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Almazole A and Almazole B, Unusual Marine Alkaloids of an Unidentified Red Seaweed of the Family Delesseriaceae from the Coasts of Senegal

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Abstract: Almazole A **1** -slowly equilibrating in solution between the major *Z* **1a** and the minor *E* **1b** forms- and almazole B **2**, unusual marine alkaloids for embodying a 2,5-disubstituted oxazole ring, were isolated from an unidentified red seaweed of the family Delesseriaceae from the coasts of Senegal.

A few, powerfully-bioactive marine metabolites embodying 2,4-dibstituted oxazole (the acetogenins hennoxazoles,[2a] kabiramide C,[2b] calyculins,[2c] bengazoles,[2d] halichondramides,[2e] mycaloide A,[2f] and ulapualide A,[2g] and the peptides orbiculamide A[3a] and keramamides B-C[3b]) or dihydrooxazole rings (the peptides ulicyclamide,[4a] ulithiacyclamide,[4a] patellamides,[4b] lissoclinamides,[4b] ascidiacyclamide,[4c] and bistratamides[4d]) have been recently isolated from tunicates, sponges, and nudibranchs that feed on them. To our knowledge, the only digression from this substitution pattern, involving substitution at the 2,5 oxazole positions, was found in the cytotoxic cyclic peptides diazonamides A and B of an ascidian.[5] Even in terrestrial taxa, 2,5-disubstituted oxazoles are unusual, rare examples being the antibiotic pimprinine of actinobateria[6a] and annulonine of rye grass.[6b]

We have now found the first case of a seaweed that produces oxazoles, and just of the most rare, 2,5-disubstituted type. Thus, work up of an unidentified red seaweed of the family Delesseriaceae from the coasts of Senegal[7] led to almazole A **1**[8] and almazole B **2**.[9] The composition $C_{21}H_{21}N_3O_3$ for **1** was revealed by MS data[8] in combination with the NMR data in the Table, which also suggested the presence of an alkylmonosubstituted and an *o*-disubstituted benzene ring. The first one was clarified to be part of a N,N-dimethylphenylalanine moiety from difference spin decoupling,[10] COSY 120[11] and HMQC.[12] Elucidation of the disubstituted benzene portion required first assessing the origin of doubling of the aromatic proton signals, which could be attributed to a slow equilibrium between two conformers, in 85:15 ratio, arising from slow rotation around the N-CHO bond. This conclusion was confirmed by irradiation at H4' (7.41 br.d) of the minor conformer **1b**, by this observing cross-saturation transfer at H4' (8.63 dd) of the major conformer **1a**.[13] The position of the formyl group (δ_C = 159.47d, $J_{C\text{-}H}$ = 180Hz, δ_H = 8.45d, J = 1.8) *ortho* to the CO- group (δ_C = 183.12s long-range coupled to H7') was established from HMBC.[14]

The three signals δ_C 167.22s, 148.94s, and 136.79 d ($J_{C\text{-}H}$ = 200Hz) and the signal δ_H 7.68s for **1** (Table) fit for a 2-alkyl-5-acyl-disubstituted oxazole ring. C2-C1" bonding rests on heterocorrelation of C2 with both H1" and $H_2$2" while heterocorrelation of the oxazole proton with C1' is compatible with C1' bonding to either C4 or C5. C1'-C5 bonding is the choice since the oxazole proton in 2-alkyl-4-acyl-substituted oxazoles was always observed

to resonate at δ_H8.0-8.2,[15a] i.e., at *ca.* 0.5 ppm lower field than in almazole. Actually, this corresponds to the well known $\Delta\delta_H$ between H4 and H5 in C2-monosubstituted oxazoles. C1'-C5 bonding is also in accordance with the observed δ_C 149.94 s for C-5. The corresponding C4 resonance for hypothetical C1'-C4 bonding would have been expected at *ca.* 10 ppm higher field[15]).

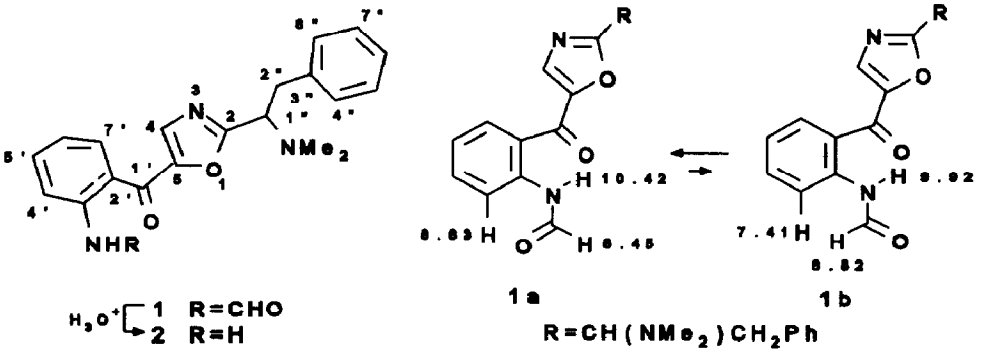

Table. NMR data ($CDCl_3$, 300 MHz) for almazole A (major conformer **1a** and, in footnotes a-e, detectable signals for the minor conformer **1b**)

Atom	**1a** δ_C(mult.)	**1a** δ_H(mult.,*J* in Hz)	**1a** Long-range ^{13}C-^{1}H[f]
2	167.22 (s)	--	7.68;4.15;3.35;3.22;2.39
4	136.79 (d)	7.68 (s)	4.15
5	148.94 (s)	--	7.68
1'	183.12 (s)	--	7.77
2'	122.77 (s)	--	8.63
3'	138.88 (s)	--	8.45;7.77;7.61
4'	122.37 (d)	8.63 (dd,8.6;1.1)[a]	7.20
5'	134.87 (d)	7.61 (ddd,8.6;7.0;1.7)[b]	7.77
6'	123.38 (d)	7.20 (ddd,8.0;7.0;1.1)	8.63
7'	130.94 (d)	7.77 (dd,8.0;1.7)[c]	7.61
1"	64.56 (d)	4.15 (dd,9.6;5.8)	7.68;3.35;3.22;2.39
2"	36.82 (t)	3.35 (dd,13.4;9.6) 3.22 (dd,13.4;5.8)	7.15;4.15;2.39
3"	137.52 (s)	--	7.20;4.15;3.35;3.22
4"-8"	128.50 (d)	7.15-7.20 (m)	3.35;3.22
5"-7"	129.02(d)	7.15-7.20 (m)	7.15-7.20
6"	126.66 (d)	7.15-7.20 (m)	7.15
CHO	159.47 (d)	8.45 (d,1.8)[d]	--
NH	--	10.42(br.s)[e]	--
NMe_2	41.70 (q)	2.39 (s)	4.15;3.35;3.22

[a]7.41(br.d,8.6) for **1b**. [b]7.59(br.t, 7.5) for **1b**. [c]7.73(br.d, 8.0) for **1b**. [d]8.82(d,11.6) for **1b**. [e]9.92(br.d,11.6) for **1b**. [f]Coupling between the C-atom in the first column and the various H-atoms.

The three partial structures could then be assembled as in structure **1**. The coupling constant values $J_{CHO\text{-}NH}$ = 1.8 and 11.6Hz in the major **1a** and minor conformer **1b**, respectively, pointed to *Z* conformation in the former and *E* conformation in the latter. This was confirmed by a 10% NOE enhancement between CHO and NH in **1a**.

With respect to almazole A **1**, the NMR spectra for almazole B **2**[9] (Table) showed the absence of the formyl

group, but otherwise supported the same connectivity pattern. The EIMS fragmentation[9] pointed to the same conclusions, which were confirmed by acid hydrolysis of almazole A **1** to almazole B **2** [16]

The conformational equilibrium for almazole A was investigated in $(CD_3)_2SO$, where chemical shifts differ from $CDCl_3$ solution but the **1a/1b** conformer population ratio at r.t. (85:15) and the proton coupling pattern are the same.[17] The two conformers were monitored through their CHO d and the H4' dd from r.t. to 391 °K (Fig., left), i.e. in the whole range from slow to fast exchange. Coalescence for the CHO signals 8.20 d of **1a** and 8.64 d of **1b** was clearly observed at 348 °K, while the H4' signal for **1b** was superimposed to other signals, so that only the trend for the 7.92 dd of **1a** is shown here (Fig., left). The best-fitting DNMR5[18]-simulated spectra, and first-order rate coefficients, are also shown in the Figure (right). Eyring analysis gave a kinetic barrier for rotation around the N-CHO bond $\Delta G^{\ddagger}$(298 °K) = 16.1±0.2 Kcal mol^{-1}. This is 3-5 Kcal mol^{-1} lower than for N,N-disubstituted amides, while there are scanty data for N-monosubstituted formamides[19] to compare.

Biogenesis of almazole A **1** can be conceived from tryptophan and N,N-dimethylphenylalanine, with oxidative pyrrole ring opening.

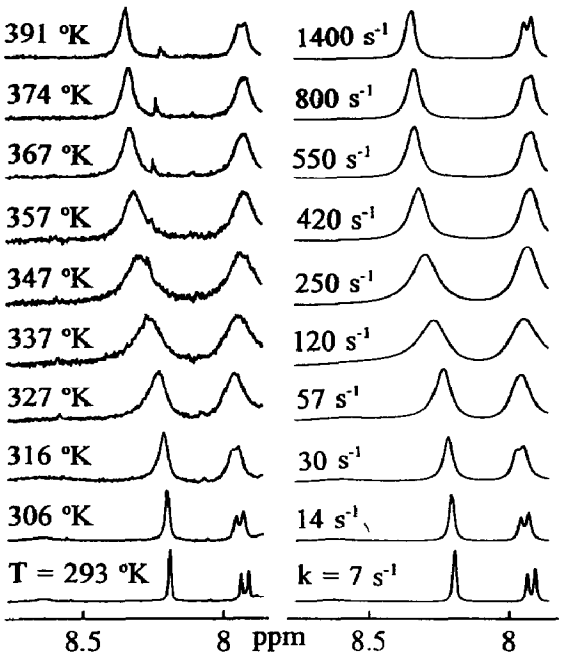

Figure. Experimental (left) and simulated (right) line shapes for H4' and formyl proton signals of almazole A **1**

Screening for biological activities and synthesis of almazoles are under way and will be reported in due course. We thank Mr. A. Sterni for recording the mass spectra and MURST (Progetti 40%) and CNR, Roma, for financial support.

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7 The alga (collection 678M), collected at low tide in May 1993 at Almadies, north of Dakar, was determined by Mr R. Lemée, Université de Nice Sophia Antipolis, to belong to the family Delesseriaceae, though not appearing in standard descriptions of the marine flora of western Africa. Identification is pending. The alga was immediately soaked in EtOH, filtered to leave 203 g of dry residue, and evaporated. The residue was hexane defatted and then AcOEt extracted to give 1.5 g of residue that was subjected to Si-60 FC (75 g) with hexane/AcOEt gradient elution. Fractions 13-16, of 40 ml each, were evaporated and subjected to LiChrosorb NH_2-HPLC with 90:10 hexane/*i*-PrOH, 5 ml min^{-1}, obtaining **2** (t_R = 9.0 min, 4 mg, 0.002%) and **1** (t_R = 9.5 min, 11mg, 0.005%).

8 **1**: colourless semisolid. UV (MeOH), $\lambda_{max}(\epsilon)$ 204 (36700), 237 (26400), 278 (18200), 320nm (6000). CD (MeOH) $\Delta\epsilon_{max}$(261nm) +2.5; (217nm) +4.9. $[\alpha]_D^{20}$ +103 (MeOH, *c*=0.155). EIMS (*m/z*,%) 364 [0.2, $(M+H)^+$], 272 [100, $(M-C_7H_7)^+$], 244 [12, $(272-CO)^+$], 148 (11). HRMS found 272.1036±0.003, calcd for $C_{14}H_{14}N_3O_3$ 272.1035. FABMS (Ar, 3-NBA) (*m/z*,%) 364 [14, $(M+H)^+$].

9 **2**: yellow semisolid. UV (MeOH), $\lambda_{max}(\epsilon)$ 203 (40320), 234 (22600), 260 (16500), 396nm (6300). CD (MeOH) $\Delta\epsilon_{max}$(255nm) +2.0; (218nm) +3.8. $[\alpha]_D^{20}$ +92 (MeOH, *c*=0.05). EIMS (*m/z*,%) 291 [0.3, $(M-NMe_2)^+$], 244 [100, $(M-C_7H_7)^+$], 120 (31). HRMS found 244.1085±0.002, calcd for $C_{13}H_{14}N_3O_2$ 244.1086. FABMS (Ar, 3-NBA) (*m/z*,%) 336 [6, $(M+H)^+$]. δ_C ($CDCl_3$) 165.60(s, C2), 134.18(d, C4) , 150.74(s, C5), C1' not det., 117.49 (s, C2'), 137.75 (s, C3'), 117.18 (d, C4'), 134.74 (d, C5'), 116.19 (d, C6'), 131.62 (d, C7'), 64.42 (d, C1"), 36.97 (t, C2"), C3" not det., 128.45 (d, C4" and C8"), 129.02 (d, C5" and C7"), 126.55 (d, C6"), 41.69 (q, NMe_2). δ_H ($CDCl_3$) 7.62 (s, H4),6.71 (dd,8.1;0.7, H4'), 7.31 (ddd,8.1;7.5;1.5, H5'),6.67 (td,7.5;0.7, H6'), 7.71(dd,7.5;1.5, H7'), 4.15 (dd,9.6;5.7, H1"), 3.34, 3.22(two dd,13.5;9.6;13.5;5.7, $H_2$2"), 7.15-7.20(m, H4", H8", H5", H7", H6"), 6.00 (br.s, NH_2), 2.38(s, NMe_2).

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16 A colourless mixture of **1** (2 mg) in 2 ml of 1.5 M HCl in EtOH turned to yellow on brief stirring at r.t. due to formation of **2**. Stirring was continued for 30 min, then 1 M KOH was added attaining pH 9 and the mixture was AcOEt extracted. FC of the residue gave a compound (1.6 mg, 74%) with spectroscopic and chiroptical data identical to natural **2**.

17 **1a**: δ_H($(CD_3)_2SO$, 300 MHz) 7.83 (H4), 7.92 (H4'), 7.62 (H5'), 7.29 (H6'), 7.63 (H7'), 4.17 (H1"), 3.21 and 3.14 ($H_2$2"), 7.15-7.25 (H4"-H8"), 8.20 (CHO), 10.27 (NH), 2.26 (NMe_2). **1b** (only signals differing from **1a**): δ_H 7.88 (H4), 7.54 (H4'), 7.60 (H5'), 7.61 (H7'), 8.64 (CHO), 9.98 (NH).

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