Synthesis of Anatoxin-a. A Constituent of Blue-Green Freshwater Algae

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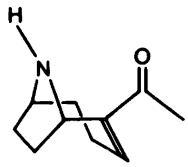
Anatoxin-a is a naturally occurring neurotoxin produced by the blue-green alga *Anabaena flos-aquae* and is a potent muscarinic and nicotinic receptor agonist. An improved synthetic pathway to racemic anatoxin-a is presented which opens possibilities for easy introduction of various side-chains for structure-activity relationship studies. Starting from 9-methyl-9-aza[4.2.1]nonan-2-one, the α,β-enone structural element was introduced using Seebach's carbonyl-"umpolung" method, followed by *N*-demethylation and dethioketalization to give pure anatoxin-a.

Toxic blooms of blue-green freshwater algae have, from time to time, been responsible for the deaths of various kinds of livestock and wildlife. The main toxic strains have been shown to belong to the genera *Microsystis, Anabaena* and *Aphanizomenon*.[1-3] A low-molecular weight toxin, anatoxin-a, has been isolated from a toxic strain of *Anabaena flos-aquae* (Lyngb.) de Breb.[4] This toxin has been shown by spectroscopic as well as X-ray methods to be the bicyclic alkaloid 2-acetyl-9-azabicyclo[4.2.1]non-2-ene (**1**).[4,5]



1

Anatoxin-a, being a potent depolarizing neuromuscular blocking agent with agonistic properties at the nicotinic and muscarinic receptors, was of great interest to us for biological testing purposes.[6-8]

A number of synthetic approaches have been made to the preparation of **1**, the first of which started from cocaine.[9] Later methodology has involved the intramolecular cyclization of 5-methylaminocycloocten-6-ol[10,11] to obtain **2** (as in the present work). Very recently, Wisemann and Lee have published an elegant synthetic scheme[12] in which **2** was obtained from 1,5-dihydroxycyclooctane in three steps and then converted to anatoxin-a via a Horner-Emmons reaction. Other approaches make use of nitrones[13], cyclization of pyrrolidine derivatives[14,15] prepared from *N*-methylpyrrole and glutamic acid [thus making available rac-, (+)- and (−)-anatoxin-a] or cleavage/transannular cyclization of 4-amino-8,8-dibromo[5.1.0]octane.[16]

Results

The purpose of this investigation was to devise a method for preparing **1** and compounds analogous to **1**, and to examine their structure-activity relationships. Accordingly, we sought a synthetic procedure in which the basic ring structure could be prepared according to a common method and various side-chains introduced at later stages of the synthesis. Of the methods described up to now, only the procedures of Edwards and coworkers[10] and Wiseman and Lee[12] fit this description. However, since the former method (the latter results appeared while preparing this manuscript) for introducing the acyl moiety seemed

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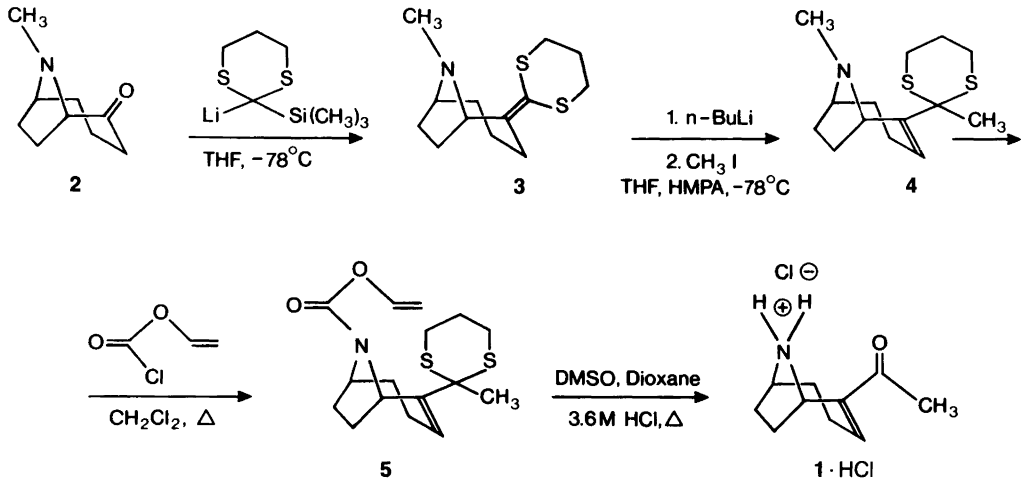

Scheme 1.

somewhat laborious from the point of view of making numbers of analogues, we decided to try an alternative method for introducing side-chains. This was accomplished (Scheme 1) by the conversion of **2** (prepared according to Ref. 10) to its corresponding ketene dithioketal (**3**), the anion of which is then alkylated. This procedure allows the introduction of both the side-chain and the endocyclic double bond in one single step. To achieve *N*-demethylation, **4** was treated with vinyl chloroformate[18] in order to substitute the methyl group for a vinyloxy group which was then removed in the next dethioketalisation step[19] to give anatoxin-a.

Discussion

Summarizing, this procedure opens possibilities for the synthesis of analogues of anatoxin-a by the facile introduction of various acyl groups using readily available reagents. Yields are generally good, considering the fact that no attempt has been made to optimize the procedures. The only draw-back in this procedure is in the preparation of the bicyclic ketone **2**, the synthesis of which has been achieved with varying results. The work reported recently by Wiseman and Lee[12] seems to have overcome these problems.

Experimental

NMR spectra were recorded on a JEOL FX 90 Q spectrometer operating at 90 MHz (^{1}H) or 22.5 MHz (^{13}C). Tetramethylsilane was used as internal standard and deuterochloroform as solvent unless otherwise stated. Mass spectra were recorded at 70 eV using a JEOL D300 spectrometer. Semi-preparative HPLC was performed with a Waters Associates pump system using a LKB 2151 Variable Wavelength Detector. Microanalyses were performed by Mikrokemi AB, Uppsala, Sweden. Melting points are uncorrected. Tetrahydrofuran was heated under reflux with, and distilled from, potassium. Hexamethylphosphoramide *(Warning: carcinogenic!)* was refluxed with, and distilled from, sodium. Reactions involving dry solvents were performed in an inert atmosphere in well-dried apparatus.

2-(1,3-Dithian-2-ylidene)-9-methyl-9-azabicyclo-[4.2.1]nonane (**3**). The lithium salt of 2-(trimethylsilyl)-1,3-dithiane was prepared by adding 25 ml of 1.6 M (40 mmol) butyllithium to a solution of the dithiane in 100 ml of tetrahydrofuran at −78 °C and allowing the temperature to slowly rise to 0 °C. After standing for 3.5 h, a tetrahydrofuran solution (30 ml) of **2** (6.0 g, 39 mmol) was added dropwise at −78 °C. The reaction mixture was allowed to attain room temperature while left overnight with stirring. The mixture was worked up by pouring into water and extracting with ether. The product was purified on a silica column using ether and then methanol as eluents to yield 6.8 g (78 %). For further purification, recrystallization from diisopropyl ether was found to be convenient. M.p. 60–62 °C. Anal.

$C_{13}H_{21}NS_2$: C, H, N. ^{1}H NMR: δ 1.2–2.3 and 2.5–3.2 (16 H, m), 2.43 (3 H, s), 3.2–3.4 (1 H, m), 4.4 (1 H, broad d). ^{13}C NMR: δ 22.7 (t), 24.65 (t), 26.67 (t), 29.59 (t), 31.61 (t), 32.26 (t), 34.54 (t), 41.62 (q), 65.16 (d), 67.57 (d), 118.16 (s), 148.53 (s). MS [*m/z* (rel. abundance/%)]: 255 (M, 90), 227 (70), 213 (100), 82 (40).

2-(2-Methyl-1,3-dithian-2-yl)-9-methyl-9-azabicyclo[4.2.1]non-2-ene (**4**). To a solution of 3.8 g (14.9 mmol) of **3** and 8 ml of hexamethylphosphoramide in 100 ml of tetrahydrofuran was added 10.5 ml of 1.6 M (16.8 mmol) butyllithium dropwise at −78 °C. After standing at room temperature for 3.5 h, 1.03 ml (2.35 g, 16.6 mmol) of methyl iodide in 30 ml of tetrahydrofuran was added at −78 °C. The reaction mixture was left overnight at room temperature prior to work-up (same procedure as for **3**). The product was purified on a silica column using ether as eluent to afford 3.5 g (85 %). An analytical sample was obtained by recrystallization from diisopropyl ether. M.p. 69–72 °C. Anal. $C_{14}H_{23}NS_2$: C, H, N. ^{1}H NMR: δ 1.0–3.0 (14 H, m), 1.67 (3 H, s), 2.40 (3 H, s), 3.3–3.5 (1 H, m), 4.08 (1 H, dd), 6.22 (1 H, broad t). ^{13}C NMR: δ 23.87 (t), 24.84 (t), 25.95 (t), 27.57 (t), 27.97 (t), 28.16 (t), 28.62 (q), 32.91 (t), 36.68 (q), 54.89 (s), 62.11 (d), 63.80 (d), 128.05 (d), 146.84 (s). MS [*m/z* (rel. abundance/%)]: 269 (M, 57), 235 (33), 195 (18), 194 (36), 163 (51), 162 (41), 96 (83), 81 (100).

2-(2-Methyl-1,3-dithian-2-yl)-9-vinyloxycarbonyl-9-azabicyclo[4.2.1]non-2-ene (**5**). To achieve *N*-demethylation, 1.0 g (3.72 mmol) of **4** was treated with 0.38 ml (4.0 mmol) of vinyl chloroformate in 100 ml of dichloromethane at 0 °C and then heated under reflux overnight. Compound **5** was obtained after evaporation and purification on a silica column using ether as eluent, yielding 1.0 g (83 %). The analytical sample was obtained by recrystallization from diisopropyl ether. M.p. 98–100 °C. Anal. $C_{16}H_{23}NO_2S_2$: C, H, N. ^{1}H NMR: δ 1.5–2.8 (17 H, m), 1.74 (s), 1.79 (s), 4.41 (1 H, d, *J* 6.3 Hz), 4.62 (1 H, m), 4.72 (1 H, broad d, *J* 14.2 Hz), 4.99 (1 H, broad d, *J* 8.8 Hz), 6.24 (1 H, dt, *J* 5.8 Hz), 7.21 (1 H, dd, J_{cis} 6.4 Hz, J_{trans} 14.2 Hz). ^{13}C NMR: δ 23.55 (t), 23.68 (t), 24.59 (t), 27.90 (t), 28.10 (t), 28.81 (t), 29.59 (q), 30.70 (t), 31.41 (t), 32.72 (t), 34.01 (t), 54.17 (s), 55.73 (d), 56.78 (d), 94.75 (t), 128.70 (d), 129.35 (d), 142.16 (d), 142.42 (d), 147.40 (s), 148.60 (s), 150.03 (s) (the NMR spectra were simplified when run at 100 °C using DMSO-d_6 as solvent). MS [*m/z* (rel. abundance/%)]: 325 (M, 53), 282 (38), 208 (100), 59 (41).

2-Acetyl-9-azabicyclo[4.2.1]non-2-ene hydrochloride (Anatoxin-a · HCl, **1** · *HCl).* Hydrolysis to anatoxin-a (**1**) was effected by heating a solution of 1.0 g (3.0 mmol) of **5** in a mixture of 6 ml of dimethyl sulfoxide, 72 ml of dioxane and 24 ml of 3.6 M hydrochloric acid under reflux for 3 h. After cooling, ice-cold 2 M sodium hydroxide was added cautiously and **1** was extracted into cold ether. The ether solution was treated with dry hydrogen chloride gas, giving **1** · HCl. The product was dried *in vacuo* at 5 °C to yield 0.4 g (64 %). Injection (portions of 300 µl) of **1** · HCl in MeOH (50 mg ml^{-1}) on a semi-preparative C-18 column (Partisil M20 10/25 ODS-2, supplied by Whatman), using 5 % 2M acetic acid in methanol as eluent, completed the purification. ^{1}H NMR: δ 1.7–2.6 (11 H, m), 2.36 (s), 4.34 (1 H, broad s), 5.20 (1 H, broad t), 7.20 (1 H, broad t), 8.3–9.9 (2 H, three broad s). ^{13}C NMR: δ 23.22 (t), 24.98 (q), 27.12 (t), 27.25 (t), 29.85 (t), 51.64 (d), 58.14 (d), 143.13 (s), 146.00 (d), 196.26 (s). MS for **1** [*m/z* (rel. abundance/%)]: 165 (M, 100), 150 (27), 136 (38), 122 (55), 94 (27), 82 (29), 68 (25).

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