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BIOACTIVE MARINE METABOLITES IX.[1] MYCALISINES A AND B, NOVEL NUCLEOSIDES WHICH INHIBIT CELL DIVISION OF FERTILIZED STARFISH EGGS, FROM THE MARINE SPONGE MYCALE SP.

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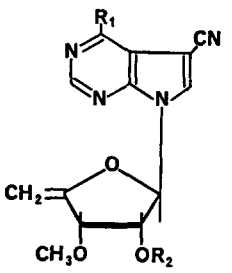
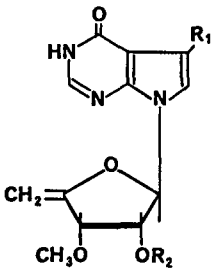
Abstract: Two novel nucleosides, mycalisines A and B, have been isolated from a marine sponge and their structures elucidated as 4-amino-5-cyano-7-(3-O-methyl-5-deoxy-β-D-erythro-pent-4-enofuranosyl)-pyrrolo[2,3-d]pyrimidine and 5-cyano-7-(3-O-methyl-5-deoxy-β-D-erythro-pent-4-enofuranosyl)-pyrrolo[2,3-d]pyrimidine-4-one, respectively. Both compounds inhibit cell division of fertilized starfish eggs.

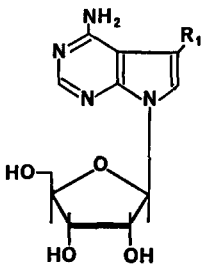
Since the Bergmann's pioneering work[2,3] on isolation of marine nucleosides from a Caribbean sponge in the 1950's, which led to the development of a recognized drug, Ara-C,[4] several biologically active nucleosides have been reported from marine organisms, including sponges,[5] gorgonians,[6] nudibranchs,[7] and seaweeds.[8] In the course of our search for bioactive marine metabolites, we found that the lipophilic extract of a marine sponge Mycale sp., collected in the Gulf of Sagami, Japan, inhibited the cell division of fertilized starfish eggs. The active compounds have been isolated and their structures elucidated as unusual nucleosides 1 and 2 which we designated mycalisines A and B, respectively.



1: $R_1 = NH_2$ $R_2 = H$
3: $R_1 = NAc_2$ $R_2 = Ac$



2: $R_1 = CN$ $R_2 = H$
4: $R_1 = CN$ $R_2 = Ac$
5: $R_1 = CONH_2$ $R_2 = H$



6: $R_1 = CN$
7: $R_1 = CONH_2$

The ethyl acetate soluble portion of an ethanol extract of the frozen sponge (9.5 kg) was subjected to silica gel flash chromatography with dichloromethane/ethyl acetate/methanol systems. The active fractions eluted with ethyl acetate/methanol(9:1) was fractionated by low pressure column chromatography on Kieselgel H(Merck) with dichloromethane/ethyl acetate(1:1). The active fractions were repeatedly purified by silica gel HPLC (CH_2Cl_2/EtOAc, 45:55) and reversed phase HPLC (50 % MeOH) to afford 10 mg of mycalisine A (1) and 48 mg of mycalisine B (2), both as colorless oil. Mycalisine A inhibited cell division of fertilized starfish (Asterina pectinifera) eggs at 0.5 μg/mL, while mycalisine B at 200 μg/mL. Both were labile at room temperature but stable when stored under a nitrogen atmosphere at -20°C.

Mycalisine A, $[\alpha]_D^{21}$=-88.0° (c=0.05, EtOH), showed UV absorption maxima [λ_{max}^{EtOH}(pH 7) 279 (ε 14000), 233sh(8900), 207(30000) nm; (pH 2) 279(11000), 233sh(15000), 207(17000) nm; (pH 12) 279 nm] comparable to those of pre Q_0 nucleoside[9] and toyocamycin(6),[10] which possess an 4-amino-5-cyano-pyrrolo[2,3-d]pyrimidine functionality.[11] This structural feature was also implied by IR absorption [2250 cm^{-1}(CN), 3430 cm^{-1}(OH) and 3300 cm^{-1}(NH)] and ^{13}C NMR signals (δ 156.6s, 153.0d, 150.3s, 130.6d, 113.8s, 103.6s, 84.5s)(Table 1).

The molecular formula of $C_{13}H_{13}N_5O_3$ was established by ^{13}C NMR signals and an MH^+ ion peak at m/z 288 in the FAB mass spectrum which gave informative fragment peaks at m/z 160 (base, aglycone + 2), 188 (aglycone + 30) and 202 (aglycone + 44).[12] These data together with color tests indicated the nucleoside nature of 1. ^{13}C NMR signals at δ 156.3s, 87.6d, 86.6t, 78.3d, 72.9d and 55.1q (Table 1) suggested that mycalisine A possessed 3-O-methyl-5-deoxy-erythro-pent-4-enofuranose as its sugar moiety, which was supported by the negative reaction to IO_4-benzidine reagent.

Table 1. ^{13}C NMR Data for Mycalisines A and B (1 and 2) (25 MHz)

	1			2		
C	δ(CD_3OD/TMS)	$^1J_{C-H}$ (Hz)	$^{2,3}J_{C-H}$ (Hz)	δ(DMSO-d_6/TMS)	$^1J_{C-H}$ (Hz)	$^{2,3}J_{C-H}$ (Hz)
1						
2	153.0 d	201		147.2 d	207	
3						
4	156.6 s		6	157.5 s		6
4a	103.6 s		— *	108.4 s		9
5	84.5 s		6	88.5 s		6
6	130.6 d	195,	4	131.3 d	198,	4
7						
7a	150.3 s			149.0 s		
1'	87.6 d	172		88.2 d	171	
2'	72.9 d	149		72.1 d	145	
3'	78.3 d	158		78.9 d	150	
4'	156.3 s			157.5 s		
5'	86.6 t	161		88.0 t	154	
CN	113.8 s		3	114.8 s		3
OMe	55.1 q	143,	4	56.6 q	142,	4

* not determined

The ^{1}H NMR spectrum (in DMSO-d_6) contained three D_2O-exchangeable protons [δ 6.96(2H, brs; $-NH_2$), 5.80(1H, brs; 2'-OH)], two protons on heterocyclic rings [δ 8.42(s; H-2), 8.27(s; H-6)], exomethylene protons [δ 4.49(d, 2.0 Hz; H-5'), 4.37(d, 2.0; H-5')], methoxyl protons [δ 3.42 (3H, s)], an anomeric proton [δ 6.33(d, 7.0); H-1'], and two carbinol protons [δ 4.89(m; H-2'), 4.24(d, 4.8, H-3')]. Irradiation of the H-1' proton at δ 6.33 sharpened H-2' proton signal, which collapsed to a doublet of doublets (7.0 and 4.8 Hz) upon addition of D_2O. Similarly, the H-2' proton was found to be coupled to carbinol protons at H-1' and H-3'. It is evident from these data that the sugar portion, 3-O-methyl-5-deoxy-erythro-pent-4-enofuranose, was β-linked to the heterocyclic base, which was secured by spectroscopic data reported for angustmycin A isolated from Streptomyces sp.[13]

Treatment of mycalisine A with acetic anhydride and pyridine yielded the acetate 3, whose EIMS spectrum showed prominent fragment ions at m/z 413(M^+), 353(M^+-60), 244(aglycone + 2) and 243(aglycone + 1). The ^{1}H NMR spectrum[14] exhibited two acetamides [δ 2.40(6H, s)] and an acetate [δ 2.15(3H, s)]. A substantial downfield shift (1.01 ppm) was observed for the carbinol proton at H-2'. The data mentioned above allowed us to assign the structure of mycalisine A to be 4-amino-5-cyano-7-(3-O-methyl-5-deoxy-β-erythro-pent-4-enofuranosyl)-pyrrolo[2,3-d]pyrimidine.

The other active compound, mycalisine B, $[\alpha]_D^{21}$=-75.9° (c=0.26, EtOH), had chemical and spectral features closely related to those of mycalisine A. FAB mass [m/z 289(MH^+)] and ^{13}C NMR spectra led to the molecular formula of $C_{13}H_{12}N_4O_4$. UV absorptions [λ_{max}^{EtOH}(pH 7) 265(ε 3000), 210(6000) nm; (pH 2) 265(3000), 214(3000) nm; (pH 12) 265sh nm] as well as an IR band at 2250 cm^{-1}(CN) indicated that the base portion of mycalisine B was 5-cyano-pyrrolo-[2,3-d]pyrimidine-4-one. This was verified by ^{13}C NMR signals (δ 157.5s, 149.0s, 147.2d, 131.3d, 114.8s, 108.4s, 88.5s) (Table 1) and the base peak at m/z 161 in EIMS. The structure of the base portion was confirmed by converting mycalisine B with 30 % H_2O_2 in 3N NaOH to the carboxamide derivative 5,[15] whose ^{1}H and ^{13}C NMR data were comparable of those for sangivamycin (7).[11,16]

The ^{1}H NMR signals (in DMSO-d_6) of the sugar moiety [δ 6.30(d, 7.1 Hz; H-1'), 4.84(dd, 7.1, 4.9; H-2'), 4.52(d, 2.2; H-5'), 4.39(d, 2.2; H-5'), 4.24(d, 4.9; H-3'), 3.43(3H, s; -OMe)] suggested that 2 has the same sugar portion as mycalisine A, which was substantiated by the ^{13}C NMR signals (Table 1) and by chemical conversion. Upon acetylation with acetic anhydride and pyridine mycalisine B gave the acetate 4, whose EIMS revealed numerous peaks at m/z 330 (MH^+), 270, 161(base) and 160, and whose ^{1}H NMR spectrum[17] closely resembled that of 3.

The dihedral angle between C(6)-N and C(1')-H(1') was estimated as ca. 130° both by the $^3J_{C-H}$ value of 4 Hz for C-6[18] (Table 1) and NOE enhancements seen for H-1' and H-2' protons upon irradiation of the H-6 proton.[19] Therefore, the negative Cotton effect observed for the p-bromobenzoate led to the D-configuration of the sugar.[20] Thus, mycalisine B is 5-cyano-7-(3-O-methyl-5-deoxy-β-D-erythro-pent-4-enofuranosyl)-pyrrolo[2,3-d]pyrimidine-4-one.

Closely related antibiotics, such as tubercidin,[21] toyocamycin[10] and sangivamycin[16] have been reported from Streptomyces sp. It is likely that our compounds, mycalisines A and B, are produced by symbiotic microorganism(s),[22] although it is surprising that we could detect no

antimicrobial activity when tested against M. ramannianus, C. albicans, S. aureus, B. subtilis, P. aeruginosa and E. coli.

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14. ^{1}H NMR δ($CDCl_3$/TMS) 7.91(s; H-2), 7.26(s; H-6), 6.68(d, 6.3 Hz; H-1'), 5.70(dd, 6.3, 5.1; H-2'), 4.73(d, 2.9; H-5'), 4.51(d, 2.9; H-5'), 4.47(d, 5.1; H-3'), 3.46(3H, s; -OMe), 2.40(6H, s; $-NAc_2$), 2.15(3H, s; -OAc).
15. ^{13}C NMR δ(DMSO-d_6/TMS) 163.0($-CONH_2$), 159.7(C-4), 157.5(C-4'), 149.2, 145.1(C-2, C-7a), 126.3(C-6), 115.7(C-5), 104.8(C-4a), 87.4, 87.0(C-5', C-1'), 78.6(C-3'), 72.2(C-2'), 56.0 (-OMe). ^{1}H NMR: δ(CD_3OD/TMS) 8.12(s; H-2), 7.93(s; H-6), 7.36(2H, brs; $-NH_2$), 6.32(d, 6.5 Hz; H-1'), 5.72(1H, brs; -OH), 4.88(m; H-2'), 4.42(d, 2.0; H-5'), 4.30 (d, 2.0; H-5'), 4.17(d, 4.6; H-3'), 3.18(3H, s; -OMe).
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17. ^{1}H NMR: δ($CDCl_3$/TMS) 8.02(s; H-2), 7.54(s; H-6), 6.53 (d, 6.6; H-1'), 5.56(dd, 6.6, 4.9; H-2'), 4.73(d, 2.9; H-5'), 4.46(d, 2.9; H-5'), 4.41(d, 4.9; H-3'), 3.44(3H, s; -OMe), 2.13(3H, s; -OAc).
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