Oxazinin-1, -2 and -3 − A Novel Toxic Compound and Its Analogues from the Digestive Glands of *Mytilus galloprovincialis*

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A chemical investigation of the digestive glands of *Mytilus galloprovincialis* from North Adriatic Sea led to the isolation of three novel compounds, oxazinin-1 (**1**), oxazinin-2 (**2**) and oxazinin-3 (**3**). Their structures, including the relative stereochemistry, were established by spectroscopic means including extensive 2D NMR and molecular mechanics calculations. The new compounds were analyzed for cytotoxic activity and oxazinin-1 (**1**) was shown to inhibit the growth of WEHI 164 and J774 cell lines in vitro.

Introduction

The escalating occurrence of poisoning after ingestion of toxic edible shellfish raises serious concerns for public health, and, in addition, causes damage to the reputation of the fishing industry. In order to prevent or minimize such damage, periodical monitoring of shellfish and structural elucidation of the involved toxins is a prerequisite. To this aim our research group has undertaken a study[1] on this kind of marine toxins through periodical analyses of shellfish from the coasts of Emilia Romagna (Italy) − an important shellfish production area − where algal bloom phenomena occur seasonally. As a part of this continuing study, we wish to report herein the isolation and structural elucidation of a novel cytotoxin, oxazinin-1 (**1**) and two structurally related compounds, oxazinin-2 (**2**) and oxazinin-3 (**3**), isolated from toxic blue mussels collected in December 1998. They are characterized by unique structural features which, to the best of our knowledge, have not been found in any other naturally occurring compound.

Results and Discussion

Compounds **1**−**3** were isolated from the digestive glands of *Mytilus galloprovincialis*, collected in Autumn 1998 from one sampling site located along the coasts of Cesenatico (Adriatic Sea) during a period of high toxicity. The animals were found to be toxic by the official mouse bioassay method for DSP (Diarrhetic Shellfish Poisoning).[2]

The homogenized digestive glands of *Mytilus galloprovincialis* were extracted with acetone and gave a residue which was dissolved in MeOH/H_2O (8:2) and extracted with *n*-hexane. The aqueous methanolic layer was subsequently partitioned between MeOH/H_2O (4:6) and CH_2Cl_2 to give a dichloromethane-soluble material which was then separated by MPLC on an ODS column using a solvent gradient system from 60% to 100% of methanol. The fraction eluted with 90% of methanol was further separated on a HW-40 SF column to afford a mixture which was purified by repeated direct- and reverse-phase HPLC. Oxazinin-1, -2 and -3 were isolated in pure form and investigated for cytotoxic activity on WEHI 164 (murine fibrosarcoma) and on J774 (murine monocyte/macrophage) cell lines in vitro. Oxazinin-1 **(1)** inhibited the growth of both the cell lines evaluated at 72 h with IC_{50} values of 16.5 μg/mL and 10.9 μg/mL, respectively.

The molecular formula of **1** was established as $C_{22}H_{21}N_3O_4$, indicative of 14 degrees of unsaturation, from the FAB MS spectrum (negative ion mode), which showed a pseudomolecular ion peak $[M - H]^-$ at m/z = 390, and by NMR spectroscopic data (Table 1). The low-field region of the ^1H NMR spectrum contained a pattern of six signals, which, according to literature data,[3] was assigned to an indole ring substituted at C-3 with a methine group resonating at δ = 5.59, the latter being long-range coupled with the indole proton at δ = 7.29 (H-2′). The ^1H NMR spectrum also contained two isolated spin systems; with the help of ^1H,^1H COSY, HOHAHA and HMQC spectroscopic data, they were confidently identified as: i) a segment comprising just two adjacent methylene groups (−C-7′′−C-8′′−); ii) a partial structure formed by a *para*-disubstituted phenyl ring linked to the moiety starting from C-6 up to a primary OH at C-7 and H−N-4 respectively, this last function being part of an amide group as indicated by the strong IR absorption band at 1661 cm^{-1} and by the ^{13}C NMR resonance at δ = 170.1 (C-3). The presence of aromatic chromophores was confirmed by UV absorption bands at λ_{max} = 216 (ε = 46000), 270 (ε = 5700), 277 (ε = 5500) and 286 nm (ε = 3500) as well as by resonances of the pertinent carbon atoms in the low-field region of the ^{13}C NMR spectrum.

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Table 1. ^{13}C and ^{1}H NMR spectroscopic data of **1** (CD_3CN) with $^{1}H,^{1}H$ COSY and $^{1}H,^{13}C$ HMBC correlations

Position[a]	δ_C	m	δ_H	m	J [Hz]	$^{1}H,^{1}H$ COSY	$^{1}H,^{13}C$ HMBC[b]
2	72.9	d	5.59	s		2′	3, 2′, 3′, 3′a, 6
3	170.1	s					
4			6.70	s			
5	59.9	d	3.71	m		6, 7a, 7b	
6	71.1	d	4.61	d	9.2	5, 2′′-6′′	2, 1′′, 2′′-6′′
7a	62.0	t	3.26	m		7-OH, 5	
7b			3.43	m		7-OH, 5	
1′			9.40	bs		2′	
2′	126.1	d	7.29	bs		1′, 2	3′, 3′a, 7′a
3′	112.3	s					
3′a	127.5	s					
4′	120.1	d	7.58	d	8.2	5′	3′, 6′, 7′a
5′	120.3	d	6.99	dd	7.5, 8.2	4′, 6′	3′a, 7′
6′	122.8	d	7.13	dd	7.5, 8.2	5′, 7′	5′, 7′a
7′	112.4	d	7.43	d	8.2	6′	5′, 3′a
7′a	137.4	s					
1′′	131.8	s					
2′′-6′′	130.0	d	7.20	d	8.4	3′′-5′′, 6	6, 4′′, 6′′-2′′
3′′-5′′	115.5	d	6.88	d	8.4	2′′-6′′	1′′, 4′′, 5′′-3′′
4′′	159.0	s					
7′′	63.9	t	4.14	t	6.0	8′′	CN
8′′	19.0	t	2.82	t	6.0	7′′	7′′, CN
CN	118.9	s					
7-OH			3.12	bs		7a, 7b	

[a] Assignment are based on DEPT, COSY, ROESY, HMQC and HMBC experiments. – [b] ^{1}H correlating with ^{13}C resonance.

At this point, in order to define the planar structure **1**, it remained to combine the above segments and assign the location of one carbon, one nitrogen and two oxygen atoms, which, based on the molecular formula, still had to be located. Three of these atoms (C, N, O) could be positioned at the ends of the dimethylene segment on account of the following evidence, while the assembly of all the part structures pointed out the location of the last oxygen atom.

An IR band at 2259 cm^{-1}, together with the ^{13}C NMR resonance at $\delta = 118.9$, suggested the presence of a cyanide functionality. It was linked at C-8′′, taking into account the chemical shift of H_2-8′′ ($\delta = 2.82$) coupled with the high-field resonance of C-8′′($\delta = 19.0$) and considering some key HMBC correlations (H-8′′/CN, H-8′′/C-7′′, H-7′′/CN). The chemical shift values of the protons and carbon of the adjacent methylene group (C-7′′), both being in the medium region of the ^{1}H and ^{13}C NMR spectra, clearly indicated its oxymethylene nature. The thus-determined $-O-CH_2-CH_2-CN$ partial structure must be linked to the phenyl ring as suggested by the intense ROESY cross peak between H_2-7′′ and the aromatic protons resonating at $\delta = 6.88$ (H-3′′ and 5′′).

The whole of the above arguments confirmed the substructures **A** and **B** shown in Figure 1. They had to be connected through the C-2–C-3 bond, as well as through an ether linkage between C-2 and C-6, as suggested by the chemical shifts values of both proton and carbon atoms at C-2 ($\delta_H = 5.59$; $\delta_C = 72.9$) and C-6 ($\delta_H = 4.61$; $\delta_C = 71.1$), and further proved by the HMBC correlations between H-6/C-2 and H-2/C-3. So, the positioning of the last oxygen atom was inferred and the planar structure **1** unambiguously determined.

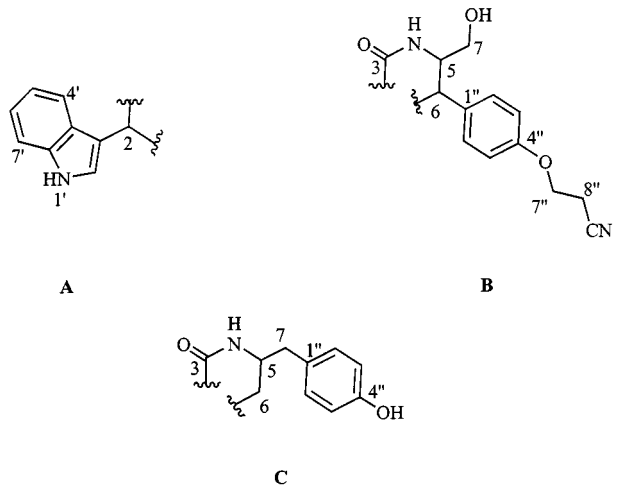
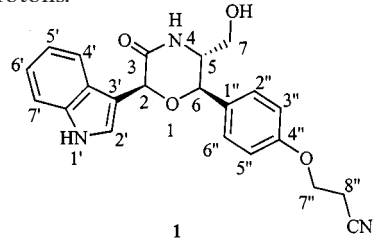


Figure 1. Partial structures leading to the structure of oxazinin-1 **(1)** and -3 **(3)**, generated from a combination of $^{1}H,^{1}H$ COSY, $^{1}H,^{1}H$ ROESY, $^{1}H,^{13}C$ HMQC and $^{1}H,^{13}C$ HMBC spectroscopic data

NMR spectroscopic data were also conclusive to assign the relative stereochemistry of **1**. The large coupling constant H-6/H-5 (9.2 Hz) pointed to a preferential chair-like conformation of the tetrahydrooxazinone ring and to a *trans* diaxial relationship between the two protons, while an intense ROESY cross peak between H-6 and H-2 was indicative of their 1,3-diaxial relationship. Additional evidence to confirm the relative stereochemistry suggested by the above NMR spectroscopic data was obtained by molecular mechanics and dynamics calculations performed in the CHARM force field. Analysis of the 126 low energy conformations provided by HTMDS/minimization (see Experimental Section for details) revealed that the tetrahydrooxazin-3-one ring preferentially assumes the chair like conformation, in which the dihedral angle H-6/C-6/C-5/H-5 is in the range 174.2°–179.9°, which fully agrees with the observed large vicinal $J_{H\text{-}5-H\text{-}6}$ value. In addition the dihedral angle H/N/C-5/H-5 is in the range 83.9°–91.4° which fully agrees with the lack of coupling observed between these two protons.



1

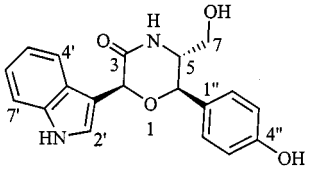
Oxazinin-2 **(2)** appeared to be closely related to **1**, so their structural differences were easily deduced on the basis of a comparison of IR, UV and, above all, NMR spectroscopic data (Table 2). In particular, the IR band at 2259 cm^{-1} and the ^{13}C NMR resonance at $\delta = 118.9$, both unique spectral features of cyanide functionality, were missing in **2**. A further notable difference was the absence in the ^{1}H and ^{13}C NMR spectra of **2** of the signals assigned to H-7′′ and H-8′′ of **1**. Since these were the only dissimilarities between

the spectra of the two compounds, the obvious conclusion appeared to be that in **2** the $-CH_2-CH_2-CN$ moiety was no longer substituted on the phenol oxygen. Further evidence consistent with the proposed structure of **2** was provided by the molecular formula obtained from the negative ion FAB MS spectrum ($C_{19}H_{18}N_2O_4$), as well as by the UV spectrum, which exhibited the bathochromic shift typical of the phenol chromophore at alkaline pH: the absorption maximum at λ_{max} = 216 nm (pH 7.0), overlapping that of the indole chromophore, was shifted by addition of OH^- to λ_{max} = 246 nm, while the band at λ_{max} = 275 nm at pH 7.0 shifted to λ_{max} = 286 nm at pH 10.0.

Table 2. ^{13}C and ^1H NMR spectroscopic data of **2** (CD_3CN) with $^1H,^1H$ COSY and $^1H,^{13}C$ HMBC correlations

Position[a]	δ_C	m	δ_H	m	*J* [Hz]	$^1H,^1H$ COSY	$^1H,^{13}C$ HMBC[b]
2	72.8	d	5.57	s		2′	2′, 3′
3	170.1	s					
4			6.64	s			
5	59.9	d	3.70	m		6, 7a, 7b	
6	71.2	d	4.56	d	9.2	5	1′′
7a	62.1	t	3.26	m		7-OH, 5	
7b			3.42	m		7-OH, 5	
1′			9.37	bs		2′	
2′	126.1	d	7.28	bs		1′, 2	
3′	112.4	s					
3′a	127.5	s					
4′	120.1	d	7.58	d	8.2	5′	
5′	120.3	d	7.00	dd	7.7, 8.2	4′, 6′	3′a, 7′
6′	122.8	d	7.14	dd	7.7, 7.9	5′, 7′	
7′	112.4	d	7.42	d	7.9	6′	
7′a	137.4	s					
1′′	130.0	s					
2′′-6′′	129.9	d	7.08	d	8.6	3′′-5′′, 6	1′′, 4′′
3′′-5′′	116.0	d	6.73	d	8.6	2′′-6′′	2′′-6′′
4′′	157.9	s					
7-OH			3.10	bs		7a, 7b	

[a] Assignment are based on DEPT, COSY, ROESY, HMQC and HMBC experiments. − [b] ^1H correlating with ^{13}C resonance.



2

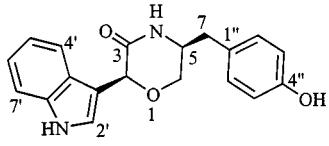
FAB MS of oxazinin-3 **(3)** contained a pseudomolecular ion peak $[M - H]^-$ at *m/z* = 321 consistent with the molecular formula $C_{19}H_{18}N_2O_3$, as confirmed by NMR spectroscopic data (Table 3). A preliminary overview of the 1D NMR spectra showed its structural similarity with **1** and, above all, with **2**, but, at the same time, highlighted some remarkable differences. So, the same 2D NMR experiments reported above for **1** and **2** were carried out for **3**. They indicated that substructure **A**, again present in **3**, was linked to a new substructure (**C**). The aliphatic portion of this substructure included new structural features which were indicated by HMBC correlations from the 7-methylene group:

Table 3. ^{13}C and ^1H NMR spectroscopic data of **3** (CD_3CN) with $^1H,^1H$ COSY and $^1H,^{13}C$ HMBC correlations

Position[a]	δ_C	m	δ_H	m	*J* [Hz]	$^1H,^1H$ COSY	$^1H,^{13}C$ HMBC[b]
2	74.7	d	5.29	s		2′	3, 2′, 3′, 3′a
3	169.8	s					
4			6.41	s			
5	54.4	d	3.87[c]			4, 7, 6a, 6b	
6a	66.2	t	3.53	dd	6.2, 11.2	5, 6b	2
6b			3.89[c]			5, 6a	
7	39.3	t	2.78	d	6.7	5, 2′′-6′′	5, 6, 1′′, 2′′-6′′
1′			9.28	bs		2′	
2′	126.1	d	7.24	bs		1′, 2	3′a
3′	113.0	s					
3′a	127.2	s					
4′	120.1	d	7.57	d	7.9	5′	6′
5′	120.2	d	7.03	dd	7.3, 7.9	4′, 6′	3′a, 7′
6′	122.7	d	7.13	dd	7.3, 7.9	5′, 7′	7′a
7′	112.3	d	7.39	d	7.9	6′	3′a
7′a	137.1	s					
1′′	131.3	s					
2′′-6′′	128.8	d	7.10	d	8.2	3′′, 5′′, 7	4′′
3′′-5′′	116.1	d	6.77	d	8.2	2′′-6′′	3′′-5′′
4′′	156.5	s					

[a] Assignment are based on DEPT, COSY, ROESY, HMQC and HMBC experiments. − [b] ^1H correlating with ^{13}C resonance. − [c] Overlapped to other signal.

H_2-7/C-1′′, H_2-7/C-2′′-6′′, H_2-7/C-5, H_2-7/C-6. The combination of **A**, **C** and one oxygen atom to build up structure **3** derived from the H/C long range correlations between H-2/C-3 and H-6a/C-2.



3

The *cis* relationship between the substituents at C-2 and C-5 was suggested by the coupling constant values H-5/H-6a (3.7 Hz) and H-5/H-6b (5.9 Hz) observed in the ^1H NMR spectrum in CD_3OD. They were inconsistent with a chair-like conformation, considered by far to be the preferential conformation of the *trans* isomer. Decisive evidence was obtained by some key interproton contacts detected through NOE difference experiments; in particular, a diagnostic enhancement was observed for the proton signal at δ = 5.46 (H-2) by irradiation at δ = 3.59 (H-6b), indicative of their *cis* relationship. In addition, the NOE observed for H-6a (while H-6b remained unaffected) by irradiation of H_2-7, proved that H-6a and the benzyl substituent lay on the same side of the tetrahydrooxazinone ring. It is to be noted that the above NOE experiments were performed in CD_3OD solution, where a better signal dispersion was observed in the midfield region of ^1H NMR spectrum than was obtained in CD_3CN.

The presence of the toxic oxazinin-1 in edible shellfish, in addition to contamination of DSP toxins,[1] increases the

potential risk to human health. Implication of these toxins in human illness has not yet been confirmed. However, we have to be vigilant on their occurrence until the potential risks to human health are better understood. An accurate analysis of toxic mussels is therefore indispensable in order to identify new toxins, other than DSP polyether toxins, and isolate a larger amount to increase knowledge of the acute and chronic risk associated with seafood.

Experimental Section

General: NMR spectra were measured on a Bruker AMX-500 spectrometer and the solvent was used as internal standard (CD_3CN: δ_H = 1.93; δ_C = 1.3 and 118.2; CD_3OD: δ_H = 3.34; δ_C = 49.0). FAB MS spectra were obtained at 70 eV on a Kratos MS 50 mass spectrometer. FT-IR spectra were recorded on a Bruker IFS-48 spectrophotometer using a KBr matrix. UV spectra were performed on a Beckman DU70 spectrometer in methanol solution. Optical rotations were measured on a Perkin−Elmer 192 polarimeter in methanol solution, using a sodium lamp at 589 nm and a 10-cm microcell. Medium-pressure liquid chromatography (MPLC) was performed on a Büchi 861 apparatus equipped with Deveosil ODS and Toyopearl HW−40 SF columns. HPLC separations were performed on a Varian 2510 apparatus, equipped with Waters 490 MS UV and RI-3 index detectors and Luna 5u C18 and Luna 5u Silica columns. UV detector, thin-layer chromatography (TLC) and biological assay were used for detection of toxins during their chromatographic purification. The wavelength of the UV detector was set at 230 nm; TLC was performed on silica gel 60 plates (Merck, precoated), with AcOEt/MeOH (95:5) as mobile phase; the oxazinins were detected by heating the plates after spraying with 50% sulfuric acid.

Collection and Extraction: Toxic mussels *M. galloprovincialis* were collected along the coasts of Cesenatico (Adriatic Sea) in December 1998 at 3 m depth, which corresponds to the upper levels of mussel farms in this area. Reference specimens were deposited at the Dipartimento di Chimica delle Sostanze Naturali, Napoli (Italy).

After collection the mussels were stored frozen at −20 °C until extraction. The digestive glands (1900 g of dry weight after extraction) were removed, homogenized with a Waring blender and extracted with acetone (twice) at room temperature. The combined extracts, after filtration, were concentrated in vacuo to give a residue which was dissolved in MeOH/H_2O (8:2) and extracted with *n*-hexane. The aqueous methanolic layer was finally partitioned between MeOH/H_2O (4:6) and CH_2Cl_2. The dichloromethane-soluble material (28.2 g) was successively chromatographed by MPLC on a Develosil ODS column using a solvent gradient system from 60% to 100% of methanol. The cytotoxic fraction eluted with 90% of methanol was purified on a Toyopearl HW−40 SF column, with 100% methanol as eluent. A cytotoxic mixture emerged from the column between 150 and 250 mL of eluent. It was rechromatographed by reverse phase HPLC, eluted with MeOH/H_2O/CH_3CN (35:50:15), and the fraction eluted at 6.44′ (fraction A) was again applied to the same column with a gradient from MeOH/H_2O (6:4) to 100% MeOH in 14 minutes, to afford pure oxazinin-2 (**2**, 2.6 mg).

Fraction B which eluted from the first RP-chromatography at 11.51′ was repurified by RP-HPLC with a linear gradient from MeOH/H_2O (55:45) to MeOH/H_2O (66:34) in 13 minutes, to obtain 18.7 mg of pure oxazinin-1 **(1)** and a mixture containing oxazinin-3 **(3)**. This mixture was purified by HPLC on silica gel eluted with AcOEt/MeOH (95:5) to afford 0.5 mg of **3**.

Molecular Mechanics and Dynamics Calculations: Calculations were carried out on an SGI Personal Iris 4D-35G computer using the force field CHARM (QUANTA 3.2 software package). All the force field calculations were carried out in vacuo (dielectric constant = 1). The conformational space of oxazinin-1 was scanned using the high temperature molecular dynamics simulation (HTDMS) technique[4] followed by energy minimization. A 100-ps molecular dynamics simulation at 2000 K provided a set of 500 conformations of **1**. Each of them was used as a starting structure for the subsequent energy minimization (1000 steps, conjugated gradient algorithm), the energy of the minimized conformations spreading over 17.7 kcal/mol. In the subsequent analysis, only the 126 conformations with a reasonably low energy (at most 2.7 kcal/mol higher with respect to the lowest energy conformer) were used.

Cytotoxicity Assay: WEHI 164 cells (murine fibrosarcoma cell line) were maintained in adhesion with Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 25 mM HEPES, penicillin (100 U/mL), streptomycin (100 μg/mL) and 2 mM L-glutamine. J774 cells (murine monocyte/macrophage cell line) were grown in adhesion on Petri dishes with a DMEM medium supplemented with 10% FBS, 25 mM HEPES, penicillin (100 U/mL), streptomycin (100 μg/mL) and 2 mM L-glutamine. All reagents for cell culture were purchased from Biowhittaker. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-phenyl-2*H*-tetrazolium bromide] was purchased from Sigma. WEHI 164, and J774 (1×10^4 cells) were plated on 96-well plates in 50 μL aliquots and allowed to adhere at 37 °C in 5% CO_2/95% air for 2 h. Thereafter the medium was replaced with 50 μL of fresh medium and 50 μL of 1:4 v/v serial dilution of the test compound was added. The cells were then incubated for 72 h. The cells′ viability was assessed through an MTT conversion assay.[5] Briefly, after 72 h of incubation, 25 μL of MTT (5mg/mL) was added to each well and the cells were incubated for an additional 3 h. After this time the cells were lysed and the dark blue crystals solubilized with 100 μL of a solution containing 50% (v/v) *N*,*N*-dimethylformamide and 20% (v/v) SDS with an adjusted pH of 4.5.[6] The optical density (OD) of each well was measured with a microplate spectrophotometer equipped with a 620 nm filter. The viability of each cell line in response to treatment with compound **1** was calculated as: % dead cells = 100 − (OD treated/OD control) × 100. The results are expressed as IC_{50} (the concentration that inhibits the cell growth by 50%).

Oxazinin-1 (1): $[\alpha]_D^{25}$ = +9.0 (MeOH). − UV (MeOH): λ_{max} (ε) = 216 nm (46000), 270 (5700), 277 (5500), 286 (3500). − IR (KBr): ν = 3478, 3341, 3185, 2930, 2259, 1661, 1623 cm^{-1}. − FAB MS: *m*/*z* = 390 $[M - H]^-$. − HRFABMS (negative mode): *m*/*z* = 390.4139 {calculated for $C_{22}H_{20}N_3O_4$ $[M - H]^-$ 390.4122}. − ^1H and ^{13}C NMR spectroscopic data (CD_3CN) are reported in Table 1.

Oxazinin-2 (2): $[\alpha]_D^{25}$ = +8.7 (MeOH). − UV (MeOH): λ_{max} (pH, ε) = 216 (7.0, 20400) and 246 nm (10.0, 8700), 275 (7.0, 3200) and 286 (10.0, 3320), 377 (700). − IR (KBr): ν = 3480, 3340, 3189, 2932, 1660, 1621 cm^{-1}. − FAB MS: *m*/*z* = 337 $[M - H]^+$. − HRFABMS (negative mode): *m*/*z* = 337.3479 {calculated for $C_{19}H_{17}N_2O_4$ $[M - H]^-$ 337.3494}. − ^1H and ^{13}C NMR spectroscopic data (CD_3CN) are reported in Table 2.

Oxazinin-3 (3): $[\alpha]_D^{25}$ = +12.0 (MeOH). − UV (MeOH): λ_{max} (pH, ε) = 220 (7.0, 20600) and 245 nm (10.0, 8500), 270 (7.0, 3100) and 285 (10.0, 3300), 375 (750). − IR (KBr): ν = 3480, 3340, 3187,

2930, 1660, 1620 cm^{-1}. − FAB MS: *m/z* = 321 $[M - H]^-$. − HRFABMS (negative mode): *m/z* = 321.3518 {calculated for $C_{19}H_{17}N_2O_3$ $[M - H]^-$ 321.3500}. − ^1H and ^{13}C NMR spectroscopic data (CD_3CN) are reported in Table 3. − ^1H NMR spectroscopic data (CD_3OD): δ = 7.67 (d, *J* = 8.1 Hz, H-4′), 7.38 (d, *J* = 8.1 Hz, H-7′), 7.29 (s, H-2′), 7.14 (dd, *J* = 7.3, 7.3 Hz, H-6′), 7.13 (d, *J* = 8.1 Hz, H-2′′-6′′), 7.05 (dd, *J* = 7.3, 7.3 Hz, H-5′), 6.79 (d, *J* = 8.1 Hz, H-3′′-5′′), 5.46 (s, H-2), 3.91 (dd, *J* = 11.8, 3.7 Hz, H-6a), 3.84 (m, H-5), 3.59 (dd, *J* = 11.8, 5.9 Hz, H-6b), 2.95 and 2.85 (AB part of an ABX system, X = multiplet δ = 3.84, J_{A-B} = 14.0 Hz, J_{A-X} = 5.9 Hz, J_{B-X} = 8.8 Hz, H_2-7′′).

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