ORGANIC LETTERS

2003
Vol. 5, No. 5
673−676

Simplakidine A, a Unique Pyridinium Alkaloid from the Caribbean Sponge *Plakortis simplex*†

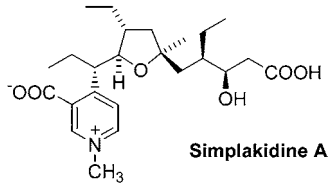
Claudio Campagnuolo,‡ Caterina Fattorusso,‡ Ernesto Fattorusso,‡ Angela Ianaro,§ Barbara Pisano,§ and Orazio Taglialatela-Scafati*,‡

Dipartimento di Chimica delle Sostanze Naturali and Dipartimento di Farmacologia Sperimentale, Università di Napoli "Federico II", Via D. Montesano 49, I-80131 Napoli, Italy

scatagli@unina.it

Received December 9, 2002

ABSTRACT



Simplakidine A

Simplakidine A, a unique 4-alkyl-substituted pyridiunium alkaloid, has been isolated from the Caribbean sponge *Plakortis simplex*. The stereostructure of simplakidine A has been determined using MS and NMR data, molecular mechanics, and an extension of the *J*-based configuration analysis. Data about the growth-inhibition activity of simplakidine A are reported.

During the last six years, our research group has devoted considerable effort to the chemical investigation of the Caribbean sponge *Plakortis simplex*, resulting in the discovery of many structurally unique and biologically active metabolites.[1] Significant examples include the immunosuppressor glycosphingolipids plakosides,[1a] the antimalarial cycloperoxide plakortin,[1b,k] and all of the series of cytotoxic polyketides of the plakortin family.[1g] Recently, we started on the analysis of the most polar fractions obtained from the organic extract of *P. simplex* and isolated plakohypaphorines A−C,[1l] the first natural iodoindoles. Further inspection of these fractions led to the isolation of a novel pyridinium alkaloid named simplakidine A (**1**); its stereostructure elucidation and biological activity are reported herein.

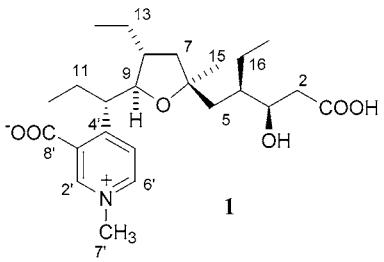
A specimen of *P. simplex* (Demospongiae, family Plakinidae, order Homosclerophorida; 57 g, dry weight after extraction) was homogenized and exhaustively extracted first with methanol and then with chloroform. The methanol extract was partitioned between *n*-BuOH and water, and subsequently the combined organic phases were subjected to chromatography over reverse-phase silica (RP18). The most polar fractions were first separated over silica gel and then rechromatographed by reverse-phase HPLC to finally yield 1.4 mg of pure simplakidine A (**1**).

The molecular formula $C_{24}H_{37}NO_6$ was assigned to simplakidine A (**1**) on the basis of the HR-nanospray-MS spectrum[2] acquired in the positive ion mode ($[M + H]^+$:

† This paper is dedicated to the memory of Professor D. John Faulkner.
‡ Dipartimento di Chimica delle Sostanze Naturali.
§ Dipartimento di Farmacologia Sperimentale.

(1) (a) Costantino, V.; Fattorusso, E.; Mangoni, A.; Di Rosa, M.; Ianaro, A. *J. Am. Chem. Soc.* **1997**, *119*, 12465. (b) Cafieri, F.; Fattorusso, E.; Taglialatela-Scafati, O.; Ianaro, A. *Tetrahedron* **1999**, *55*, 7045. (c) Cafieri, F.; Fattorusso, E.; Taglialatela-Scafati, O.; Ianaro, A. *Tetrahedron* **1999**, *55*, 13831. (d) Costantino, V.; Fattorusso, E.; Mangoni, A.; Di Rosa, M.; Ianaro, A. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 271. (e) Costantino, V.; Fattorusso, E.; Mangoni, A.; Di Rosa, M.; Ianaro, A. *Tetrahedron* **2000**, *56*, 1393. (f) Costantino, V.; Fattorusso, E.; Imperatore, C.; Mangoni, A. *Tetrahedron* **2000**, *56*, 3781. (g) Fattorusso, E.; Taglialatela-Scafati, O.; Di Rosa, M.; Ianaro, A. *Tetrahedron* **2000**, *56*, 7959. (h) Costantino, V.; Fattorusso, E.; Imperatore, C.; Mangoni, A. *Tetrahedron* **2001**, *57*, 4045. (i) Costantino, V.; Fattorusso, E.; Imperatore, C.; Mangoni, A. *Eur. J. Org. Chem.* **2001**, 4457. (j) Campagnuolo, C.; Fattorusso, E.; Taglialatela-Scafati, O.; Ianaro, A.; Pisano, B. *Eur. J. Org. Chem*, **2002**, 61. (k) Fattorusso, E.; Parapini, S.; Campagnuolo, C.; Basilico, N.; Taglialatela-Scafati, O.; Taramelli, D. *J. Antimicrob. Chemother.* **2002**, *50*, 833. (l) Campagnuolo, C.; Fattorusso, E.; Taglialatela-Scafati, O. *Eur. J. Org. Chem.* **2003**, 284.

10.1021/ol027437r CCC: $25.00
Published on Web 02/08/2003



1

m/*z* 436.2687, calcd *m*/*z* 436.2699; $[M + 2Na - H]^+$: *m*/*z* 481.2630, calcd *m*/*z* 481.2625). Proton resonances showed distribution in three different regions of the ^1H NMR spectrum of **1** (500 MHz, CD_3OD, Table 1): a series of partially overlapped multiplets were confined between δ 0.80 and 2.40, four signals were present between δ 3.70 and 4.40, whereas two mutually coupled doublets (δ 8.20 and 8.68) and a singlet (δ 8.80) resonated in the region of the spectrum characteristic of aromatic signals. The presence of an aromatic chromophore in the structure of simplakidine A (**1**) was further indicated by the UV absorption at λ_{max} 272 nm and by the presence of five signals between δ 129 and 163 in the ^{13}C NMR spectrum of **1** (62 MHz, CD_3OD, Table 1). The remaining 19 carbon signals present in the spectrum were assigned, with the aid of DEPT experiments, to five methyls, six methylenes, five methines, and one unprotonated carbon, all of the sp^3 type, while two unprotonated carbons resonated at δ 169.3 and 181.6. These latter resonances were ascribable to one carboxylate and one carboxylic acid group, as suggested by IR absorptions at ν_{max} 1625 and 1710 cm^{-1}, respectively.

Table 1. NMR Data of Simplakidine A (**1**) Recorded in CD_3OD

carbon no.	δ H, mult., *J* in hertz	δ C, mult.
1		181.6, C
2a	2.41, dd, 14.8, 3.0	42.5, CH_2
2b	2.27, dd, 14.8, 10.2	
3	4.39, ddd, 10.2, 3.0, 1.5	72.1, CH
4	1.50, overlapped	45.1, CH
5a	1.85, dd, 14.1, 7.4	43.1, CH_2
5b	1.49, overlapped	
6		83.9, C
7a	2.00, overlapped	46.9, CH_2
7b	1.56, overlapped	
8	2.14, overlapped	42.7, C
9	3.72, dd, 8.1, 1.3	86.0, CH
10	3.95, ddd, 10.4, 3.9, 1.3	46.4, CH
11a	2.12, overlapped	21.6, CH_2
11b	1.99, overlapped	
12	0.80, t, 7.3	12.6, CH_3
13a	1.57, overlapped	24.0, CH_2
13b	1.32, overlapped	
14	0.97, t, 7.3	12.7, CH_3
15	1.11, s	26.3, CH_3
16a	1.69, m	27.2, CH_2
16b	1.31, overlapped	
17	1.00, t, 7.3	13.1, CH_3
2′	8.80, s	144.6, CH
3′		142.7, C
4′		163.0, C
5′	8.20, d, 6.2	129.6, CH
6′	8.68, d, 6.2	144.4, CH
7′	4.34, s	48.0, CH_3
8′		169.3, C

Inspection of $^1H-^1H$ COSY NMR spectrum of **1** allowed us to arrange all the multiplets of the sp^3 region within two spin systems (Figure 1): the first fragment, spanning from C-2 to C-5, comprises the oxymethine C-3 (δ_H 4.39) and an ethyl branching at C-4; the second fragment, connecting C-7 to C-12, possesses an ethyl group linked at C-8 and encompasses the methines C-9 (δ_H 3.72) and C-10 (δ_H 3.95). With the establishment of all the one-bond $^1H-^{13}C$ connectivities through the HMQC spectrum, the correct interpretation of gradient-HMBC data (Figure 1) became possible. Diagnostic g-HMBC cross-peaks of the unprotonated oxygenated carbon at δ 83.9 (C-6) with H_3-15 (δ 1.11) and with both H_2-7 and H_2-5 allowed us to join the two fragments deduced above. Moreover, the g-HMBC cross-peak H-9/C-6 is indicative of the linkage of both C-6 (δ 83.9) and C-9 (δ 86.0) to the same oxygen atom, thus building a tetrahydrofuran ring. Finally, cross-peaks of H_2-2 and H-3 with the signal at δ 181.6 confirmed the linkage of the carboxylic group at C-2.

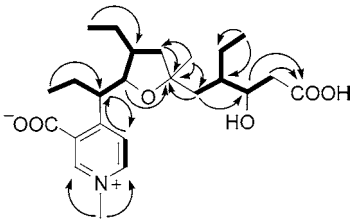


Figure 1. Spin systems deduced by COSY spectrum (bold linkages) and key g-HMBC correlations (H → C arrows) of simplakidine A.

With these data in our hands and bearing in mind the molecular formula, the final assembling of the carbon framework of simplakidine A (**1**) required the elucidation of an aromatic $C_7H_6NO_2$ subunit, linked at C-10 and comprising a carboxylate group. This fragment was disclosed to be a 4-substituted *N*-methyl-nicotinic acid on the basis of g-HMBC evidence corroborated by excellent agreement with literature values.[3] In particular, the *N*-methyl singlet at δ 4.34 (H_3-7′) showed g-HMBC cross-peaks with two protonated carbons at δ 144.6 (C-2′, HMQC coupled with δ_H 8.80, s) and 144.4 (C-6′, HMQC coupled with δ_H 8.68, d, $J = 6.2$ Hz), thus ruling out substitution at both C-2′ and C-6′. The

(2) Wilm, M. S.; Mann, M. *Int. J. Mass Spectrom. Ion Processes* **1994**, *136*, 167.

(3) Sciuto, S.; Chillemi, R.; Piattelli, M. *J. Nat. Prod.* **1988**, *51*, 322.

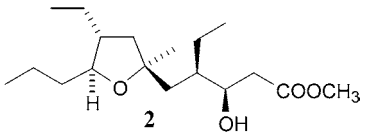
spatial proximity of H_3-7′ with both H-2′ and H-6′, evidenced through the ROESY experiment, further supported the above conclusion.

Since H-6′ exhibited vicinal coupling with the signal at δ 8.20 (H-5′, d, J = 6.2 Hz), only the 3,4-disubstitution remained possible. The g-HMBC cross-peaks H-10/C-5′ and H-5′/C-10 allowed us to link C-10 at the pyridinium carbon C-4′ and, therefore, the remaining carboxylate group must be placed at C-3′.

Taking into account all the above data, the planar structure of simplakidine A (**1**) was completely assembled. The nonaromatic part of this molecule closely parallels the structure of plakortethers A−E (e.g., plakortether B, **2**),[1j] a class of polyketides structurally related to plakortin that we recently obtained from the apolar fractions of the organic extract of *P. simplex*.

Of the sp^3 carbon atoms of simplakidine A (**1**), 6 of the 17 are asymmetric centers, and determination of their stereochemistry represented a particularly challenging task. Unfortunately, only one of these carbons (C-3) appeared to be amenable to derivatization with chiral auxiliary reagents, a procedure generally used to determine the absolute stereochemistry of natural products.[4]

As a consequence, stereochemical elucidation of simplakidine A (**1**) might greatly rely on the comparison of its spectral data with those of a model compound, plakortether B (**2**), whose (3*R*,4*R*,6*R*,8*R*,9*S*)-configuration has been unambiguously defined by a three-step semisynthesis from plakortin.[1j] In particular, we observed that the small coupling constant $J_{H-3/H-4}$ (1.5 Hz) of simplakidine A (**1**), as well as the chemical shift values of the relevant protons, are almost identical to the corresponding parameters measured for plakortether B (**2**) in the same solvent. This strongly suggested that simplakidine A (**1**) and the model compound plakortether B (**2**) actually share the same relative configuration about the C-3/C-4 bond. The relative configuration was promoted to the absolute configuration since standard application of a modified Mosher method[5] to the secondary alcohol C-3 of simplakidine A (see Supporting Information) enabled us to assign the (*R*)-configuration to this center, the same of plakortether B (**2**). Consequently, the (*R*)-configuration at C-4 of the model compound **2** was extended to C-4 of simplakidine A (**1**).



2

The relative configuration at the three tetrahydrofuran asymmetric centers C-6, C-8, and C-9 of **1**, easily deduced on the basis of the ROESY cross-peaks of H_3-15 with both H-9 and H_2-13, is the same as that detected for **2**. Also in this case, useful information to upgrade the relative configuration of this region to the absolute configuration came from comparison of ^{1}H and ^{13}C NMR spectra of **1** with those of the model compound, plakortether B (**2**). In this regard, ^{1}H coupling constants and ^{1}H and ^{13}C chemical shifts of the entire C-3/C-6 fragment of simplakidine A (**1**) appeared to be almost superimposable to the parallel values obtained for plakortether B (**2**) in the same solvent (see Supporting Information). This is a strong indication that the two molecules share the same relative configuration of this fragment. Therefore, the establishment that both C-3 and C-4 of simplakidine A (**1**) possess the same absolute configuration as plakortether B (**2**) allowed the absolute configuration of C-6 and, consequently, of C-8 and C-9 of **1** to be assigned as that of the corresponding carbons of **2** (6*R*,8*R*,9*R*).

Determination of relative geometry around the C-9/C-10 bond required additional spectral analysis. The small value of $^3J_{H-9/H-10}$ (1.3 Hz) indicates that a dominant staggered rotamer exists around the C-9/C-10 axis, and this constitutes the first prerequisite for applying the *J*-based configuration analysis recently developed by Murata et al.[6] This NMR-based method allows elucidation of the relative configuration in acyclic structures on the basis of $^3J_{H,H}$ and $^{2,3}J_{C,H}$ values. In our case, the four required heteronuclear coupling constants (Figure 2) were qualitatively evaluated through analysis of the PS-HMBC spectrum.[7]

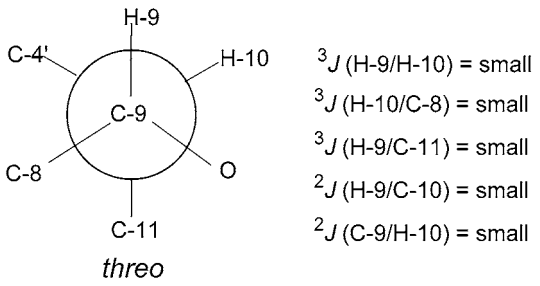


Figure 2. Application of Murata's method to the C-9/C-10 bond of simplakidine A (**1**).

The obtained pattern of *J* values appeared perfectly consistent and indicated a *threo* stereochemical relationship between C-9 and C-10 of simplakidine A (**1**), as reported in Figure 2, thus implying the (*S*)-stereochemistry at C-10. Anyway, since the Murata method has been originally developed and reported only to determine relative stereochemistries between oxygen- and methyl-substituted[6] (and successively extended to halogen-substituted[8]) methines, the above application needed to be verified by additional independent experimental evidence.

To this aim, we tried to take advantage of some key cross-peaks of the ROESY spectrum of **1**, namely, H-5′/H-9, H-5′/

(4) Seco, J. M.; Quiñoá, E.; Riguera, R. *Tetrahedron: Asymmetry* **2001**, *12*, 2915.
(5) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092.
(6) Matsumori, N.; Kaneno, D.; Murata, M.; Nakamura, H.; Tachibana, K. *J. Org. Chem.* **1999**, *64*, 866.
(7) Matsumori, N.; Murata, M.; Tachibana, K. *Tetrahedron* **1995**, *51*, 12229.
(8) Ciminiello, P.; Fattorusso, E.; Forino, M.; Di Rosa, M.; Ianaro, A.; Poletti, R. *J. Org. Chem.* **2001**, *66*, 578.

H_2-11, and H-5′/H_2-13, verifying if these correlations were compatible with only one of the two possible relative geometries about the C-9/C-10 bond. The two alternative diastereomers ((3*R*,4*R*,6*R*,8*R*,9*R*,10*S*)- and (3*R*,4*R*,6*R*,8*R*,9*R*,10*R*)-) of simplakidine A (**1**) were both subjected to a conformational search (InsightII, Accelrys, San Diego) by systematically varying the torsional angles H-9/C-9/C-10/H-10 and H-10/C-10/C-4′/C-5′ in order to check all the possible orientations of the pyridinium ring with respect to the tetrahydrofuran ring. Resulting conformations were geometrically optimized using the esff force field and a quasi-Newton−Raphson minimization method until the maximum RMS derivative was less than 0.001 kcal/mol. A distance-dependent dielectric constant set to the value of methanol ($\epsilon = 32.70$) was used during the calculations to simulate the same environment embedding the molecule during the NMR analysis. The obtained conformers were filtered taking into account both of the following criteria: (i) interatomic distances in agreement with the above cited ROESY dipolar couplings and (ii) a dihedral angle H-9/C-9/C-10/H-10 in accordance with the measured value of $^3J_{H-9/H-10}$ (1.3 Hz). Interestingly, as a result of this analysis, only the (3*R*,4*R*,6*R*,8*R*,9*R*,10*S*)-diastereomer appeared to fit all of the experimental evidence. In addition to confirming the relative configuration at C-9/C-10 and, therefore, the assignment of (*S*)-configuration to the latter carbon atom, this study suggests that, most likely, the Murata *J*-based configuration analysis has a broader application than that described by the author.[6] In our case, for example, this method gave a correct prediction of the relative configuration for a carbon linking an electron-withdrawing aromatic ring (pyridinium).

The incredible pool of secondary metabolites disclosed for the sponge *P. simplex* is now enriched with simplakidine A (**1**), a unique example of a pyridinium alkaloid. This molecule possesses a C_{17} polyketide moiety, sharing with plakortin the carbon backbone and the absolute stereochemistry of the corresponding asymmetric centers, in turn linked to a pyridinium ring. Although pyridinium alkaloids are not rare in marine sponges, the structural diversity within this class of compounds is somewhat limited. One type of pyridinium derivatives comprises macrocyclic oligomeric structures with linear alkyl chains linked at positions C-3 and N-1 of the pyridinium unit (e.g., halitoxin).[9] Another structural class includes homarine or trigonelline derivatives substituted at C-3 or C-2, respectively, with simple alkyl chains (e.g., sulcatin).[10] To the best of our knowledge, simplakidine A (**1**) constitutes a unique example of a trigonelline nucleus substituted at position C-4 with a complex polyketide-deriving moiety.

Simplakidine A (**1**) exhibited weak cytotoxicity toward RAW 264-7 (murine macrophages) with 30% growth inhibition at 60 μg/mL. Plakortether B (**2**) showed, against the same cell line, a much higher activity (50% inhibition at 9.5 μg/mL). Most likely, simplakidine A (**1**) is too polar to cross the cell membrane.

Acknowledgment. Financial support was provided by M.I.U.R., PRIN 2001. NMR and MS facilities were provided by "Centro di Ricerca Interdipartimentale di Analisi Strumentale". The authors thank Prof. J. R. Pawlik for the opportunity to participate in the expedition to the Caribbean Sea, during which *P. simplex* was collected, and Prof. M. Pansini for identifying the organism.

Supporting Information Available: NMR spectra, tables of one- and two-dimensional NMR data, physical constants, isolation procedure, and molecular mechanics details for Simplakidine A (**1**). This material is available free of charge via the Internet at http://pubs.acs.org.

OL027437R

(9) Schmitz, F. J.; Hollenabeak, K. H.; Campbell, D. C. *J. Org. Chem.* **1978**, *43*, 3916.
(10) Aiello, A.; Fattorusso, E.; Menna, M.; Iuvone, T. *J. Nat. Prod.* **2000**, *63*, 517.