton is probably because this proton lies directly above the hydroxybenzene ring and is shielded by the induced ring current of the benzene ring. H-2α lies in the same plane as the C4-carbonyl, which results in a significant lower field-shift (δ 4.48) than H-2β (δ 3.25). The conformation of H1α-H2α-H2β-3NH was determined on the basis of NOE data and proton coupling constants—i.e. no NOE was observed from H-2β to H-1α, where the dihedral angle was almost 90° ($J_{1,2} = 0$ Hz).

The primary and stereochemical structure of sanjoinine-G1 was also confirmed by a totally synthetic approach [14].

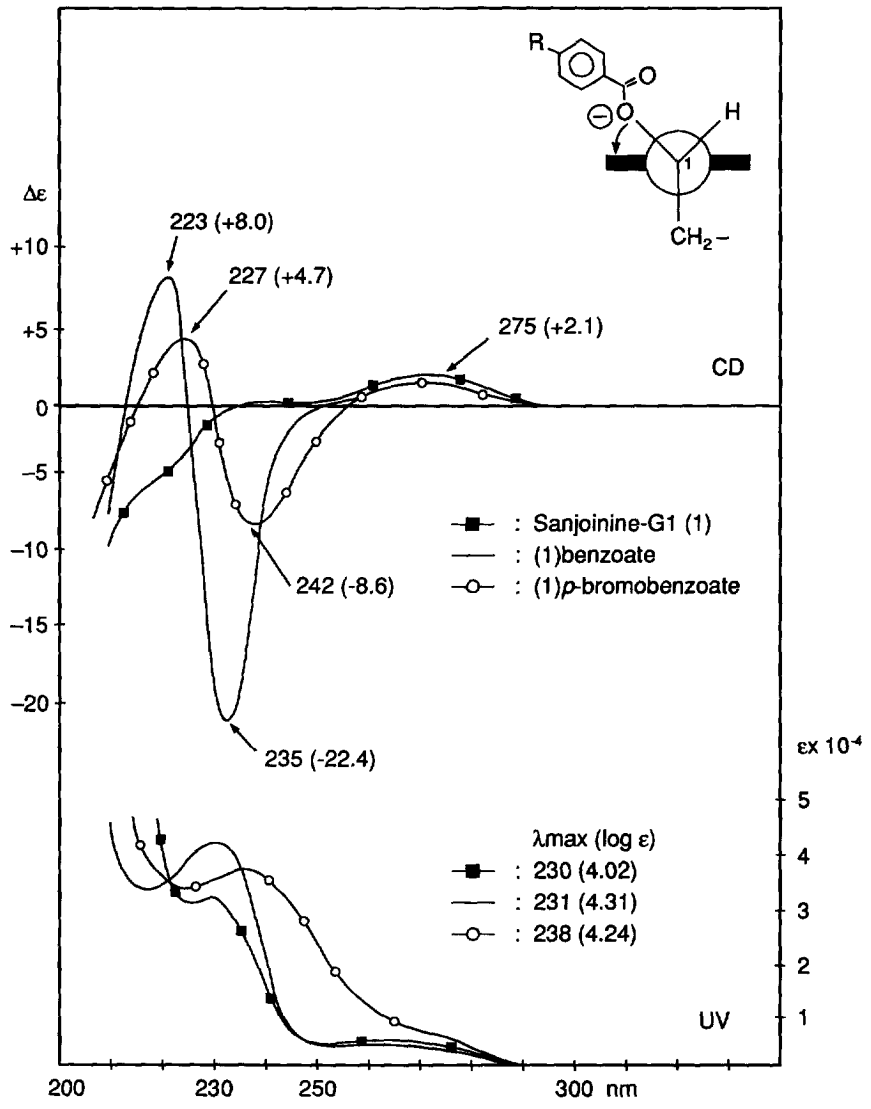

Fig. 1. CD and UV spectra of compounds **1**–**3**.

Table 2. Steady-state ^{1}H-NOE measurement of compound **2**

Irradiation (δ)	Observation (δ)	NOE enhancement (%)
H1α (6.35)	H13α (7.12)	11
	H2α (4.48)	3.0
H2α (4.48)	H13α (7.12)	3.7
	H1α (6.35)	6.1
	H2β (3.25)	13.7
H2β (3.25)	H2α (4.48)	9.0
3N-H (5.34)	H5β (3.98)	12.1
	H2β (3.25)	3.0
H5β (3.98)	3N-H (5.34)	7.4
6N-H (5.78)	H12α (6.89)	1.4
	H8α (4.28)	13.5
H8α (4.28)	6N-H (5.78)	8.7
H9β (4.79)	H16β (6.95)	13.1
	H21 (1.92)	4.8
	H22 (pro *R*) (1.12)	0.9

EXPERIMENTAL

General. Mps: uncorr; ^{1}H NMR (360 MHz) was recorded in $CDCl_3$. Chemical shifts are given in δ values relative to TMS as int. standard. MS were measured at 70 eV.

Isolation of compound **1**. Crude alkaloids were extracted by usual methods. Extensive chromatography and repeated prep. TLC of the crude bases of the seeds of *Z. vulgaris* var. *spinosus* (60 kg) with $CHCl_3$–MeOH (10:1), cyclohexane–EtOAc–MeOH (30:15:4) and EtOAc–Et_2O–$CHCl_3$ (10:10:1) yielded compound **1** (21 mg, 3.5×10^{-5}%). More details are given in ref. [2].

Compound **1**. Mp 236–238°. $[\alpha]_D^{20}$ −68.6° ($CHCl_3$; *c* 0.175). UV λ_{max} nm: 232, 278, (log ε 3.97, 3.3). IR ν_{max}^{KBr} cm^{-1}: 3300, 3270, 2770, 1670, 1230 cm^{-1}. HR-

MS *m/z* 552.3273 ($[M]^+$, calcd for $C_{31}H_{44}N_4O_5$, 552.3311); EI-MS *m/z* (rel, int): 552 $[M]^+$ (0.01), 537 $[M-Me]^+$ (0.03), 509 $[M-43]^+$ (0.02), 491 $[M-H_2O-43]^+$ (0.03), 461 $[M-C_6H_5CH_2]^+$ (2.4), 443 $[M-H_2O-C_6H_5CH_2]^+$ (0.05), 210 (0.1), 208 (0.2), 207 (0.3), 195 (0.6), 190 (0.6), 189 (0.4), 182 (0.4), 167 (0.4), 153 (0.5), 148 (100), 135 (1.1), 97 (2.5), 86 (2.3). ^{1}H NMR in Table 1.

Acetylation of compound **1**. Compound **1** (8 mg) was derivatized as a monoacetate by Ac_2O–pyridine. Needles ($CHCl_3$–MeOH), mp 282–285°. MS *m/z* 594 $[M]^+$. ^{1}H NMR ($CDCl_3$): δ 2.18 (3H, *s*, O-$COCH_3$).

Benzoylation of compound **1**. **1** (3 mg each) in pyridine was treated with benzoic anhydride or *p*-bromobenzoylchloride to yield compounds **2** and **3**. ^{1}H NMR of compound **2**: Table 2; UV and CD of compounds **2** and **3**: Fig. 1.

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