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RIGIDIN, A NOVEL ALKALOID WITH CALMODULIN ANTAGONISTIC ACTIVITY FROM THE OKINAWAN MARINE TUNICATE *EUDISTOMA* CF. *RIGIDA*

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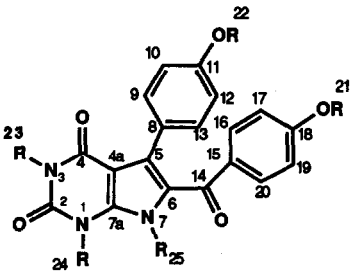
Summary: A novel pyrrolopyrimidine alkaloid, rigidin (**1**), with calmodulin antagonistic activity has been isolated from the Okinawan marine tunicate *Eudistoma* cf. *rigida*. The structure was elucidated on the basis of spectral data of **1** and its pentamethyl derivative (**2**).

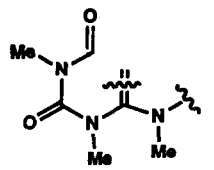
In our continuing search for bioactive compounds from Okinawan marine organisms,[1] we have encountered a purple-colored compound tunicate *Eudistoma* cf. *rigida* to result in the isolation of the two novel cytotoxic 24-membered macrolides, iejimalides A and B.[2] In this communication we wish to report the isolation and structure elucidation of a novel pyrrolopyrimidine alkaloid, named rigidin (**1**), with potent calmodulin antagonistic activity from the same tunicate.

The tunicate was collected at Ie Island, Okinawa, by SCUBA (-5 ~ -15 m) and kept frozen until used. The methanol extract was partitioned between toluene and water. The aqueous layer was subsequently extracted with chloroform, ethyl acetate, and *n*-butanol. The ethyl acetate soluble material was subjected to a silica gel column ($CHCl_3$/*n*-BuOH/AcOH/H_2O, 3:6:1:1) followed by a Sephadex LH-20 column ($CHCl_3$/MeOH, 1:1) to give rigidin (**1**, 0.0015% wet weight) as a purple solid: mp >300 °C.

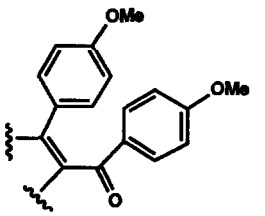
The molecular formula, $C_{19}H_{13}N_3O_5$, of **1** was established by HRFABMS (*m/z* 364.0917, M+H$^+$, Δ -1.6 mmu). Elemental analysis suggested that rigidin (**1**) was isolated as a free base. The UV absorption indicated the presence of phenol chromophore(s).[3] The IR spectrum was indicative of the presence of hydroxyl and unsaturated carbonyl groups.[3] The ^{1}H NMR spectrum of **1** showed five NH/OH protons (δ 9.27 ~ 11.78 in DMSO-d_6) and eight aromatic protons in two A_2B_2 systems (δ 7.29, 2H, *J*=8.6 Hz; 6.94, 2H, *J*=8.6 Hz; 6.48, 2H, *J*=8.6 Hz; 6.44, 2H, *J*=8.6 Hz). The ^{1}H-^{1}H COSY spectrum showed a weak but



1 R=H
2 R=Me



A



B

Table 1. ^{1}H and ^{13}C NMR data for pentamethyl rigidin (**2**) in $CDCl_3$

Position	δ_C (m)	δ_H (m)	J (Hz)	H coupled with C
1				
2	152.40 (s)			H_3-23, H_3-24
3				
4	158.67 (s)			H_3-23
4a	99.57 (s)			
5	129.23 (s)			H_2-9,13
6	129.63 (s)			H_3-25
7				
7a	142.81 (s)			H_3-24, H_3-25
8	123.87 (s)			H_2-10,12
9,13	132.12 (d)	7.09 (d)	8.8	H_2-10,12
10,13	113.16 (d)	6.61 (d)	8.8	H_2-9,13
11	158.94 (s)			H_2-9,13, H_3-22
14	186.79 (s)			H_2-16,20
15	130.72 (s)			H_2-17,19
16,20	132.23 (d)	7.55 (d)	8.8	
17,19	112.83 (d)	6.59 (d)	8.8	
18	163.12 (s)			H_2-16,20, H_3-21
21	55.38 (q)	3.74 (s)		
22	55.16 (q)	3.74 (s)		
23	28.29 (q)	3.38 (s)		
24	33.92 (q)	3.85 (s)		
25	35.78 (q)	3.94 (s)		

clear cross peak between the protons at δ 11.21 (H-23 or H-24) and 10.64 (H-24 or H-23), which may be assigned to a W-coulpling. Since no further structural information was available from the NMR data of **1** itself, the structure determination was mostly carried out with its pentamethyl derivative (**2**). **2** was obtained as major product on methylation of **1** with CH_2N_2 in methanol. The ^1H NMR of **2** (Table 1) showed no longer NH/OH protons, but five singlet methyls at 3 ~ 4 ppm region, along with eight A_2B_2 aromatic protons just as in **1**. The EIMS of **2** showed the molecular ion at *m/z* 433 and a sole fragment ion at *m/z* 135. The HREIMS established the molecular formula of **2** as $C_{24}H_{23}N_3O_5$ (Found *m/z* 433.1610, Δ-2.8 mmu). Among five methyls in the ^{13}C NMR of **2** (Table 1) two resonating at δ 55.16 and 55.38 were assgned to methoxyls, and the remaining three were all assigned to N-methyls due to their higher-field resonances (δ 28.29, 33.92, and 35.78). Long-range ^1H-^{13}C couplings observed by HMBC[4] experiments (Table 1) suggested the presence of two *p*-methoxyphenyl moieties by the following correlations: H_3-22/C-11, H_2-10,12/C-8, H_2-9,13/C-11; H_3-21/C-18, H_2-16,20/C-18 and H_2-17,19/C-15. This was also confirmed by NOE experiments. Irradiation of the H_3-22 signal resulted in 5.4% NOE for H_2-10,12, while irradiation of the H_3-21 signal caused 6.1% NOE for H_2-17,19. In the HMBC spectrum the H_2-9,13 also coupled with an sp^2 carbon at δ 129.23 (C-5), which showed no coupling to other protons and therefore should be located at four-bond away from any other proton. The H_2-16,20, however, showed a cross peak to the lowest field carbon (δ186.79), which should be assigned to a cross-conjugated carbonyl group (C-14). The intense fragment ion peak at *m/z* 135 was ascribable to the methoxybenzoyl substituent ($C_8H_7O_2$). The observation of 2.1% NOE of H_2-9,13 on irradiation of H_2-16,20 strongly suggested the presence of partial structure **B**. The assignment of partial structure **A** consisting of N-1 ~ C-4, C-7a and N-7 atoms was straightforward: the methyl at δ 3.38 (H_3-23) coupled to C-4 (δ 158.67) and C-2 (δ 152.40), the latter in turn coupled to the methyl at δ 3.85 (H_3-24) in the HMBC spectrum. Similarly, the H_3-24 and H_3-25 (δ 3.94) showed common cross peak to a carbon at δ 142.81 (C-7a).

Partial structures **A** and **B** accounted for 23 carbons, and only one carbon and three unsaturated degrees remained to be assigned. Since the carbon at δ 129.63 (C-6) coupled to H_3-25 protons, it should be connected directly to 7-N atom (three bond away from Me protons) and must be C-6. Therefore the remaining carbon (C-4a) has a chemical shift of δ 99.57 and connected to C-7a and C-5 to constitute a pyrrolopyrimidine moiety. Thus the structure of pentamethyl rigidin was concluded to be **2** and that of rigidin itself was assigned to **1**.

Rigidin (**1**) exhibited potent calmodulin antagonistic activity.[5,6] The value of the 50% inhibitory concentration of calmodulin-activated brain phosphodiesterase was 5 x 10^{-5} M. Although pyrrolo[2,3d]pyrimidine type compounds have been found in some nucleoside antibiotics,[7] isolated from strains of *Streptomyces*, there are no precedents in marine sources.[8] Interestingly, pyrrolo[2,3d]pyrimidine-2,4-diones have been synthesized and some of them showed weak affinity for the benzodiazepine receptor.[9]

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3. UV (MeOH) λmax 401 (ε 2500), 346 (2500), 285 (4300), and 240 nm (sh); (MeOH+KOH) λmax 401 (ε 2500), 346 (4500), 285 (4300), and 240 nm (sh); (MeOH+HCl) λmax 552 (ε 200), 356 (2500), 276 (4500), and 232 (sh) nm; IR (KBr) 3200, 1700, 1610, 1420, and 1270 cm^{-1}.
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