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Assignment of the absolute stereochemistry of oxazinin-1: application of the 9-AMA shift-correlation method for β-chiral primary alcohols

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Abstract—Reported herein is the assignment of the absolute stereochemistry of oxazinin-1, a cytotoxic compound recently isolated from the digestive glands of *Mytilus galloprovincialis* of the North Adriatic Sea. The recent 9-AMA shift-correlation method for assigning absolute configuration to β-chiral primary alcohols was employed and preparatory Molecular Mechanics calculations were carried out.

1. Introduction

Worldwide infestation of bivalve shellfish by microalgal toxins poses serious threats to both human health and shellfish industries. In the course of our studies on marine toxins from edible mussels of the North Adriatic Sea, we have recently reported the isolation from the digestive glands of *Mytilus galloprovincialis* and the structural elucidation of the unprecedented toxic oxazinin-1 (**1**), together with two analogues oxazinin-2 (**2**), and -3 (**3**)[1] (Scheme 1). The determination of the structure and the relative stereochemistry of **1**–**3** was based on spectroscopic evidence including extensive 2D NMR and Molecular Mechanics (MM) calculations. Oxazinin-1, investigated for cytotoxic activity, was shown to inhibit the growth of WEHI 164 (murine fibrosarcoma) and J774 (murine monocyte/macrophage) cell lines in vitro.[1] On account of the unprecedented structural features of **1** and its bioactivity, it was considered worthwhile to characterize it as thoroughly as possible, the assignment of the absolute configuration being an unavoidable step.

2. Results and discussion

On account of the very limited amount of available material, the best way to achieve this goal appeared to be the derivatization of oxazinin-1 with enantiomers of an appropriate auxiliary reagent followed by the comparison of the ^{1}H NMR spectrum of the resulting diastereomers. In this regard, it is to be noted that **1** has three chiral centers at C2, C5, and C6, respectively (the relative stereochemistry was established as 2*S*,5*R*,6*R*/2*R*,5*S*,6*S*[1]), none of which directly supporting a hydroxyl, primary amine, or carboxylic acid group, generally required for the above type of analysis. However, oxazinin-1 contains a β-chiral primary alcoholic group and a method for the assignment of the absolute configuration of this functionality has been recently proposed by Riguera et al.[2] This method utilizes 9-anthrylmethoxyacetic acid (9-AMA, **4**) as an auxiliary reagent (Scheme 2). 9-AMA Derivatization, when compared with that of the usual shift reagents (MPA, MPTA etc.), assures a greater efficiency in converting the aromatic shielding into high-field shifts, due to a reduction of the flexibility of the molecule.

According to the authors, the above method can be safely employed for the assignment of the absolute configuration of most β-chiral primary alcohols; however, if the chiral center is part of a ring, as in **1**, MM or ab initio calculations must be performed in order to ensure that the molecule exhibits appropriate conformational behavior. In particular, the rotation around O–C_α–CO–O, C_α–CO–O–C1′, CO–O–C1′–C2′ and O–C1′–C2′–L1 bonds must be analyzed (Fig. 1).

To keep the study within practical limits, Riguera et al.[1] suggested to override the rotational mobility around Cα–CO and CO–O bonds by assuming these skeletal fragments to be almost rigid.[3–5] The remaining two bonds (C1′–C2′ and O–C1′) are very flexible and each bond can give rise to three low-energy rotamers (two *gauche* and one

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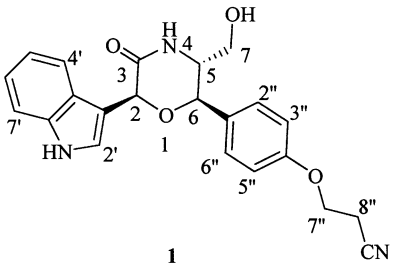

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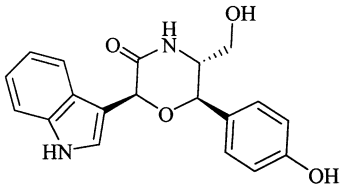

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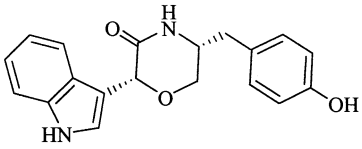

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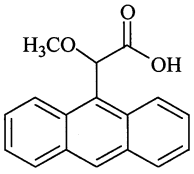
Scheme 1.



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Scheme 2.

anti), thus resulting in nine rotamers in all. Among these, three were established to be the main ones ($g-/a$, a/a and $g+/a$), all having an *anti* conformation around the O–C1′ bond and, therefore, rotation around this bond does not need to be considered. As shown in Fig. 1, only the a/a conformer allows the assignment of the stereochemistry at C2, since only in this case can the anthryl ring differently shield L1 and L2 in (*R*)-(**5**) and (*S*)-9-AMA (**6**) derivatives (Scheme 3), respectively. This results in appreciable average shielding increments in ^{1}H NMR spectra. As a consequence, the method of Riguera et al. is considered reliable providing that the a/a conformer is the most populated one.

The predominance of the a/a form is directly connected with a significant difference of conformational energy between *gauche* and *anti* forms around the C1′–C2′ bond (ΔE^{ag}); consequently, the reliability of the method requires the calculation of ΔE^{ag}. It is to be noted that, following the Riguera et al procedure, the underestimation of the stability of the *anti* forms associated with the use of MM force fields with respect to ab initio calculations can be balanced by the introduction of a correction factor of -5.8 KJ mol^{-1} to the calculated ΔE^{ag}.

Taking into account the above prerequisites, we performed a systematic conformational search (SCS) on **5** and **6** (by assuming 2*S*,5*R*,6*R*configuration for oxazinin-1) to generate all theoretically possible conformations of these compounds. In considering the rotatable angles to be scanned during SCS, we applied all the simplifications previously reported in the method of Riguera et al. Since in **5** and **6** the rotation around C5–C7 bond (corresponding to C2′–C1′ in model reported in Fig. 1) can be affected by

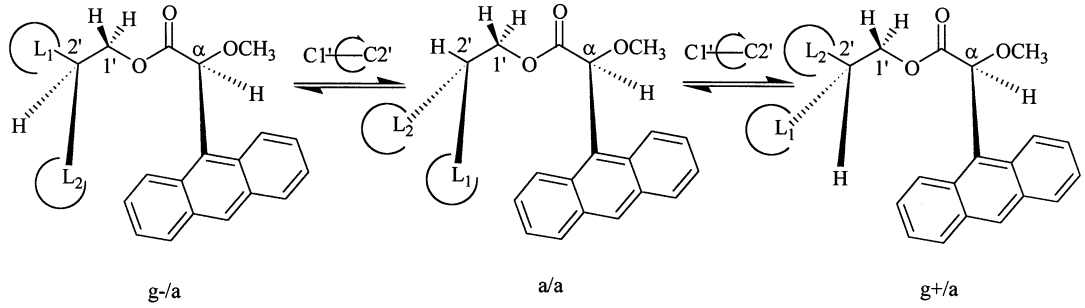
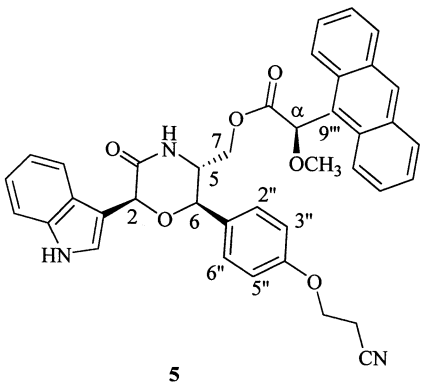


Figure 1. Main rotamers in the alcohol moiety of a 9-AMA ester of a β-chiral primary alcohol by rotation around the C1′–C2′ bond. The symbols $g+/a$, a/a, and $g-/a$ identify each conformer, defining rotation around C1′–C2′ bond and O–C1′ bond, respectively; $g+$ or $g-$ means a 120° turn (clockwise or counterclockwise, respectively) from the a position.



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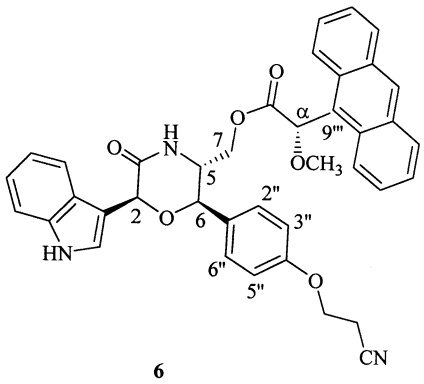

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Scheme 3.

Table 1. Comparison of MM (cff91) data (KJ mol^{-1}, values corrected by -5.85 KJ mol^{-1} as suggested by Riguera et al.) for the main conformers of **5** and **6**

Conformer	**5**	**6**
g/*a*[a]	11.45	0.58
a/*a*	0.00	0.00

[a] *g*+/*a* for **5**, *g*−/*a* for **6**.

Table 2. Selected ^{1}H NMR data of **5** and **6** (CD_3CN)

Position	**5** (ppm)	**6** (ppm)	**1** (ppm)
6	4.76	4.71	4.61
2″-6″	7.28	7.26	7.20
3″-5″	6.86	6.85	6.88
7a	3.85	3.76	3.26
7b	4.22	4.08	3.43

tetrahydrooxazinone ring conformation and by rotation around C6–C1″ and C_α–C9‴ bonds, our SCS studies included also these conformational parameters. The resulting conformations were geometrically optimized by molecular mechanics calculations (cff91 force field[6]) and their energies were evaluated in order to establish which conformer is the most stable one (Table 1).

Following the procedure of Riguera et al., our molecular mechanics results indicated the *a*/*a* form as the most stable for both (*R*)- and (*S*)-9-AMA esters of oxazinin-1, and consequently the anthryl ring can differently shield L1 and L2 allowing the assignment of the absolute configuration of the β-chiral primary alcoholic group.

Once the MM calculations showed (*R*)- and (*S*)-9-AMA-oxazinin esters to possess the conformational prerequisites for the application of the Riguera et al. method, they were synthesized by treatment of **1** with (*R*)- and (*S*)-9-anthrylmethoxyacetic acid chlorides in the presence of triethylamine. HRFAB MS spectra (negative ion mode) of **5** and **6** showed the expected molecular ions at *m*/*z* 637.2217 and 637.2219 (calcd 637.2213, error max 25 ppm) respectively corresponding to $C_{39}H_{32}N_3O_6$. In the ^{1}H NMR spectrum of both derivatives the methylene protons at C-7 were downfield shifted (from δ 3.26/3.43 in **1** to 3.85/4.22 in **5** and to 3.76/4.08 in **6**, respectively).

The stereochemical assignment was based on the chemical shift differences of the protons at C6, C2″ (6″), and C3″ (5″). As reported in Table 2, the observed values for compound **5** are down field shifted with respect to the corresponding ones of **6**. As shown in Fig. 2, where the shielding effect of anthryl ring on the above protons are clear, the observed NMR data are fully consistent with the absolute stereochemistry 2*S*,5*R*,6*R* for oxazinin-1.

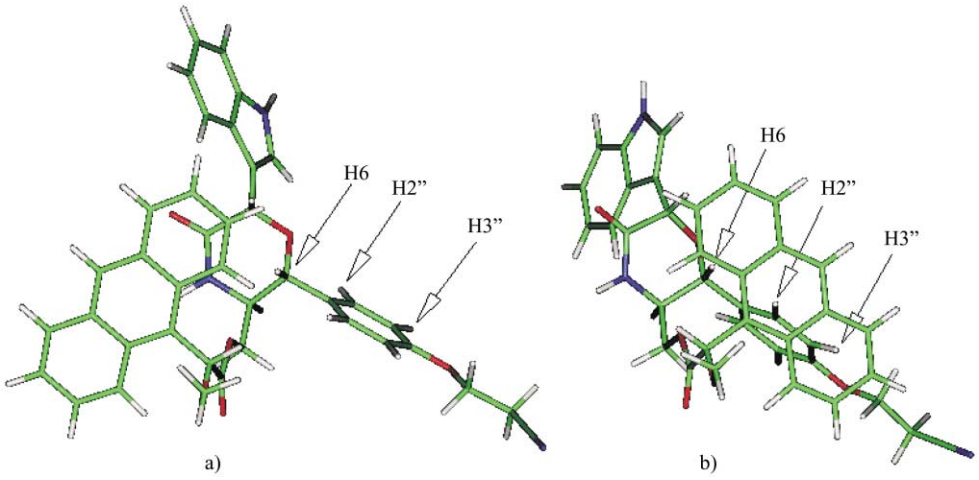

Figure 2. (a) Low energy rotamer of (*R*)-9-AMA ester of (2*S*,5*R*,6*R*) oxazinin-1. (b) Low energy rotamer of (*S*)-9-AMA ester of (2*S*,5*R*,6*R*) oxazinin-1.

3. Experimental

3.1. General

^{1}H NMR spectra were measured on a Brüker AMX-500 spectrometer and the solvent was used as internal standard (CD_3CN: ^{1}H δ 1.93). FAB MS spectra were obtained at 70 eV on a Kratos MS 50 mass spectrometer.

3.2. Molecular Modeling

All molecular mechanics calculations were run on a Silicon Graphics Indigo2 workstation. Initial molecular geometries were originated from the Builder Module of Insight2000 (MSI, San Diego). SCSs were carried out on the structures of (*R*)-9-AMA and (*S*)-9-AMA esters of oxazinin-1 (**5** and **6**, respectively), using the SEARCH routine within Sybyl 6.6 (Tripos, Saint Louis). All torsional angles were scanned using 30° increments within a 0–359° range. In the case of the torsional angle about the ester bond, the increment was set to 180°. When the torsional angle was associated to the rotation of the *para*-substituted phenyl ring, the absolute interval of variation was restricted to 180°. Torsional angles included in rings were analyzed using the Ring Search module, by increment of 10° using 0–359° as interval of variation. The permissible variance on the distance between the ring closure atoms was set to 0.3 Å, while the permissible variance on the valence angles about the ring closure atoms was set to 10°. Starting conformations were considered with zeroed torsional angles. A 0.750 Van der Waals Radii Scaling Factor was used to soften steric contacts in the rigid rotamers. All theoretically possible conformations were generated without evaluating their conformational energy. Resulting structures files were transferred in Insight2000 software to perform energetic calculations. All the conformations were geometrically optimized (Discover module) using the cff91 force field.[6] Energy minimizations were performed in vacuo with a distance dependent dielectric constant ($\epsilon=1^*r$) using conjugate gradient as minimization algorithm[7] until the maximum RMS derivative was less than 0.001 kcal mol^{-1}. Resulting output conformers were clustered into a smaller number of families according to the values of their torsional angles and energetically evaluated.

3.2.1. (2*S*,5*R*,6*R*) Oxazinin-1 (*R*)-9-AMA ester 5. An excess of oxalyl chloride was added to a solution of (*R*)-9-AMA (2.4 mg, 0.0061 mmol), $[\alpha]_D^{25}=-131.2$ (EtOH) in dichloromethane at 0°C and the mixture was stirred for 2 h at room temperature. The mixture was evaporated under nitrogen flow, dissolved in DMF (100 μl) and added of a solution of oxazinine-1 (7.4 mg, 0.028 mmol), $[\alpha]_D^{25}=+9.0$ (MeOH) and triethylamine (10 μl) in DMF (100 μl). The resultant mixture was stirred overnight at room temperature. H_2O and 1 M HCl were added (pH 7) and the mixture was extracted with EtOAc. The ester was contained in the organic layer. HRFAB MS (negative ion mode) $[M-H]^-$ 637.2217 (calcd 637.2213, error max 25 ppm). ^{1}H NMR (500 MHz, CD_3CN) δ 4.00 (1H, m, H-5), 4.76 (1H, d, J=9.2 Hz, H-6), 3.85 (1H, m, H-7a), 4.22 (1H, m, H-7b), 7.28 (2H, d, J=8.6 Hz, H-2″, 6″), 6.86 (2H, d, J=8.6 Hz, H-3″-5″).

3.2.2. (2*S*,5*R*,6*R*) Oxazinin-1 (*S*)-9-AMA ester 6. An excess of oxalyl chloride was added to a solution of (*S*)-9-AMA (2.8 mg, 0.0072 mmol), $[\alpha]_D^{25}=+133.7$ (EtOH) in dichloromethane at 0°C and the mixture was stirred for 2 h at room temperature. The mixture was evaporated under nitrogen flow, dissolved in DMF (100 μl) and added of a solution of oxazinine-1 (9.4 mg, 0.035 mmol), $[\alpha]_D^{25}=+9.0$ (MeOH) and triethylamine (10 μl) in DMF (100 μl). The resultant mixture was stirred overnight at room temperature. H_2O and 1 M HCl were added (pH 7) and the mixture was extracted with EtOAc. The ester was contained in the organic layer. HRFAB MS (negative ion mode) $[M-H]^-$ 637.2219 (calcd 637.2213, error max 25 ppm). ^{1}H NMR (500 MHz, CD_3CN) δ 3.85 (1H, m, H-5), 4.71 (1H, d, J=8.6 Hz, H-6), 3.76 (1H, m, H-7a), 4.08 (1H, m, H-7b), 7.26 (2H, d, J=8.6 Hz, H-2″, 6″), 6.85 (2H, d, J=8.6 Hz, H-3″-5″).

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