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Note

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Absolute Configuration of Franganine

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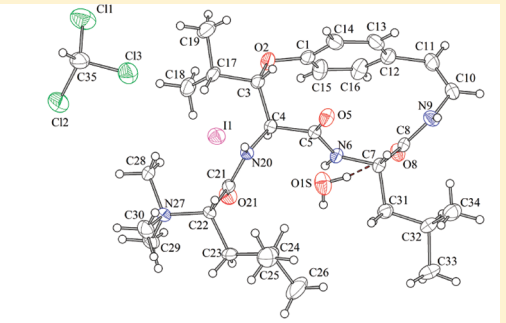
Supporting Information

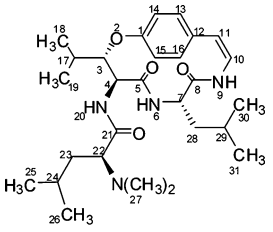
ABSTRACT: The absolute configuration of franganine (**1**), a cyclopeptide alkaloid isolated from the methanol root bark extract of *Discaria americana*, was established on the basis of detailed NMR spectroscopic data and X-ray diffraction analysis of its salt (**2**).



Tri-N-methylfranganine methiodide (**2**)

Cyclopeptide alkaloids are polyamidic bases found in many plant families, mainly of the Rhamnaceae species.[1,2] These molecules are composed of 13-, 14-, and 15-membered macrocycles that, as general structural elements, bear two amino acids, a common amino acid (Phe, Leu, Ile, Val, Ala, Pro, Trp), as an α-amino acid residue, and a β-hydroxyamino acid unit (usually 3-hydroxyproline, 3-hydroxyleucine, or 3-phenylserine), which is connected to a styryl fragment via an ether bridge. A side chain, usually a peptidogenic amino acid with an *N*-monomethyl or *N,N*-dimethylated terminus, is attached to the β-hydroxyamino acid.[1,2] Interestingly in this type of structure, the β-hydroxyamino acid, 3-hydroxyleucine or 3-phenylserine, can exist in the *erythro* (D,L) and *threo* (D,L) forms, allowing the formation of diastereoisomeric cylopeptide alkaloids. As the biological activity of a substance may be closely related to its stereochemistry, as in the case of the diastereoisomeric alkaloids scutianine M and condaline A,[3] it is interesting to determine unequivocally the configuration of all their stereogenic centers.

In continuation of a project on the determination of the configuration of the stereogenic centers of representative members of this group of alkaloids,[4,5] we now report the determination of the absolute configuration of franganine (**1**), a 14-membered cyclopeptide alkaloid isolated from *Discaria americana* Gill. & HooK.[6] The absolute configuration of the constituent amino acid units *N,N*-dimethylleucine and leucine was previously determined by enantioselective GC.[4] The absolute configuration of the β-hydroxyamino acid (β-hydroxyleucine), not found in the hydrolysate of franganine, was not determined by this method. In the present work, the absolute configuration of all stereogenic centers of **1** was determined by X-ray diffraction of its $N(CH_3)_3^+I^-$ salt (**2**).



Franganine (**1**)

Because the ^1H and ^{13}C NMR spectroscopic data are important tools for establishing the relative and absolute configuration of the β-hydroxyamino acid, we compare this method with the absolute data obtained by X-ray diffraction. In the ^1H NMR spectrum of some 14-membered cyclopeptide alkaloids, such as lasiodine B, scutianine D, scutianine H, discarine A, and discarine B, the *erythro* form of the β-hydroxyleucine group shows a $J_{3,4}$ value of ca. 8.0 Hz, whereas the *threo* β-hydroxyleucine group shows a $J_{3,4}$ of ca. 2.0 Hz.[2,7−10] The $^3J_{3,4}$ values provide only the relative configuration (*erythro/threo*) of this unit, but not the absolute configuration (L- or D-*erythro/threo* β-hydroxyleucine). However, the ^{13}C NMR data may provide additional data that can be used to determine the absolute configuration of this unit.[2,9,10] For the L-*erythro* series, the signals of the α-(C-4) and β-(C-3) carbons appear at ca. δ_C 55.0 and 81.5, respectively, whereas for the D-*erythro* series, they appear at ca. δ_C 53.0 and ca. 87.0, respectively. Therefore, the $^3J_{3,4}$ values together with the carbon chemical shifts of C-3 and C-4 should be sufficient for the determination of the absolute configuration of the β-

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hydroxyleucine unit in the *erythro* form, but nothing has been reported for the *threo* series. Because the NOE correlations between H-3, H-4, H-6, and H-7 have been used in several studies to determine the relative configuration of this unit, in this work we are comparing the validity of this method, since the absolute configuration of franganine was determined by X-ray diffraction.[11]

The single-crystal X-ray diffraction study shows that the crystal structure of **2** (Figure 1) is composed of one cation of

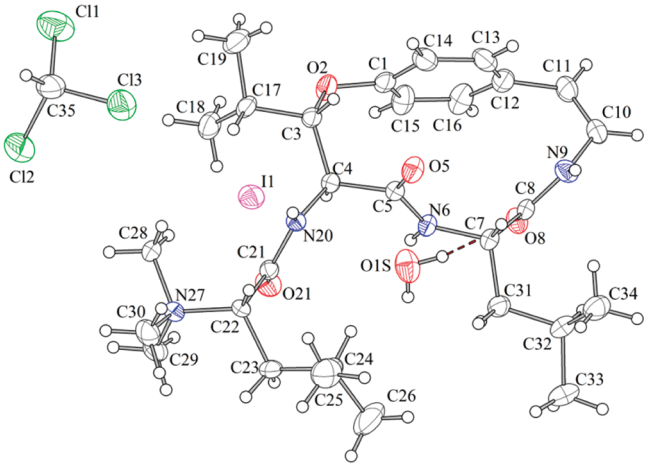

Figure 1. Structure of **2** at the 30% probability level showing atom-numbering scheme.

the trimethylammonium derivative of franganine with its iodide anion together with single H_2O and $CHCl_3$ solvate molecules in the asymmetric unit. All distances and angles are normal. Analysis using Mogul[12] with Mercury[13] gives the largest *z*-score of 1.070 for the N-9−C-10 bond and 1.752 for the C-5−C-4−N-20 angle. The two N−H and one C═O group of the franganine molecule participate in intermolecular hydrogen bonding with the solvate water molecule and the iodide ion. The resonance scattering of the heavy chlorine and iodine atoms within the structure are sufficient to determine the absolute configuration of **2** using 4953 Bijvoet pairs (78.1%).[14,15] The Flack *x* parameter was refined, giving a value of 0.02(2), which indicates an enantiomorphically pure crystal of the correct handedness.

From the solution NMR data, it is evident that the *β*-hydroxyleucine unit has a relative *erythro* configuration based on the $^3J_{3,4}$ values of 8.4 Hz in methanol-d_4, 7.2 Hz in $CDCl_3$, and 8.0 Hz in DMSO-d_6, which by the Karplus equation indicated dihedral angles of ca. 20° and 150°. In the ^{13}C NMR spectra of **1** and **2** in methanol-d_4 (Table 1), the chemical shifts of C-3 (δ_C 80.7 and 79.4, respectively) and of C-4 (δ_C 55.4 and 55.8, respectively) suggest an L-*erythro* absolute configuration, indicative of a dihedral angle near 150°. The configuration attributed to C-3 and C-4 via NMR data is consistent with the absolute configuration obtained by X-ray diffraction. Thus, franganine possesses *S* absolute configuration at C-3, C-4 (L-*erythro*), C-7, and C-22.

In the NOESY spectra of **1**, in $CDCl_3$ (Figure SI5) or DMSO-d_6 (Figure SI11), and of **2** in methanol-d_4 (Table 1), there was a cross-peak between H-3 and H-4 that suggests a *syn* relationship, which corresponds to a *threo* configuration for the hydroxyamino acid, contrary to the X-ray diffraction data. The X-ray diffraction data show that the dihedral angle between H-3 and H-4 is 157° (*anti*), and the distance between H-3 and H-4 is 2.836 Å. This short distance could explain the correlation observed in the NOESY spectra, even if H-3 and H-4 are *anti* oriented. Another explanation for the observed cross-peak between H-3 and H-4 would be the presence of zero-quantum coherence artifacts in the NOESY spectrum, common at mixing times of 100 ms or less for small molecules (MW ≤ 600 Da).[16] In this work, the experiment was conducted with the mixing time of 200 ms and no attempt was made to suppress *J*-cross-peaks.

Table 1. NMR Spectroscopic Data for Compounds 1 and 2 (in Methanol-d_4, 400 MHz)

	1			2		
position	δ_C[a]	δ_H, mult. (*J* in Hz)	NOESY	δ_C[a]	δ_H, mult. (*J* in Hz)	NOESY
1	156.4			157.5		
2						
3	80.7	4.88, dd (2.0, 8.4)	4,18,14	79.4	4.92, dd (1.6, 8.8)	4
4	55.4	4.45, d (8.4)	3,6,20	55.8	4.54, d (8.8)	3
5	171.1			171.3		
6						
7	51.6	4.08, dd (8.0, 4.0)	30,10,11 11	52.3	4.11, dd (6.0, 7.2, 10.0)	30
8	170.8			166.3		
9						
10	128.2	6.71, d (7.6)	7	128.2	6.81, d (7.6)	
11	125.6	6.20, d (7.6)		127.2	6.02, d (7.2)	
12	128.5			129.7		
13	130.2	6.97		130.2	6.98	
14	121.0	7.0		121.0	7.0	
15	117.9	7.0		116.8	7.0	
16	129.4	7.0		131.0	7.0	
17	28.8	2.16, m	18,19	30.2	2.12, dd (2.8, 12.4)	
18	19.6	1.19, d (6.4)		20.6	1.19, d (6.8)	
19	14.2	0.98, d (6.8)		15.2	1.01, d (6.4)	
20						
21						
22	66.3	3.01, dd (6.0, 8.8)		74.8	3.97, dd (2.4, 11.6)	
23	38.2	1.54, m		36.7	1.75, m	
23′		1.42, m			1.34, m	
24	24.9	1.49, m	25, 26	25.8	1.43, m	
25	21.4	0.87, d (5.6)		22.7	0.95, d (6.0)	
26	22.5	0.87, d (5.6)		23.3	0.88, d (6.0)	
27	41.0	2.30, s		43.2	3.24, s	
28	40.8	1.38		43.1	1.33	
28′		1.25			1.28	
29	24.3	1.38	31, 32	26.0	1.37	
30	21.1	0.84, d (6.0)	30,7	21.2	0.86, d (6.8)	
31	22.1	0.78, d (6.0)		22.7	0.83, d (6.8)	

[a]Assignments confirmed by $^1H-^1H$ COSY, $^1H-^{13}C$ HMBC, and NOESY acquired with a mixing time of 200 ms.

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EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were determined with a MQAPF-301 apparatus and are uncorrected. Optical rotations were recorded on a Perkin-Elmer 341 digital polarimeter. Low-resolution ESIMS were recorded on an Agilent LC/MS/MS model 6460. ^{1}H and ^{13}C NMR spectra were recorded at 400.1/100.6 MHz on a Varian Mercury Plus AS-400 spectrometer using $CDCl_3$, methanol-d_4, and DMSO-d_6 as solvents and TMS as internal standard. Thin-layer chromatography was performed on precoated TLC plates (Merck, silica 60 F-254), by spraying with Dragendorff's reagent and 10% H_2SO_4/EtOH, followed by heating.

Plant Material. The root bark of *D. americana* was collected in May 2007 at Santana do Livramento, RS, Brazil (30°,53′,27″ S, 55°,31′,58″ W) and authenticated by Prof. Adelino Alvarez Filho from the Botany Department of Universidade Federal de Santa Maria, RS, Brazil, and where a specimen sample (SMDB 3296) is deposited.

Extraction and Isolation. Dried ground bark (1.8 kg) of *D. americana* was extracted with MeOH in a Soxhlet apparatus for 12 h. The resulting MeOH extract was filtered and concentrated under vacuum to obtain a crude residue (420.4 g). This residue was dissolved in H_2O (300 mL) and acidified with 2 N HCl to pH 2−3. The acidic solution was exhaustively extracted with Et_2O (5 × 300 mL) to yield the acidic ether extract (17.0 g). The aqueous solution was made basic with NH_4OH (pH 8−9) and extracted with Et_2O to yield the basic ether extract (4.9 g). A portion of the basic ether extract (1.5 g) was applied to Si gel CC (120 g), which was eluted with $CHCl_3$ containing increasing amounts of MeOH (up to 20%), to give 15 fractions, A−O. Fractions B and C ($CHCl_3$/MeOH, 99:1) were combined (114.0 mg) and subjected to preparative TLC ($CHCl_3$/MeOH, 99:1, two elutions) to yield franganine (112.0 mg).

*Franganine (**1**):* white powder (MeOH/Et_2O); mp 248−249 °C [lit.[3] mp 248 °C]; TLC 0.60 ($CHCl_3$/MeOH, 98:2); $[\alpha]_D^{25}$ −302 (*c* 0.1, $CHCl_3$); ^{1}H and ^{13}C NMR, see Table 1; ESIMS *m*/*z* 501 $(M + H)^+$.

*Tri-N-methylfranganine Methiodide (**2**).* **2** was obtained from methylation of franganine (10 mg) with MeI (0.5 mL) in acetone (2 mL) and stirring under reflux for 2 h. The mixture was allowed to stand at room temperature overnight to ensure complete crystallization of the quaternary product. Recrystallization in MeOH/$CHCl_3$ (1:1) afforded yellow crystals of tri-*N*-methylfranganine methiodide suitable for X-ray diffraction: mp 196−197 °C; ^{1}H and ^{13}C NMR in methanol-d_4, see Table 1.

X-ray Crystal Structure. A single-crystal X-ray diffraction experiment was undertaken to determine the structure of **2** and its absolute configuration using the resonant scattering of the heavy iodine/chlorine atoms.[14,15] The experiment was carried out at room temperature on a crystal mounted on a glass fiber in a Bruker X8 Kappa APEX II CCD diffractometer using Mo Kα graphite-monochromatized radiation (λ = 0.710 73 Å). In 11.79 h, 2123 frames were collected, from which 80 173 reflection intensities were determined in an orthorhombic unit cell using SAINT[17] to a θ_{max} of 30.62° (0.70 Å resolution). Data were corrected for absorption effects using the multiscan method (SADABS).[18] The structure was solved and refined using the Bruker SHELXTL software package,[19] using the space group $P2_12_12_1$, with $Z = 4$ for the formula unit, $C_{30}H_{50}Cl_3IN_4O_5$. Tables 1−7 (SI), which summarize the sample, crystal, collection, and refinement data of the X-ray experiment, ^{1}H and ^{13}C NMR spectra (in $CDCl_3$, methanol-d_4, and DMSO-d_6), and GIAO ^{13}C NMR chemical shift calculation on franganine (**1**) are given as Supporting Information.

ASSOCIATED CONTENT

Supporting Information

Crystallographic data have been deposited at the Cambridge Crystallographic Data Centre under CCDC deposition number CCDC 873084. Copies of the data can be obtained free of charge on application to the CCDC, 12 Union Road, Cambridge CB2 IEZ, UK. Fax: +44 (0)1223 762911 or e-mail: deposit@ccdc.cam.ac.uk. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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