Biomimetic Synthesis of Ascididemin and Derivatives

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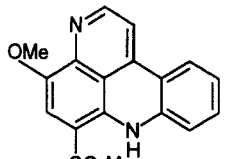
A two-step biomimetic synthesis of the pentacyclic pyrido[2,3,4-*kl*]acridine marine alkaloid ascididemin (**3a**) from quinolinequinone **5a** and *N*-trifluoroacetamidokynuramine (**4**) is described. The crucial step (**6** to **7**) involves the simultaneous formation of two pyridine rings in a process which might well offer an explanation for the biogenetic synthesis in marine organisms. The preparation of substituted ascididemins by either starting from substituted quinolinequinones, e.g., **5b** to afford 11-methoxyascididemin (**3b**), or by nitration of **3a** to the mono 1- or 3-nitroascididemins (**8** and **9** respectively) is reported.

To date the largest family of marine alkaloids characterized has been based on the pyrido[2,3,4-*kl*]acridine skeleton.[1-8] As a group these N-metabolites show a broad range of biological properties.[9] Most recently we have reported three new syntheses of pyrido[2,3,4-*kl*]acridines.[10-12] One[11] is a short, biomimetic synthesis starting from kynuramine (earlier shown by us to also be a marine natural product[13]) and hydroquinones. This biomimetic synthesis was used by us to prepare a variety of pyrido[2,3,4-*kl*]acridines including eilatin (**1**), a unique biologically active heptacyclic marine alkaloid.[7,12] A different synthesis of norsegolin (**2**), the structurally simplest of the group,[7] was most recently reported by McKillop.[14]



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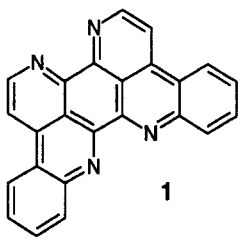

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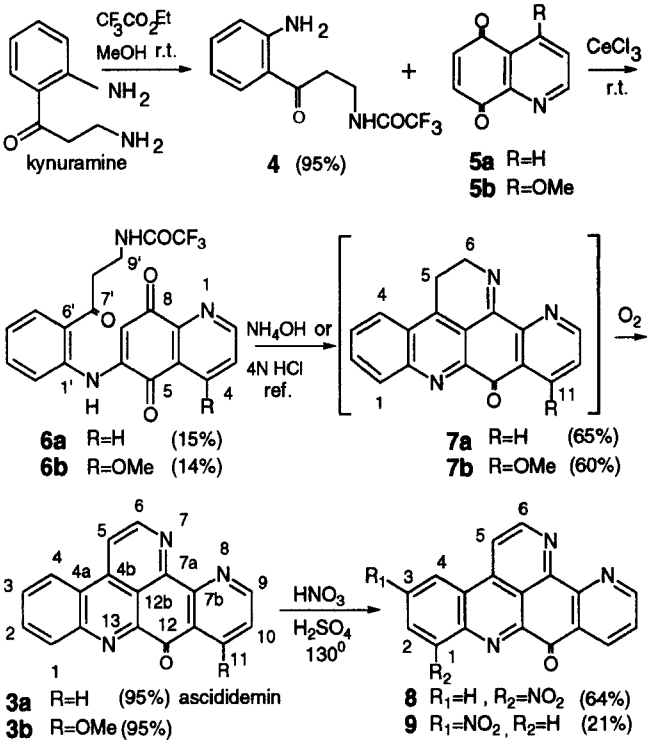
Recent reports by Moody and Kubo of the synthesis of the powerful antineoplastic alkaloid ascididemin (**3a**)[4,15,16] brought us to describe our biomimetic two-step synthesis of **3a**, and derivatives, from *N*-trifluoroacetamidokynuramine (**4**)[12] and quinolinequinone **5a**[17] (compound **5a** was also one of the starting materials of Bracher's first synthesis of ascididemin[18]). The described synthesis might offer the basis of the biogenetic synthesis of **3a** and other pyrido[2,3,4-*kl*]acridine alkaloids and may also be employed in the preparation of other members of these biologically interesting active compounds.

In the event, reacting equimolar quantities of *N*-trifluoroacetamidokynuramine (**4**)[12] with quinolinequinone **5a**[17] under oxidative conditions[11,12,18] ($CeCl_3 \cdot 7H_2O$, r.t., 18 h) afforded adduct **6a**. The structure of the latter oxidative amination product was confirmed mainly by NMR spectroscopy. Most important was a hetero COSY experiment (HMBC) which *inter alia* determined the C6 attachment of the kynuramine moiety to the quinone (correlations between: H-7/C5 and C8a; H-2/C3, C4 and C8a; and H-3 to C4a).



Under either basic (NH_4OH–MeOH 1 : 10, 0.5 h) or acidic conditions (4 N HCl, 1 h) compound **6a** afforded as an intermediate 5,6-dihydroascididemin (**7a**) (60%), which was characterized spectroscopically, and transformed directly by air oxidation (48 h) to ascididemin (**3a**).[4]

In a similar way, starting from 4-methoxyquinoline-5,8-quinone (**5b**),[19] 11-methoxyascididemin (**3b**) was prepared via **6b** and **7b**.

Preliminary studies have shown that nitration of **3a** with a mixture of fuming HNO_3–concentrated H_2SO_4 (130 °C, 1.5 h) afforded a ca. 3 : 1 mixture of 1-nitro- and 3-nitroascididemin (compounds **8** and **9**, respectively). The location of the nitro substituent in **8** and **9**, which were not separated, was determined by COSY and difference NOE experiments. Interestingly, in the case of **9** the signal of H-4, *ortho* to the C3 nitro group was found to resonate as low as $\delta = 9.7$.

It can be expected that isomers **8** and **9** will be suitable starting materials for a whole variety of derivatives of ascididemin which will be obtained from the corresponding diazonium salts or by other electrophilic attacks at C1 or C3.

Another starting material for the preparation of ascididemin derivatives for structure–activity relationship studies is the 5,6-dihydro derivative which will enable other substitutions at the 5 and/or 6 position.

IR spectra were recorded on a Nicolet 205 FT-IR spectrophotometer. Low resolution mass spectra were recorded on a Finnigan-4021 mass spectrometer. ^1H and ^{13}C NMR spectra were recorded on Bruker AMX-360 and ARX-500 spectrometers. All chemical shifts are reported with respect to TMS ($\delta = 0$). Coupling constants, J, are in Hz. HRMS were taken on a MAT 711 instrument. TLC for all compounds was performed on silica gel and eluted with MeOH–EtOAc, 1:9.

2-(3-Trifluoroacetamidopropanoyl)aniline (4):

Kynuramine[20] (700 mg, 4.2 mmol) in MeOH (10 mL) was reacted with ethyl trifluoroacetate (2.84 g, 20 mmol) at r.t. for 4 h. The MeOH was then removed and the residue taken into 2% MeOH in CH_2Cl_2 (50 mL). The organic phase was washed with aq $NaHCO_3$ and brine. The dried (Na_2SO_4) solution was evaporated to afford a red oil (1 g) which was not further purified.

MS: m/z (%) = 260 (M^+, 18), 147 ($M^+ - H_2N\,COCF_3$, 27).

^1H NMR (360 MHz, $CDCl_3$): δ = 7.62 (d, J = 8, H-3), 7.24 (t, J = 8, H-4), 6.98 (t, J = 8, H-5), 6.61 (d, J = 8, H-6), 3.76 (m, H_2-3'), 3.19 (t, H_2-2').

6-[2-(3-Trifluoroacetamidopropanoyl)anilino]-5,8-quinoline Quinone (6a):

Quinone **5a**[17] (160 mg, 1 mmol) was reacted with compound **4** (260 mg, 1 mmol) in EtOH (50 mL) in the presence of $CeCl_3 \cdot 7H_2O$ (390 mg, 1.05 mmol) at r.t. for 18 h. Most of the EtOH was removed under vacuum, H_2O was added and the mixture extracted with CH_2Cl_2 (3 × 50 mL).

Chromatography of the residue after evaporation of the solvent (vacuum liquid chromatography on silica gel-H, 1% MeOH in EtOAc afforded compound **6a** (62 mg, 15%) an orange amorphous powder; mp 213 °C. R_f = 0.85

MS: m/z (%) = 417 (M^+, 25), 306 (M^+–$NCOCF_3$, 28).

IR (KBr): ν = 1717, 1650, 1579, 1536, 1307, 1191 cm^{-1}.

^1H NMR (500 MHz, $CDCl_3$–CD_3OD, 9:1): δ = 9.02 (dd, J = 5.2, 1, H-2), 8.48 (dd, J = 8, 1, H-4), 7.96 (d, J = 8, H-5'), 7.65 (m, H-3,2',4'), 7.22 (t, J = 8, H-3'), 6.92 (s, H-7), 3.73 (t, J = 6, H-9'), 3.35 (t, J = 6, H_2-8').

^{13}C NMR: δ = 200.9 (s, C7'), 181.8 (s, C5), 180.1 (s, C8), 162.1 (q, $\underline{C}OCF_3$), 154.6 (d, C2), 147.2 (s, C4b), 143.2 (s, C6), 138.2 (s, C1'), 134.0 (d, C3), 133.9 (d, C3'), 131.3 (d, C5'), 127.1 (s, C4a), 125.8 (d, C4), 125.7 (s, C6'), 123.2 (d, C4'), 120.9 (d, C9), 105.8 (d, C7), 39.0 (t, C9'), 34.5 (t, C8').

Ascididemin (3a) via 5,6-Dihydroascididemin (7a):

Method A: To a solution of **6a** (31 mg, 0.074 mmol) in MeOH (5 mL) was added NH_4OH 28%, (0.5 mL), and the mixture was stirred for 0.5 h at r.t. Water (30 mL) was added, and the diluted mixture extracted with CH_2Cl_2 (3 × 30 mL). The extract was washed with brine, dried and evaporated to afford 5,6-dihyroascididemin (**7a**), as a yellow amorphous powder (20 mg). R_f = 0.45.

^1H NMR (500 MHz, $CDCl_3$): δ = 9.15 (dd, J = 4.8, 1.6, H-9), 8.76 (dd, J = 8, 1.6, H-11), 8.57 (d, J = 8, H-4) 8.48 (d, J = 8, H-1), 7.94 (t, J = 8) and 7.50 (t, J = 8) (H-2,3), 7.84 (dd, J = 8, 4.8, H-10), 4.06 (m, H_2-6), 3.93 (m, H_2-5).

Stirring a solution of **7a** which was not further purified (13 mg, 0.044 mmol) in MeOH (5 mL) for 48 h, under air afforded ascididemin (**3a**, 12 mg, 60%, from **6a**) identical in all respects with the lit. data[4]).

^1H NMR ($CDCl_3$–CD_3OD): δ = 9.26 (d, J = 5), 9.16 (bd, J = 4), 8.78 (d, J = 8), 8.70 (d, J = 8), 8.62 (d, J = 8), 8.56 (d, J = 5), 8.02 (t, J = 8), 7.95 (t, J = 8), 7.69 (dd, J = 4,8).

Method B: A solution of **6a** (31 mg, 0.074 mmol) in 4 N HCl (5 mL) was refluxed for 1 h. Ice (10 g) was added, the mixture was basified with NH_4OH (28%, 10 mL) and then extracted with CH_2Cl_2 (3 × 30 mL). The organic layer was washed with brine, dried and evaporated to afford 5,6-dihydroascididemin (**7a**) (14 mg) that was not further purified. Refluxing of a solution of the latter **7a** in 4N HCl (5 mL) for 2 h under air afforded, after workup as described above and chromatography (eluting with EtOAc:MeOH 95:5), ascididemin (**3a**, 13 mg, 60% from **6a**), R_f = 0.35.

Compound 6b:

Compound **5b**[19] (190 mg, 1 mmol), under the same conditions as described for **5a**, afforded compound **6b** (63 mg, 14%); an orange powder, mp 226 °C. R_f = 0.85.

MS: m/z (%) = 449 ($M^+ + 2H$, 24)[4], 447 (M^+, 4), 336 ($M^+ - NCOCF_3$, 8).

IR (KBr): ν = 1712, 1649, 1628, 1610, 1571, 1561, 1545, 1449, 1254, 760 cm^{-1}.

^1H NMR (500 MHz, $CDCl_3$–CD_3OD, 9:1): δ = 8.80 (d, J = 5.2, H-2), 7.96 (d, J = 8, H-5'), 7.58 (m, H-12, 14), 7.20 (t, J = 8, H-3'), 7.07 (d, J = 5.2, H-3), 7.74 (s, H-7), 4.08 (s, OMe), 3.71 (t, J = 6, H_2-9'), 3.26 (t, J = 6, H_2-8').

^{13}C NMR: δ = 201.3 (s, C7'), 180.1 (s, C5), 166.0 (s, C4), 155.6 (d, C2), 150.1 (s, C4b), 140.0 (s, C1'), 134.8 (d, C3'), 131.6 (d, C5'), 123.5 (d, C4'), 122.1 (s, C6'), 120.8 (d, C2'), 117.0 (s, C4a), 110.5 (d, C3), 105.2 (d, C7), 56.7 (q, OMe), 36.5 (t, C9'), 34.5 (t, C8').

11-Methoxyascididemin (3b) via 5,6-Dihydro-11-methoxyascididemin (7b):

Starting from compound **6b** (30 mg, 0.067 mol) afforded compound **3b** via **7b** (12 mg, 56%) under the same conditions as described for ascididemin (**3a**).

Compound **7b**, amorphous yellowish powder. R_f = 0.40.

MS: m/z (%) = 317 ($M^+ + 2H$, 1).

^1H NMR (500 MHz, $CDCl_3$): δ = 8.87 (d, J = 4.8, H-9), 8.52 (d, J = 8, H-4), 8.48 (d, J = 8, H-1), 7.91 (t, J = 8.0) and 7.84 (t, J = 8) (H-2 and 3), 7.19 (d, J = 4.8, H-10), 4.14 (s, OMe), 3.92 (m, 4H).

11-Methoxyascididemin (**3b**), amorphous powder, mp > 300 °C.

R_f = 0.30.

MS: m/z (%) = 315 ($M^+ + 2H$, 2)[4], 313 (M^+, 2).

IR (KBr): ν = 1672, 1663, 1655, 1640, 1610, 1575, 1490, 1415, 1238, 1010, 815 cm^{-1}.

^1H NMR (500 MHz, $CDCl_3$–CD_3OD, 9:1): δ = 9.20 (d, J = 5.5, H-6), 8.89 (d, J = 5.8, H-9), 8.61 (d, J = 8, H-4), 8.54 (d, J = 8, H-1), 8.47 (d, J = 5.5, H-5), 7.93 (t, J = 8, H-2), 7.85 (t, J = 8, H-3), 7.09 (d, J = 5.8, H-10), 4.20 (s, OMe).

^{13}C NMR: δ = 167.5 (s, C11), 155.2 (d, C9), 149.5 (d, C6), 147.0 (s, C13a), 144.0 (s, C7a), 143.2 (s, C12a), 138.8 (s, C4b), 132.4 (d, C1), 131.9 (d, C2), 130.5 (d, C3), 123.9 (s, C4a), 122.9 (d, C4), 119.5 (s, C11a), 117.2 (d, C12b), 117.0 (d, C5), 110.1 (d, C10), 56.6 (q, OMe).

1- and 3-Nitroascididemin (8 and 9):

Ascididemin (**3a**) (22 mg, 0.067 mmol) in a solution of conc. H_2SO_4/fuming HNO_3 (1:1, 1 mL) was heated for 15 h at 130 °C. Ice (5 g) was then added and the resulting mixture was extracted with CH_2Cl_2 (3 × 20 mL), the organic phase was washed with aq. $NaHCO_3$ and brine, dried ($MgSO_4$), and then evaporated. The crude product was passed through a silica gel-H column eluted with MeOH–EtOAc (1:20) to afford a mixture (15 mg, 55%) of 1-nitro and 3-nitroascididemin (**8** and **9** in a ca. 3:1 ratio, respectively).

Compound **8**, yellow powder.

^1H NMR (500 MHz, $CDCl_3$): δ = 9.47 (d, J = 5.7, H-6), 9.28 (dd, J = 4.3, 1.8, H-9), 9.01 (d, J = 8.2, H-4), 8.86 (dd, J = 8, 1.8, H-11), 8.68 (d, J = 5.7, H-5), 8.39 (d, J = 8.2, H-2), 8.15 (t, J = 8.2, H-3), 7.81 (dd, J = 8, 4.3, H-10).

Compound **9**. R_f = 0.25.

MS: m/z = 328.

^1H NMR: δ = 9.70 (brs, H-4), 9.60 (d, J = 5.4, H-6), 9.35 (d), 8.90 (d), 8.77 (d, J = 5.4, H-5), 8.30 (d), 8.18 (t), 7.84 (dd). The signal assignments were based on a COSY experiment and d-NOE's (the most important NOE being the one between H-5 and H-6 and a strong one between H-5 and H-4).

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