Agelastatin A, a New Skeleton Cytotoxic Alkaloid of the Oroidin Family. Isolation from the Axinellid Sponge *Agelas dendromorpha* of the Coral Sea

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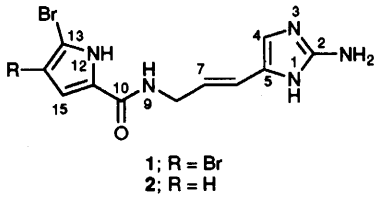
Agelastatin A, isolated from the axinellid sponge *Agelas dendromorpha* of the Coral Sea, is a new-skeleton alkaloid with, unusually for the oroidin family to which it belongs, marked cytotoxicity toward tumour cells in culture.

Initially isolated from the Mediterranean axinellid sponge *Agelas oroides*,[1a] oroidin **1** is historically the central example[1b,2] in a series of similar alkaloids, like hymenidin **2**,[3] or the hypothesized precursor of dimerized,[2] or 6,15,[4] 4,15–5,9,[4b,5] 4,12–5,9,[6] or 4,9–4,12[7] cyclized alkaloids isolated from marine sponges, mostly of the order Axinellida. Isolated from *Agelas dendromorpha*, we report here the novel alkaloid agelastatin A **3**, which may be viewed to descend biogenetically from a hymenidin-like precursor along the new cyclization mode 4,8–7,12.

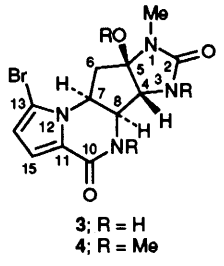
A. dendromorpha, collected by dredging at Les Trois Bancs, −260 m, New Caledonia, was freeze-dried (12 g), extracted with EtOH, and partitioned between H_2O and CH_2Cl_2. The aqueous layer was evaporated to give 2.6 g of residue, which was subjected to TLC to give agelastatin A **3** (0.15 g, 1.2% on dry sponge residue), which proved to be markedly cytotoxic.†

Treatment of **3** with MeI in dry KOH–Me_2SO led to the incorporation of three methyl groups, giving, after HPLC (Merck Si-60, CH_2Cl_2/EtOH 97 : 3, 5 ml min^{-1}, t_R = 12.8 min) pure **4**, the ^{13}C and ^1H NMR spectra of which, in combination with MS data,‡ revealed the composition $C_{15}H_{19}BrN_4O_3$ and the presence of four unsaturated bonds. Two of these were revealed as a 1,4-disubstituted cisoid diene system from typical coupling J = 4.2 Hz, while the other two showed up as amide carbonyl groups from typical $\delta_{C=O}$ values. Thus, agelastatin must be tetracyclic.

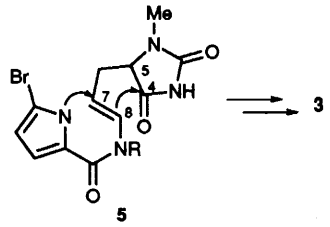
From now on, NMR data for either **3**§ or **4**‡ are used alternatively as most appropriate. Thus, connectivities C(6)–C(7)–C(8) were best supported with natural **3** by large interproton couplings, while C(6) could be connected to C(5) and the latter to C(4) on the basis, respectively, of ^1H–^{13}C correlation data and 10% nuclear Overhauser effect (NOE) at H–C(4) on irradiation at MeO. Closure of the cyclopentane ring was based on both ^1H–^{13}C correlation between C(5) and H–C(8) and small (<0.5 Hz) J coupling between H(4) and H(8) with **3**; for the latter the C(4)–N(1) fragment was also suggested by ^1H–^{13}C correlation within the couples H–N(3)···C(2), H–C4···C2, and MeN(1)···C(2). The imidazolidone ring could be closed on the basis of large deshielding of C(5) in either **3** or **4** (δ 93–98), which demands bonding of C(5) to both O and N. The diene system, identified above, was extended to C(10)–N(9) on the basis of ^1H–^{13}C correlations between either C(10) and MeN(9) in **4**, or C(11) and H–N(9) in **3**. The oxopyrazine–pyrrole system fused to the cyclopentane ring was based both on typical pyrrole and bromopyrrole δ_c values for C(11) and C(13),†,§ and the observation of deshielded C(7) and C(8), which require bonding to N. This was further supported by selective INEPT (insensitive nuclei enhanced by polarisation transfer) irradiation at H(7) which brought about magnetiza-



1; R = Br
2; R = H



3; R = H
4; R = Me



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Scheme 1

† **3**, contaminated, as far as can be deduced from ^1H NMR spectra, by a few percent of the difficultly removable 13,14-dibromo analogue, proved to be powerfully cytotoxic toward KB cells in culture with EC_{50} value between 0.5 and 0.1 μg ml^{-1} and, which is probably an expression of the same property, to inhibit *in vitro* the concanavaline A-(lymphocyte T) or LPS-induced (lymphocyte B) proliferation of murine spleen cells.

‡ Data for **4**: $[\alpha]_D^{20}$ −84.3 (EtOH, *c* 0.3 g per 100 ml); UV (EtOH) λ_{max} 279 (11900), 232 (8400), 203 (12400); IR (KBr) 1700, 1635, 1550 cm^{-1}; ^{13}C NMR (75.43 MHz; CD_3OD) δ (rel. to $SiMe_4$) 26.65 [s, Me-N(1)], 162.21 [s, C(2)], 30.71 [q, Me-N(3)], 67.18 [d, C(4)], 98.41 [s, C(5)], 52.16 (q, OMe), 41.59 [t, C(6)], 55.12 [d, C(7)], 66.50 [d, C(8)], 33.06 [q, Me-N(9)], 161.97 [s, C(10)], 125.70 [s, C(11)], 108.03 [s, C(13)], 115.72 [d, C(14)], 117.73 [d, C(15)]; ^1H NMR (299.94 MHz; CD_3OD) δ ($SiMe_4$) 2.81 [s, Me-N(1)], 2.98 [s, Me-N(3)], 4.30 [br.s, H(4)], 3.14 (s, MeO), 2.12 [br.t, H_β(6)], 2.67 [br.dd, H_α(6)], 4.67 [m, H(7)], 4.24 [br.d, H(8)], 3.18 [s, Me-N(9)], 6.33 [d, H(14)], 6.89 [br.d, H(15)] (*J* values as for **3** in CD_3OD); EI-MS *m/z* 384/382 (M^+, 10.0/10.5%), 303 ([M − Br]$^+$, 68), 271 ([M − Br − MeOH]$^+$, 22), 228 (45), 125 (100); HREI-MS *m/z* 382.063 ± 0.0025, calculated for $C_{15}H_{19}N_4O_3{}^{79}Br$ 382.064; 384.061 ± 0.0027, calculated for $C_{15}H_{19}N_4O_3{}^{81}Br$ 384.062.

§ Data for **3**: ^{13}C NMR (CD_3OD) δ 25.79 [q, MeN(1)], 163.00 [s, C(2)], 68.98 [d, C(4)], 97.24 [s, C(5)] 41.58 [t, C(6)], 55.96 [d, C(7)], 63.76 [d, C(8)], 162.65 [s, C(10)], 125.71 [s, C(11)], 108.80 [s, C(13)], 115.37 [d, C(14)], 117.59 [d, C(15)]; [$(CD_3)_2SO$] δ 23.50 [q, Me-N(1)], 158.58 [s, C(2)], 65.02 [d, C(4)], 93.34 [s, C(5)], 38.93 [t, C(6)], 52.49 [d, C(7)], 60.25 [d, C(8)], 157.69 [s, C(10)], 123.52 [s, C(11)], 104.49 [s, C(13)], 111.83 [d, C(14)], 113.36 [d, C(15)]; ^1H NMR (CD_3OD) δ 2.81 [s, Me-N(1)], 3.89 [br.s, $J_{4,6\beta}$, $J_{4,6\alpha}$, $J_{4,7}$ and $J_{4,8}$ <0.5 Hz, H(4)], 2.10 [br.t, J_{gem} 12.9, $J_{6\beta,7}$ 12.3, $J_{6\beta,8}$ 0.6, $J_{6\beta,4}$ <0.5 Hz, H_β(6)], 2.65 [br.dd, J_{gem} 12.9, $J_{6\alpha,7}$ 6.6, $J_{6\alpha,8}$ 0.9, $J_{6\alpha,4}$ <0.5 Hz, H_α(6)], 4.60 [m, $J_{7,6\beta}$ 12.3, $J_{7,6\alpha}$ 6.6, $J_{7,8}$ 5.4, $J_{7,4}$ and $J_{7,15}$ <0.5 Hz, H(7)], 4.09 [br.d, $J_{8,7}$ 5.4, $J_{8,6\alpha}$ 0.9, $J_{8,6\beta}$ 0.6, $J_{8,4}$ <0.5 Hz, H(8)], 6.33 [d, $J_{14,15}$ 4.2, H(14)], 6.92 [br.d, $J_{15,14}$ 4.2, $J_{15,7}$ <0.5 Hz, H(15)]; [$(CD_3)_2SO$] δ 2.64 [s, Me-N(1)], 7.11 [d, $J_{3,4}$ 2.1 Hz, HN(3)], 3.77 [br.d, $J_{4,3}$ 2.1 Hz, H(4)], 1.92 [br.t, J_{gem} 12.6, $J_{6\beta,7}$ 12.0 Hz, H_β(6)], 2.46 [br.dd, J_{gem} 12.6, $J_{6\alpha,7}$ 6.0 Hz, H_α(6)], 4.36 [m, $J_{7,6\beta}$ 12.0, $J_{7,6\alpha}$ 6.0, $J_{7,8}$ 5.4 Hz, H(7)], 3.96 [br.d, $J_{8,7}$ 5.4 Hz, H(8)], 8.02 [s, HN(9)], 6.34 [d, $J_{14,15}$ 4.2 Hz, H(14)], 6.74 [d, $J_{15,14}$ 4.2 Hz, H(15)].

tion transfer to both C(13) and C(11), thus also establishing the position of Br. We based 4,5- and 7,8-*cis* fusions on differential NOEs within MeO···H(4) and H(7)···H(8), respectively, and 4,8-*trans* fusion on small coupling between H(4) and H(8). Extensive differential NOE within the couples of protons MeN(1)···MeO, H(4)···MeN(3), H(4)···Me(9), H_2(6)···H(7), H(8)···MeN(3), and H(14)···H(15) with both **3** and **4** confirmed these structural deductions.

Biogenesis of agelastatin A may be imagined, as in Scheme 1, from enzyme-driven C(8) attack at C(4) in hymenidin-like precursor **5** and pyrrole nitrogen attack at developing positive C(7), followed by re-functionalization at C(4) and C(5).

Oroidin-family alkaloids have already shown antibacterial,[2,4a] antifungal,[2] antiserotonergic,[3,8] α-adrenoceptor blocking,[4a,9] and mild cytotoxic[10] activities. Agelastatin A emerges in this family of alkaloids as the first example of markedly cytotoxic agent toward tumour cells.

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