Tetrahedron Vol. 41, No. 17, pp. 3447 to 3453, 1985
Printed in Great Britain.

0040-4020/85 $3.00 + .00
Pergamon Press Ltd.

THE USE OF NITRONES IN THE SYNTHESIS OF ANATOXIN-a, VERY FAST DEATH FACTOR

JOSEPH J. TUFARIELLO,* HAROLD MECKLER and
K. PUSHPANANDA A. SENARATNE
Department of Chemistry, State University of New York at Buffalo, Buffalo, NY 14214, U.S.A.

(*Received in Switzerland* 31 *December* 1984)

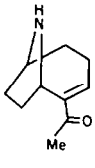
Abstract—The synthesis of anatoxin-a (**1**) was completed by using the cycloaddition of 1-pyrroline 1-oxide (**2**) onto dienol (**6**), a reaction which proceeded with high stereoselectivity, regioselectivity, and site selectivity. The resultant adduct (i.e. **7**) was oxidized to a second nitrone (i.e. **8**) which undergoes a second closure to afford cycloadduct **9a** with regiospecificity. The conversion of **9a** into anatoxin-a hydrochloride (**1** · HCl) was both direct and efficient.

Various species of marine algae have been harvested by man for centuries. The principal micro-algae that have been used for human and animal consumption are enkaryotic chlorophytes such as *Chorella* and *Scenedesmus*. Another class of micro-algae, the cyanophytes, have also been used as a source of food because they have a high protein content and good digestibility. Their rapid growth on waste nutrients make the cyanophytes an attractive source of inexpensive food. The main drawback in using these bloom-forming cyanophytes as food relates to the presence of toxic strains, indistinguishable from non-toxic strains.[1] The most common of these toxic bloom-forming cyanophytes are *Microcystis aeruginosa* Kutz emend Blekin, *Anabaena flos-aquae* (Lyngb.) de Breb. and *Aphanizomenon flos-aquae* (L.) Ralfs.[1]

The toxic strains of *Anabaena flos-aquae* were first examined in the 1950s. The animal loss at Storm Lake, Iowa, in the fall of 1952 was substantial and forced the closing of the lake to public recreation.[2] The toxin was designated "very fast death factor" (VFDF).[3]

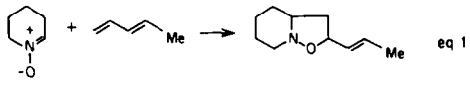
The pure alkaloid has a minimum lethal dose (intraperitoneal mouse assay) of 0.3 mg kg^{-1} (4–5 min survival). A pharmacologic study of both natural and synthetic anatoxin-a reveals that they exhibit both muscarinic and nicotinic activity.[1]

The structure of VFDF was shown to be 2-acetyl-9-azabicyclo[4.2.1]non-2-ene (anatoxin-a; **1**), an assignment which was confirmed by X-ray crystallography.[4,5] There have been several synthetic explorations directed toward this interesting alkaloidal system.[6–9]

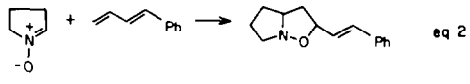


1

It was our belief that nitrone cycloaddition chemistry could be applied to the synthesis of anatoxin-a. A retrosynthetic analysis is included in Scheme 1. The point of embarkation for this approach was expected to involve the cycloaddition of 1-pyrroline 1-oxide (**2**) with 3,5-hexadiene-2-one (**3**). Previous work in our laboratories suggested that such a reaction should exhibit the high site-selectivity and regioselectivity which is the keystone of this approach. Thus, we knew that the reactions illustrated in Eqs (1) and (2) proceed



eq 1

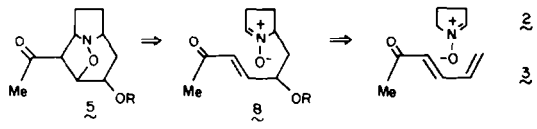


eq 2

as depicted.[10] Moreover, the experimental observations are supported by perturbation molecular orbital calculations.[11] Unfortunately, yields of adduct from the reaction of **2** with the dienone **3** were very low, presumably as a result of the tendency of the latter to undergo concomitant polymerization. To circumvent this problem, 3,5-hexadien-2-ol (**6**)[12] was substituted for the dienone in the reaction with 1-pyrroline 1-oxide.

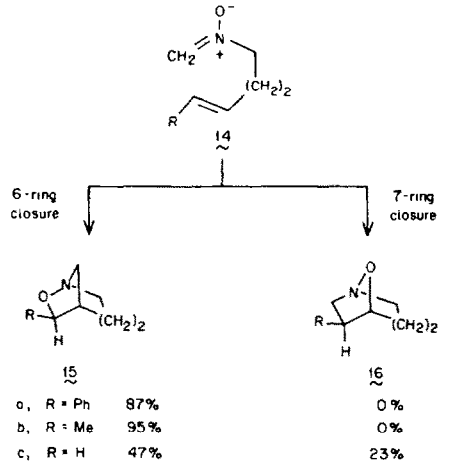
Previous work in our laboratories relating to the stereochemistry of nitrone–diene cycloaddition reactions suggests that such cycloaddition processes should proceed principally through an *exo*-transition state to afford **7**. When this adduct was oxidized with manganese dioxide, only one product (i.e. **4**) could be detected. The PMR spectrum for this compound exhibited a clean quartet at δ 4.71 (J = 6.7 Hz), a doublet of doublets at δ 6.61–6.85 (J = 16.1, 6.7 Hz), and a doublet at δ 6.24 ppm (J = 16.1 Hz). These signals correspond to the proton at the 5-position of the isoxazolidine ring (i.e. methine) and the protons associated with the *trans*-α,β-unsaturated ketone, respectively.

The oxidative cleavage of the isoxazolidine **7** with *m*-chloroperbenzoic acid (MCPBA) affords the less substituted nitrone **8**, exclusively.[13,14] The second, intramolecular cycloaddition reaction was induced by refluxing the nitrone in methylene chloride. The structural assignment of the cycloadduct (i.e. **9**) entailed important implications with regard to the proposed synthetic strategy (cf. Scheme 1). It has already been noted[15] that N-alkenyl nitrones can cyclize to produce either the product of 6-membered ring closure (i.e. **15**) or

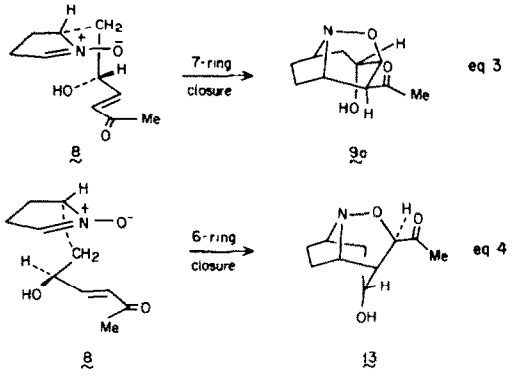


Scheme 1.

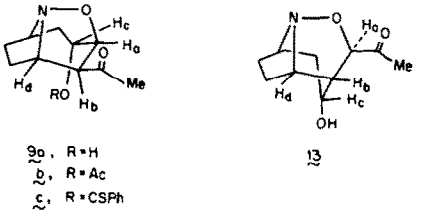
that of 7-membered ring closure (i.e. **16**). Thus the intramolecular cycloaddition reactions of **14a** and **14b** leads to the exclusive formation of **15a** and **15b**, respectively, via 6-membered ring closure. Even **14c**, which incorporates a monosubstituted alkenyl moiety, displays this mode of closure preferentially.[15] This is



remarkable since, for intermolecular nitrone cycloadditions to such alkenes, the observed[16] regiochemical preference involves C—C bond formation between the carbon of the nitrone functionality and the less substituted carbon of the recipient alkene grouping, a circumstance which would result in 7-membered ring closure in the case of **14c**. Clearly, nitrone **8** can undergo either 6- or 7-membered ring closure (cf. Eqs 3 and 4).

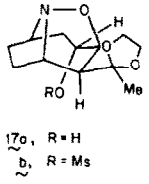


We believed that **8** might overcome the normal predilection for 6-membered ring closure because of the natural tendency of nitrones, in intermolecular cycloaddition reactions, to afford the adduct with the nitrone oxygen bound to the β-carbon of an α,β-unsaturated carbonyl system.[13,16–18] Indeed, when this reaction was attempted, we could only identify a single cycloadduct from **8** (i.e. **9a**). The PMR spectrum



of this adduct consisted of a doublet at δ 4.38 (J = 5.5 Hz, 1H), a complex pattern at δ 3.71–4.23 (2H), another complex pattern at δ 3.38–3.60 (1H), a doublet at δ 3.00 (J = 6.0 Hz, 1H), a doublet centered at δ 2.50 (J = 5.5 Hz, 1H), a complex pattern centered at δ 1.39–2.20 (6H), and a sharp singlet at δ 2.20 (3H) ppm. When the free hydroxyl group was acetylated (Ac_2O, pyr) to afford **9b**, the signal at δ 3.50 (H_c) was transformed into a pattern centered at δ 5.09 ppm. The doublet at δ 4.38 moved to δ 4.51 ppm (H_a) and the doublet at δ 2.50 (OH) ppm vanished. When the hydroxyl group was functionalized as a thiobenzoate (**9c**), the shifts of the PMR patterns were even more pronounced. The complex pattern centered at δ 3.50 was transformed into an eight-line pattern centered at δ 5.97, and the downfield doublet (H_a) shifted to δ 4.79 ppm. The fact that H_a appears as a doublet is associated with the virtual 90° dihedral angle involving H_a and H_b. Indeed, when **9a** was converted into the corresponding ethylene ketal, the doublet (H_b) at δ 3.00 was shifted upfield to δ 2.39 ppm. These assignments were confirmed by spin decoupling experiments. The accumulated data suggests that **13** can be ruled out as the structure for the cycloadduct since ketalization would be expected to shift H_a, associated with the signal at lowest field, to markedly higher fields. Moreover, H_a and H_c would not be expected to be coupled in contradiction to observation. Finally, the subsequent transformation of **9** to anatoxin-a is a compelling argument in regard to the accuracy of the assignment.

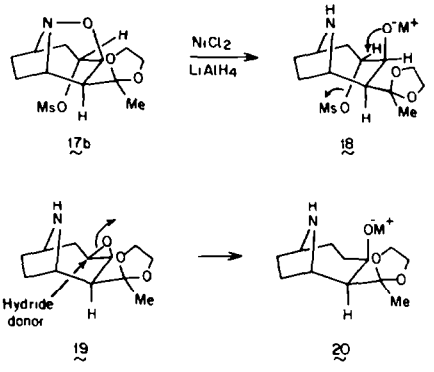
Since the second cycloaddition proceeded by 7-membered ring closure, the basic 7-azabicyclo[4.2.1]nonane ring system of the natural product now was assembled. The cycloadduct was converted into the 1,3-dioxolane **17a** prior to attempted removal of the unwanted OH function. Our initial attempts to remove the OH moiety involved conversion to the corresponding methanesulfonate **17b**, followed by attempted displacement with lithium



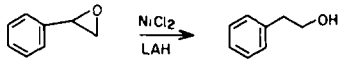
triethylborohydride. This reaction led to O—S bond cleavage rather than the requisite C—O bond cleavage. Indeed, all attempts to induce an S_N2 displacement of the mesylate function of **17b** were unsuccessful. These attempts included reaction with halide and phenyl selenate anions. Such displacements are apparently inhibited by steric factors as well as anion–lone pair repulsion at the stage of the S_N2 transition state. Attempts to remove the thiobenzoate grouping in **9c** with tributylstannane[19] also failed to result in formation of the desired dethiobenzoylated product.

Ashby and Lin reported[20] that complex hydride reducing agents, $NiCl_2$–$LiAlH_4$ and $CoCl_2$–$LiAlH_4$ were able to reductively remove primary halides and sulfonates and secondary halides in high yield without rearrangement. When mesylate **17b** was treated with 3 equiv of a 1:1 molar mixture of $NiCl_2$–$LiAlH_4$, a mixture of products was formed, of which **10** was the predominant constituent. Clearly, the mesylate

function was reductively removed and the N—O bond of the isoxazolidine was cleaved, an added bonus. When anhydrous cobalt(II) chloride was used instead of nickel(II) chloride, the yield of isolated product was attenuated considerably.

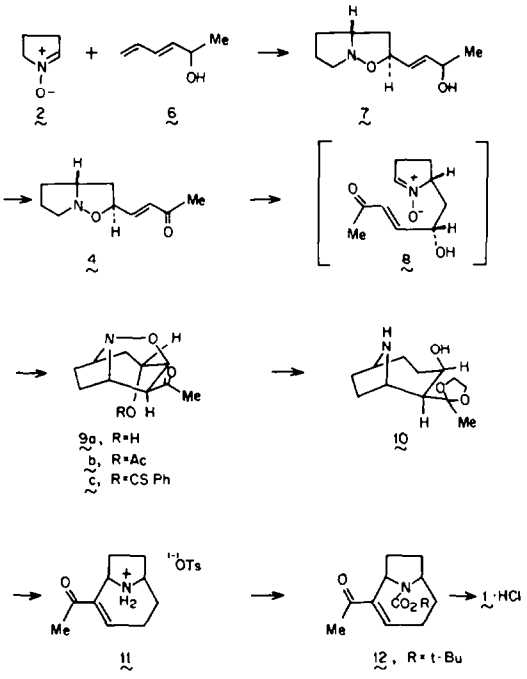


We suspected that the mesylate was initially removed by the "nickel hydride", followed by N—O bond cleavage; however, we found that secondary mesylates



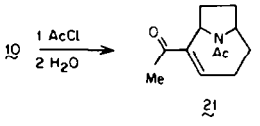
(e.g. cyclododecyl mesylate, cyclohexyl mesylate, 2-octyl mesylate) were unaffected by this reagent. This suggested the possibility that the isoxazolidine ring was cleaved initially. Indeed, the results in Table 1 suggest clearly that isoxazolidines are cleaved efficiently by the complex hydride reagent. Thus, although mechanistic variants can be envisioned, we suggest that N—O bond cleavage of **17b** leads to **18** which spontaneously results in oxirane formation. Attack of a hydride donor from the less hindered direction then produces **20**, which upon aqueous work-up gives **10**. In contrast, we found that when styrene oxide is treated with $LiAlH_4$–$NiCl_2$ under the same conditions, the product isolated (95%) is β-phenylethanol. This latter product is also produced when the same oxide is exposed to Raney nickel. Lithium aluminum hydride alone gives α-phenylethanol.

The cleaved adduct **10** was exhaustively acetylated with acetyl chloride. An aqueous work-up resulted in both deketalization and deacetoxylation to afford N-acetyl anatoxin-a (**21**). The PMR, IR and mass spectra



Scheme 2.

of the synthetic derivative match those of the same derivative of the natural product.[21] To secure anatoxin-a itself, the amino alcohol **10** was treated with



a stoichiometric amount of *p*-toluenesulfonic acid in acetone to induce both *trans*-ketalization and dehydration. To effect purification, the crude salt (i.e. **11**) was then treated with 2 equiv of sodium bicarbonate and di-t-butyldicarbonate according to the procedure of Tarbell *et al.*[23] to afford the t-butyl carbamate (t-BOC) of anatoxin-a. Chromatographic separation of **12** was followed by hydrolytic removal of the labile

Table 1. The reduction of isoxazolidines with $NiCl_2$–$LiAlH_4$

Substituents*	Product, % yield
(1) R_1, R_2 = Ph, R_3 = H, R_4 = n-Bu	92
(2) R_1, R_2 = —$(CH_2)_4$—, R_3 = H, R_4 = Ph	87
(3) R_1, R_2 = —$(CH_2)_4$—, R_3 = H, R_4 = Ph CH_3I salt	76
(4) R_1, R_2 = —$(CH_2)_4$—, R_3 = H, R_4 = n-Bu	97
(5) R_1, R_2 = —$(CH_2)_4$—, R_3, R_4 = Ph	95

*The isoxazolidines were prepared by nitrone–alkene cycloaddition reactions.

t-BOC group to give anatoxin-a hydrochloride (**1**·HCl). The PMR, IR and mass spectra of this material were virtually identical to those of the salt of the natural toxin.[21]

EXPERIMENTAL

All m.ps (determined on a Mel-Temp capillary tube apparatus) and b.ps are reported, uncorrected, in °C. IR spectra have been determined on either a Perkin–Elmer 1310 or 727 spectrometer and calibrated using the 6.238 band of polystyrene. All IR absorptions are reported in μm: w, m, and s indicate the relative intensity of the absorption as weak, medium and strong, respectively. PMR spectra were recorded on a Varian T-60, JEOL MH-100 or Varian EM-390, spectrometer, using TMS as an internal standard. The position of the peaks are reported in ppm down field relative to TMS. Notations s, d, t, q, m, and cp designate singlet, doublet, triplet, quartet, multiplet and complex patterns, respectively. The integration number and coupling constant(s) are shown in parentheses. Mass spectra were recorded on a Hitachi–Perkin–Elmer RMU-6E spectrometer. The ionizing voltage was 70 V with a current of 80 A. All samples were introduced via direct inlet. If higher probe temps were needed, they were noted in parentheses. UV spectra were recorded on a Cary 14 spectrometer. Values are reported in nm.

Combustion analyses were performed by Atlantic Microlabs, Atlanta, Georgia. High resolution mass spectra were recorded on an AEI-MS-902 instrument by Dr T. Wachs at the Mass Spectroscopy Laboratory, Cornell University, Ithaca, New York.

Column chromatography was performed on both silica gels and alumina. Baker Analyzed Silica Gel (40–140 mesh) was used for samples larger than 250 mg and EM Reagents Silica Gel 60 (70–230 mesh) was used for samples smaller than 250 mg. Baker Analyzed Aluminum Oxide, Neutral was adjusted to Brockmann Activity III by adding (*ca*) 10% (w/w) water to the aluminum oxide which had been activated for 24 hr at 125°. Preparative TLC was performed on either Analtech Woelm Silica Gel GF (2000 μm, 20 × 20 cm) or Analtech Aluminum Oxide GF (1000 μm, 20 × 20 cm) preparative TLC plates, activated at 110° for 3 hr prior to use.

N-*hydroxypyrrolidine*. This was prepared according to previously discussed procedures[10,17] and was obtained in 32% yield; b.p. 53–55°/5 mmHg (lit.[17] b.p. 65–72°/20 mmHg).

2,4-*Pentadienal*. The title compound was prepared following the procedure of Woods and Sanders.[25] To a stirred soln of 46 ml of 85% phosphoric acid in 200 ml water was added 46.2 g (0.36 mol) of 2-ethoxy-5,6-dihydropyran. When the soln became homogeneous, it was added dropwise to a soln of 60 ml of 85% phosphoric acid in 500 ml water already undergoing steam distillation. The steam distillation was continued until the distillate no longer had the characteristic odor of pentadienal and failed to quench the short wave fluorescence on a TLC plate. The aqueous distillate was saturated with NaCl, extracted with CH_2Cl_2 (2 × 100 ml CH_2Cl_2/200 ml aqueous distillate), dried over Na_2SO_4, filtered and the solvent was removed at reduced pressure (10°/150 mmHg). The yield was determined to be 11.2 g (38%) by PMR comparison with a 50 l benzene sample and the product was used crude in the following reaction, PMR (100 MHz, $CDCl_3$) δ 5.33 (s, CH_2Cl_2), 5.48–6.17 (cp, 3H), 6.45–6.60 (dd, 1H, J = 10.2, 8.0 Hz), 6.91–7.15 (dd, 1H, J = 10.2, 4.0 Hz), 7.31 (s, added benzene) and 9.48 ppm (d, 1H, J = 8.0 Hz).

3,5-*Hexadiene-2-ol* (**6**). The title compound was prepared using a modification of the procedure of Woods and Schwartzman.[12] To a cooled soln of freshly prepared MeMgI (from 38.5 g (0.27 mol) MeI and 6.51 g (0.27 mol) dried, crushed Mg turnings in 250 ml dry ether) was added dropwise the crude 11.2 g (0.14 mol) 2,4-pentadienal diluted in 50 ml with dry ether. The reaction was warmed to room temp and stirred for 1 hr, quenched with sat NH_4Cl and filtered. The ether soln was extracted with brine, dried over $MgSO_4$, filtered and the solvent removed *in vacuo*, leaving a light yellow oil which was distilled to afford 11.8 g (86%) of a colorless oil, b.p. 73–75°/32 mmHg (lit.[12] b.p. 65–65.6°/20 mmHg), IR (neat film) 2.89–3.03 (br, s), 3.10 (w), 3.22 (s), 3.35 (m), 5.35 (w), 5.78 (w), 6.01 (m), 6.89 (s), 7.43 (m), 8.35 (s), 8.85 (s), 9.42 (s) and 10.8 μm (m); PMR (100 MHz, $CDCl_3$) δ 1.22 (d, 3H, J = 7.2 Hz), 3.94 (s, 1H, exchanges with D_2O), 4.23 (m, 1H), 4.94–5.76 (cp, 3H) and 5.81–6.20 ppm (cp, 2H).

(*E*)-4-(*Hexahydropyrrolo*[1,2-b]isoxazol-2-yl)-3-buten-2-*ol* (**7**)

A soln of **2** in 40 ml of dry benzene was prepared from 5 g (0.05 mol) of N-hydroxypyrrolidine and 30 g of yellow mercuric oxide. To this was added 4.75 g (0.053 mol) of 3,5-pentadien-2-ol, 150 ml of dry benzene and the resulting soln was heated to reflux for 3 hr under N_2. Upon cooling to room temp, the solvent was removed at reduced pressure. The crude product was chromatographed on silica gel (ethylether eluent), from which 5.88 g (70%) of the adduct was obtained, IR (neat film) 3.00 (s, broad), 3.4 (s), 3.49 (m), 6.1 (s), 6.88 (m), 7.22 (w), 8.67 (w), 9.29 (m), 10.3 μm (m); PMR (100 MHz, $CDCl_3$) δ 1.19 (d, 3H, J = 6 Hz), 1.50–2.09 (cp, 6H), 3.00 (t, 1H), 3.30 (m, 1H), 3.60 (s, broad 1H), 4.1–4.2 (cp, 2H), 5.3–5.7 ppm (m, 2H).

(*E*)-4-(*Hexahydropyrrolo*[1,2-b]*isoxazol*-2-*yl*)-3-*buten*-2-*one* (**4**)

A 600 ml round bottom flask, equipped with an efficient stirrer and N_2 flow, was charged with a soln of 4.0 g (0.022 mol) of **7** in 300 ml of alumina dried CH_2Cl_2. A mixture of 12.6 g (0.14 mol, 6.5 equiv) of active MnO_2 and 12.6 g of celite 545 (Fisher) was added in one portion and the reaction was stirred for 24 hr. The grey Mn suspension was filtered through a $MgSO_4$ pad. The resultant powder was reslurried in CH_2Cl_2 and filtered. This was repeated until the washings were colorless and the solvent removed *in vacuo* to leave a brown oil. This oil was chromatographed on silica gel (EtOAc eluent) to give 3.78 g (96%) of a yellow–orange oil which crystallized in the freezer, but melted at room temp, IR (neat film) 3.39 (s), 3.50 (m), 5.95 (s), 6.15 (m), 6.90 (m), 7.00 (m), 7.35 (s), 8.0 (s), 8.6 (m), 10.3 (m) and 13.8 μm (m); PMR (90 MHz, $CDCl_3$) δ 1.50–2.17 (cp, 6H), 2.27 (s, 3H), 3.18–3.30 (cp, 2H), 3.61–3.92 (cp, 1H), 4.54–4.81 (q, J = 13.8, 7.2 Hz, 1H), 6.24 (d, J = 15 Hz, 1H) and 6.61–6.85 ppm (dd, J = 15, 7.2 Hz, 1H); mass spectra (70 eV), *m/e* (M^+) 181, high-resolution mass spectrum *m/e* 181.1110, calc for $C_{10}H_{15}NO_2$ 181.1103.

Hexahydro-3-*hydroxy*-2,7-*methano*-2H-*pyrrolo*[1,2-b]-[1,2]-*oxazin*-9-*yl methyl ketone* (**9a**)

To a cooled (0°), well stirred, soln of 1.00 g (5.52 mmol) of **4** in 40 ml of alumina dried CH_2Cl_2 was added 1.14 g (6.62 mmol, 1.2 equiv) of purified[24] *m*-chloroperbenzoic acid. This soln was stirred for exactly 20 min and then 4 ml of a suspension of K_2CO_3 in sat K_2CO_3 aq was added. The CH_2Cl_2 was removed at reduced pressure and the solids were stirred for 20 min in fresh CH_2Cl_2. The suspension was filtered through a sintered glass funnel, washed with fresh CH_2Cl_2 (5 × 100 ml) and dried over Na_2SO_4 for 3 hr. After the soln was filtered, the volume was adjusted by the addition of fresh CH_2Cl_2 to a total of 600 ml and refluxed in the dark for 18.5 hr. The solvent was removed *in vacuo* leaving a light yellow oil which crystallized on standing. The resulting solid was chromatographed on silica gel (EtOAc eluent) giving 893 mg of light yellow crystals (m.p. 115–121°) which were recrystallized from hot benzene to give 778 mg (71%) of white crystals; m.p. 133–134°, IR (KBr pellet) 3.15 (s, br), 3.38 (s), 5.70 (s), 6.65 (s), 6.80 (s), 7.18 (s), 7.6 (m), 8.2 (s), 9.1 (s), 10.3 (s), 11.5 (m), 12.5 (m) and 13.0 μm (m); PMR (90 MHz, $CDCl_3$) δ 11.39–2.20 (cp, 6H), 2.20 (s, 3H), 2.50 (d, J = 5.5 Hz, 1H), 3.00 (d, J = 6 Hz, 1H), 3.38–3.63 (cp, 1H), 3.71–4.23 (cp, 2H) and 4.38 ppm (d, J = 5.5 Hz, 1H); mass spectrum (70 eV), *m/e* (M^+) 197. (Found: C, 60.90; H, 7.69; N, 7.07. Calc for $C_{10}H_{15}NO_3$: C, 60.91; H, 7.61; N, 6.81%.)

Hexahydro - 3 - acetoxy - 2,7 - methano - 2H - pyrrolo[1,2-b] - [1,2] - oxazin - 9 - yl methyl ketone (**9b**)

To a soln of 150 mg (0.76 mmol) of **9a** in dry pyridine (3 ml) was added Ac_2O (1 ml) which was stirred overnight. After the pyridine was removed in a N_2 stream, the product was dissolved in dry toluene. The toluene was removed under reduced pressure. The solid was dissolved in $CHCl_3$ (100 ml), extracted with $NaHCO_3$ aq and dried ($MgSO_4$). After filtration, the solvent was removed *in vacuo*, leaving 173.3 mg (95%) of white crystals which were recrystallized from CH_2Cl_2–pentane, m.p. 77–77.5°; IR ($CHCl_3$) 3.33 (s), 3.37 (s), 3.48 (w), 5.76 (s), 5.83 (s), 6.79 (w), 7.02 (w), 7.29 (m), 7.63 (w), 7.81 (m), 8.06 (s), 8.23 (s), 8.47 (m), 7.94 (m), 9.66 (s), 10.8 (w), 13.3 (s) and 15.5 μm (m); PMR (90 MHz, $CDCl_3$) δ 1.30–2.30 (cp, 12H, contains two 3H singlets at 2.03 and 2.17), 2.98 (d, J = 6.0 Hz, 1H), 3.41–3.69 (cp, 1H), 3.71–3.92 (cp, 1H), 4.49 (d, J = 5.2 Hz, 1H) and 4.98–5.20 ppm (cp, 1H).

Hexahydro - 3 - hydroxy - 2,7 - methano - 2H - pyrrolo[1,2-b] - [1,2] - oxazin - 9 - yl methyl ketone methanesulfonate (ester) (**17b**)

Following the procedure of Crossland and Servis,[26] the title compound was prepared. To a cooled soln of 265 mg (1.35 mmol) of **9a** in 50 ml of alumina dried CH_2Cl_2 was added 245 mg (0.337 ml) of Et_3N and 186 mg (0.126 ml) of methanesulfonyl chloride. The soln was stirred for 1 hr, then was washed with water (2 × 50 ml), $NaHCO_3$ aq (1 × 50 ml), and dried over $MgSO_4$. After filtration, the solvent was removed at reduced pressure leaving a light yellow oil which was chromatographed on silica gel (EtOAc eluent) giving 263 mg (71%) of white crystals, m.p. 114–116°; IR ($CHCl_3$) 3.31 (m), 3.36 (m), 3.46 (w), 5.80 (s), 6.01 (w), 6.76 (w), 6.87 (w), 7.03 (w), 7.30 (s, br), 7.75 (w), 8.13 (m), 8.47 (s), 10.0 (m), 10.3 (s), 11.5 (m), 11.7 (m) and 12.2 μm (m); PMR (100 MHz, $CDCl_3$) δ 1.27–2.43 (cp, 9H, contains a 3H singlet at 2.20 ppm), 3.00 (cp, 4H), 3.42–3.74 (cp, 1H), 3.78–3.95 (cp, 1H), 4.62 (d, J = 5 Hz) and 4.79–5.02 ppm (7 lines, 1H).

O - *Hexahydro - 3 - hydroxy - 2,7 - methano - 2H - pyrrolo[1,2-b] - [1,2] - oxazin - 9 - yl methyl ketone thiobenzoate* (**9c**)

The title compound was prepared following the procedure of Barton and McCombie.[19] To a suspension of 150 mg (0.76 mmol) of **9a** in 5.0 ml dry THF and 0.5 ml dry CH_2Cl_2 was added a CH_2Cl_2 soln of phenylimidoylchloride methochloride (freshly prepared from 1.1 g phosgene and 147 mg (0.99 mmol) of N,N-dimethylbenzamide in 2 ml dry CH_2Cl_2) which was stirred for 1 hr at room temp. To this soln was added 0.25 ml dry pyridine and H_2S (bubble for 20 min, stir for 20 min, bubble for 15 min and stir for 30 min). Water (10 ml) was added and the layers were separated. The aqueous layer was extracted with $CHCl_3$ (2 × 30 ml). The combined organic layers were extracted with water (30 ml), $NaHCO_3$ aq (30 ml), dried over $MgSO_4$ and filtered. The solvents were removed at reduced pressure. The remaining yellow solid was chromatographed on silica gel (prep TLC, EtOAc eluent) and recrystallized from CH_2Cl_2–pentane to afford 127 mg (53%) of yellow needles, m.p. 143–144°; IR ($CHCl_3$) 3.37 (m), 5.82 (s), 6.25 (m), 6.87 (s), 7.33 (m), 7.58 (s), 7.84 (s), 7.94 (s), 8.10 (s), 8.30 (s), 8.47 (s), 9.1 (m), 9.3 (s), 9.5 (s), 9.8 (s), and 10.6 μm (w); PMR (90 MHz, $CDCl_3$) δ 1.32–2.58 (cp, 9H, contains a 3H singlet at 2.17) 3.11 (d, 1H, J = 4.5 Hz), 3.53–3.80 (cp, 1H), 3.82–4.09 (cp, 1H), 4.79 (d, 1H, J = 4.0 Hz), 5.82–6.13 (cp, 1H), 7.23–7.58 (cp, 4H) and 8.02–8.18 ppm (cp, 2H).

Hexahydro - 3 hydroxy - 2,7 - methano - 2H - pyrrolo[1,2-b] - [1,2] - oxazin - 9 - yl methyl ketone ethylene ketal (**17a**)

To a stirred suspension of 300 mg (1.5 mmol) of **9a** in benzene (50 ml) was added 140 mg (1.6 mmol) ethylene glycol, 300 mg (1.8 mmol) *p*-toluenesulfonic acid monohydrate. The resultant soln was refluxed for 12 hr through a Dean–Stark apparatus. After cooling, sat K_2CO_3 aq (10 ml) was added and the two-phase mixture was stirred for 1 hr. The layers were separated and the aqueous layer was extracted with $CHCl_3$ (4 × 60 ml). The combined organic layers were dried over $MgSO_4$, filtered and the solvent removed *in vacuo*, leaving 409 mg of a light red solid. This material was chromatographed on silica gel (2% MeOH in EtOAc eluent) affording 354 mg (97%) of white crystals, m.p. 126–127.5°; IR (CH_2Cl_2) 2.79 (m), 2.82–3.24 (br, m), 3.38 (s), 3.45 (s), 6.85 (m), 7.20 (m), 8.23 (m), 8.71 (m), 9.5 (s), 10.6 (s), 11.6 (w) and 12 μm (w); PMR (90 MHz, $CDCl_3$) δ 0.82–1.32 (cp, 1H), 1.32 (s, 3H), 1.43–2.28 (cp, 5H), 2.39 (d, 1H, J = 6 Hz), 3.30–3.67 (cp, 2H), 2.98 (s, 4H), 3.99–4.15 (cp, 2H), 4.73–4.91 (s, 1H); mass spectrum (70 eV), *m/e* (M^+) 241.

O - *Hexahydro - 9 - (2 - methyl - 1,3 - dioxolan - 2 - yl) - 2,7 - methano - 2H - pyrrolo[1,2-b] - [1,2] - oxazin - 3 - ol methanesulfonate (ester)* (**17b**)

To a stirred, cooled (0°) soln of 354 mg (1.47 mmol) of **17a** in CH_2Cl_2 (40 ml) was added 327 mg (3.24 mmol, 0.45 ml) of Et_3N and 247 mg (2.16 mmol, 0.167 ml) of methanesulfonyl chloride. The soln was stirred for 1 hr and washed with water (2 × 10 ml), sat $NaHCO_3$ aq (2 × 10 ml) and then dried over $MgSO_4$. After filtration, the solvent was removed at reduced pressure and the resultant brown oil was chromatographed on silica gel ($CHCl_3$ eluent) to give 385 mg of a clear glass which slowly crystallized on standing. Recrystallization from CH_2Cl_2–isooctane gave 442 mg (94% yield) of white crystals, m.p. 116–117°; IR ($CHCl_3$) 3.30 (m), 3.38 (s), 3.49 (m), 6.8 (w), 6.91 (w), 7.31 (s), 8.5 (s), 10.3 (s), 10.7 (s), 11.7 (s) and 12.3 μm (w); PMR (90 MHz, $CDCl_3$) 1.33 (s, 3H), 1.38–2.31 (cp, 6H), 2.38 (d, J = 6 Hz, 1H), 3.09 (s, 3H), 3.41–3.70 (cp, 2H), 3.98 (s, 4H), 4.35 (d, J = 6 Hz, 1H) and 4.80–5.06 ppm (7 lines, 1H); mass spectrum (70 eV), *m/e* (M^+) 319. (Found: C, 48.52; H, 6.59; N, 4.33. Calc for $C_{13}H_{21}NO_6S$: C, 48.89; H, 6.63; N, 4.39%.)

2 - (2 - *Methyl - 1,3 - dioxalan - 2 - yl*) - 2 - *azabicyclo*[4.2.1] - *nonan - 3 - ol* (**10**)

The title compound was prepared following the general procedure of Ashby and Lin.[20] To an oven dried 25 ml round bottom flask containing 244 mg (1.88 mmol) anhyd nickel(II) chloride under an Ar atmosphere, was added 252 mg (1.88 mmol) of **17b**. The flask was sealed under a slight positive pressure of Ar. Anhyd THF (8.0 ml) was added. The flask cooled to −78° where 74 mg (1.88 mmol) of lithium aluminum hydride were added in one portion. The flask was allowed to warm slowly to −40° (until the reaction subsided). The soln was then allowed to warm to room temp where it was stirred for 16 hr. Water (5 ml) was added. This soln was stirred for 5 min, saturated with K_2CO_3, extracted with pure ether (5 × 20 ml), and dried over $MgSO_4$. After filtration, the solvent was removed *in vacuo* to give 95.6 mg of a colorless oil which consisted of three very polar spots on TLC which resisted chromatographic separation. The product was carried forward unpurified; IR (neat film) 2.71–3.39 (w, br), 3.04 (m), 3.42 (s), 3.49 (s), 6.85 (m), 7.01 (m), 7.27 (m), 7.45 (w), 7.55 (w), 7.06 (w), 7.92 (w), 8.35 (m), 8.78 (s), 9.9 (s), 9.5 (s), 10.2 (m), 10.6 (m), 11.2 (w), 11.7 (w), 12.1 (w), 12.6 (w) and 14.7 μm (w); PMR (90 MHz, $CDCl_3$) δ 1.11–2.28 (cp, 9H), 1.47 (s, 3H), 3.41–3.78 (cp, 4H), 3.90 (s, 4H), and 4.05–4.21 ppm (cp, 1H); mass spectrum (70 eV), *m/e* (M^+) 227.

2,9 - *Diacetyl - 9 - azabicyclo*[4.2.1] - *non - 2 - ene*(N - *acetyl anatoxin-a*) (**21**)

A soln of 20 mg of **10** in acetyl chloride (3 ml) was stirred at room temp for 0.5 hr, then cooled to 0°. Water (1 ml) was slowly added, saturated with $NaHCO_3$ and extracted with $CHCl_3$ (2 × 20 ml). After drying ($MgSO_4$) and solvent removal, the remaining oil was chromatographed on silica gel (preparative TLC, 50% $CHCl_3$–EtOAc, multiple development 6 ×), leaving a colorless oil which afforded 14.3 mg (77%) of white crystals (recrystallized from CH_2Cl_2–pet. ether as colorless plates), m.p. 77.5–78.5°; IR ($CHCl_3$) 3.34 (m), 6.00 (m), 6.18 (s), 7.17 (w), 7.32 (w), 7.93 (m) and 8.1–8.3 (m); NMR (90 MHz, $CDCl_3$) δ 1.49–2.60 (cp, 18H contains two 3H singlets at 1.94 and 2.31), 4.59–4.97 (cp, 1H), 5.21 (d, J = 8 Hz, 1H) and 6.92 ppm (t, J = 6 Hz, 1H); mass spectrum (70 eV), *m/e* (M^+) 207; UV (EtOH) λ_{max} = 226 nm, ε = 10,700.

Spectra match those supplied by Dr O. E. Edwards

(Division of Biological Sciences, National Research Council of Canada, Ottawa, Ontario, Canada K1A 0R6).

t-Butyl-1-(9-azabicyclo[4.2.1]-non-2-ene-2-yl)ethanone carbamate (**23**)

To a stirred soln of 83 mg of **10** in dried acetone (10 ml) was added *p*-toluenesulfonic acid (80 mg). The soln was heated to reflux for 15 hr. After cooling and removal of the solvent, the remaining red oil was dissolved in $CHCl_3$ (5 ml), treated with $NaHCO_3$ aq (93.6 mg in 2 ml water) and stirred for 5 min. NaCl (250 mg) was added. After it dissolved, a soln of di-t-butoxydicarbonate (130 mg in 5 ml $CHCl_3$) was added. The two-phase mixture was heated to reflux for 1.5 hr and, after cooling, the aqueous layer was extracted with $CHCl_3$ (3 × 15 ml). After drying ($MgSO_4$) and removal of the solvent at reduced pressure, the remaining oil was chromatographed on silica gel (20% $CHCl_3$ in CH_2Cl_2 eluent) giving 42.2 mg (42.5% from **17b**) of a colorless oil; IR (neat film) 3.35 (m), 3.40 (m), 5.68 (w), 5.92 (s), 6.11 (w), 6.77 (w), 6.87 (w), 7.14 (s), 7.38 (m), 7.46 (m), 7.94 (m), 8.12 (m), 8.52 (s), 9.01 (s), 9.41 (w), 9.85 (w), 10.1 (w), 10.7 (w), 11.6 (w), 11.9 (w) and 13.0 μm (w); PMR (90 MHz, $CDCl_3$) 1.41 (s, 9H), 1.51–2.49 (cp, 11H, contains a 3H singlet at 2.30), 4.22–4.44 (broad hump, 1H), 5.12 (d, J = 8 Hz, 1H) and 6.78 ppm (t, J = 6 Hz, 1H); mass spectrum (70 eV): *m/e* (M^+) 265; UV (ethanol) λ_{max} 226 nm; ε = 10,250.

1-(9-Azabicyclo[4.2.1]-non-2-en-2-yl)ethanone hydrochloride: (**1** · HCl) (*anatoxin-a hydrochloride*)

A soln of 3 N HCl in EtOAc (2 ml conc HCl dissolved in 6 ml EtOAc) was added to 42.0 mg (0.16 mmol) of **23** and stirred at room temp for 2 hr. The solvent was removed at reduced pressure and the remaining light red oil was dissolved in $CHCl_3$. This soln was passed down a column of anhyd Na_2SO_4. The solvent was removed and the resulting oil was chromatographed on silica gel (10% MeOH–$CHCl_3$ eluent) giving 41 mg (> 100%) of colorless glass which still contained a little $CHCl_3$; IR ($CHCl_3$) 2.74 (w), 2.77–3.07 (w, br), 3.40 (s), 3.50–4.54 (s, br), 5.84 (w, shoulder), 5.98 (s), 6.08 (m), 6.29 (m), 6.78 (m), 6.97 (m), 7.09 (m), 7.84 (m), 8.06–8.23 (m, br), 8.58 (vw), 8.92 (vw), 9.3 (w), 10.3 (w), 10.9 (w) and 11.2 μm (w); PMR (90 MHz, $CDCl_3$) δ 1.69–2.71 (cp, 11H contains a 3H singlet at 2.3), 4.20–4.40 (cp, 1H), 5.18 (d, J = 9 Hz, 1H), 7.11 (t, J = 6 Hz, 1H) and 7.65 ppm (s, br 2H); mass spectrum (70 eV): *m/e* (M^+) 165 85°; UV (ethanol) λ_{max} 226 nm; ε = 8370.

These spectra match those supplied by Dr O. E. Edwards.[21]

Typical $NiCl_2$–$LiAlH_4$ reduction of an isoxazolidine

This reaction was usually run on 100 mg (*ca*) of isoxazolidine. To a stirred suspension of anhyd nickel(II) chloride (3 equiv) in 5 ml dry THF was added a soln of isoxazolidine (1 equiv) in 2.5 ml dry THF. The flask was cooled to −78° (dry ice–acetone) and lithium aluminum hydride (3 equiv) was added. The flask was allowed to warm to −35°, at which point, the orange–grey mixture turned to a dark brown suspension and gas evolution was observed. After the initial vigorous reaction, the flask was allowed to warm to room temp and the suspension was stirred for another 16 hr. The reaction was quenched with water (2 ml), saturated with K_2CO_3 and extracted with ether (2 × 25 ml). The combined ether solns were dried over $MgSO_4$ and filtered. The solvent was removed *in vacuo* to afford the crude product which was chromatographed and recrystallized to afford the purified product.

2-Octyl methanesulfonate. To a cooled (0°) soln of 1.0 g (7.7 mmol) 2-octanol and 1.5 g (14.8 mmol, 2.1 ml) Et_3N in 100 ml CH_2Cl_2 was added, dropwise, a soln of 0.97 g (0.74 ml, 8.46 mmol) methanesulfonyl chloride in 30 ml CH_2Cl_2. The soln was stirred for 30 min and then washed with ice water (50 ml), 10% HCl (50 ml), sat $NaHCO_3$ aq (50 ml) and sat NaCl aq (50 ml). The CH_2Cl_2 soln was dried ($MgSO_4$) and filtered. The solvent was removed *in vacuo*. The remaining oil was chromatographed on silica gel (CH_2Cl_2 eluent) to afford 1.2 g (73%) of a colorless oil; IR (neat) 3.3 (w), 3.39 (s), 3.49 (m), 6.81 (m), 7.05 (w), 7.35 (s), 7.46 (s), 8.41 (s), 8.92 (w), 10.2 (m), 10.5 (s), 12.1 (w) and 12.4 μm (w); PMR (60 MHz, $CDCl_3$) δ 0.92 (cp, 3H), 1.10–1.85 (cp, 13H), 3.00 (s, 3H) and 4.62–4.95 (m, 1H).

Cyclohexyl mesylate. The title compound was prepared following the same procedure as O-octyl mesylate and was obtained in 61% yield; IR (neat) 3.28 (w), 3.40 (s), 3.49 (s), 6.90 (m), 7.05 (w), 7.41 (br, s), 7.93 (w), 8.43 (s), 9.08 (w), 13.7 (s), and 12.9 (s); PMR (60 MHz, $CDCl_3$) 1.05–2.10 (cp, 11H), 3.00 (s, 3H) and 4.65–4.83 ppm (cp, 1H).

Cyclododecyl mesylate. The title compound was prepared following the same procedure as for 2-octyl mesylate and was obtained in 65% yield; IR ($CHCl_3$) 3.28 (w), 3.39 (s), 3.59 (m), 6.78 (m), 6.90 (m), 7.05 (w), 7.38 (s), 7.49 (s), 8.51 (s), 10.3 (m), 10.9 (s), 11.3 (m) and 12.1 μm (w); PMR (60 MHz, $CDCl_3$) δ 1.35–1.96 (cp, 22H), 3.00 (s, 3H) and 4.63–5.05 ppm (cp, 1H).

Attempted reduction of secondary mesylates with $NiCl_2$–$LiAlH_4$

To a stirred suspension of anhyd nickel (II) chloride (3 equiv) in 5 ml dry THF was added a soln of secondary mesylate (1 equiv) in 2.5 ml dry THF. The flask was cooled to −78° (dry ice–acetone) and lithium aluminum hydride (3 equiv) was added. The flask was allowed to warm to −35° (*ca*) at which point, the orange–grey mixture turned to a dark brown suspension and gas evolution was observed. After the initial vigorous reaction, the flask was allowed to warm to room temp and the suspension was stirred for another 16 hr. The reaction was quenched with water (2 ml), saturated with K_2CO_3 and extracted with ether (2 × 25 ml). The ether soln was dried ($MgSO_4$) and filtered. The solvent was removed *in vacuo*, leaving quantitative recovery of a material which was identical (PMR, IR and TLC) with the starting secondary mesylate.

2-Phenylethanol. To a stirred suspension of 324 mg (2.5 mmol) anhyd nickel(II) chloride in dry THF (7.5 ml) was added 100 mg (0.833 mmol) of styrene oxide. The flask was cooled to −78° (dry ice–acetone) and lithium aluminum hydride (95 mg, 2.5 mmol) was added. The flask was allowed to warm to −35° (*ca*) at which point, the orange–grey mixture turned to a dark brown suspension and gas evolution was observed. After the initial vigorous reaction, the flask was allowed to warm to room temp and the suspension was stirred for an additional 15 hr. The reaction was quenched with water (2 ml), saturated with K_2CO_3 and extracted with ether (2 × 2 ml). The combined ether extracts were dried ($MgSO_4$), filtered and the solvents removed *in vacuo* to afford a light yellow oil which was chromatographed on silica gel (CH_2Cl_2 eluent) to afford 96.7 mg (95%) of a colorless oil; IR (neat film) 2.74–3.19 (br, s), 3.21 (w), 3.25 (w), 3.26 (m), 3.30 (m), 3.40 (m), 3.47 (m), 6.21 (w), 6.68 (m), 6.77 (m), 9.03 (w), 9.27 (m), 9.60 (br, s), 11.0 (w), 11.7 (w), 13.4 (m) and 14.4 μm (s); PMR (90 MHz, $CDCl_3$) δ 1.78–2.15 (br, s, 1H), 2.71–2.94 (t, 2H, J = 7.4 Hz) 3.69–3.87 (t, 2H, J = 7.4 Hz) and 7.22 ppm (s, 5H). IR spectra matched Aldrich Infrared No. 515H.[27] PMR spectrum matches Aldrich NMR No. 5. 20 c.[28]

Acknowledgements—We thank Nicholas Saccamano, Joseph Koslowski, David Scherer, Bridget McCourtney, and John Brinkman for technical assistance. We are grateful to the National Institutes of Health (GM 25303) for financial support.

REFERENCES

[1] P. R. Gorham and W. W. Carmichael, *Pure Appl. Chem.* **52**, 165 (1975).
[2] E. T. Rose, *Proc. Iowa Acad. Sci.* **60**, 738 (1953).
[3] P. R. Gorham, J. McLachlan, U. T. Manner and W. K. Kim, *Verh. Int. Verein. Limnol.* **15**, 796 (1964).
[4] J. P. Devlin, O. E. Edwards, P. R. Gorham, N. R. Hunter, P. K. Pike and B. Stavric, *Can. J. Chem.* **55**, 1357 (1977).
[5] L. S. Huber, *Acta Crystallogr.* **B28**, 2577 (1972).
[6] H. F. Campbell, O. E. Edwards and R. J. Kolt, *Can. J. Chem.* **55**, 1372 (1977).
[7] H. F. Campbell, O. E. Edwards, J. W. Elder and R. J. Kolt, *Pol. J. Chem.* **53**, 27 (1979).
[8] H. Rapoport and H. A. Bates, *J. Am. Chem. Soc.* **101**, 1259 (1979).

[9] J. S. Petersen, G. Fels and H. Rapoport, *Ibid.* **16**, 4539 (1984).
[10] G. B. Mullen, Ph.D. Dissertation, State University of New York at Buffalo (1977).
[11] K. N. Houk, J. Sims, R. E. Duke, R. W. Strozier and J. K. George, *J. Am. Chem. Soc.* **95**, 7287 (1973).
[12] F. G. Wood and L. H. Schwartzman, *J. Am. Chem. Soc.* **70**, 3394 (1948).
[13a] J. J. Tufariello and G. B. Mullen, *J. Am. Chem. Soc.* **100**, 3638 (1978); [b] J. J. Tufariello, G. B. Mullen, J. J. Tegeler, E. J. Trybulski, S. C. Wong and Sk. A. Ali, *Ibid.* **101**, 2435 (1979).
[14] N. A. LeBel, M. E. Post and D. Hwang, *J. Org. Chem.* **44**, 1819 (1979).
[15] W. Oppolzer, S. Siles, R. L. Snowden, B. H. Bakker and M. Petrzilka, *Tetrahedron Lett.* 4391 (1979).
[16a] R. Huisgen, *Angew. Chem. Int. Ed. Engl.* **2**, 565 (1963); [b] D. St. C. Black, R. Crozier and V. Davis, *Synthesis* 205 (1975).
[17] J. J. Tufariello and J. P. Tette, *J. Org. Chem.* **40**, 3866 (1975).
[18] J. J. Tufariello, *Accts. Chem. Res.* **12**, 396 (1979).
[19] D. H. R. Barton and S. W. McCombie, *J. Chem. Soc. Perkin Trans. I* 1574 (1975).
[20] E. C. Ashby and J. J. Lin, *J. Org. Chem.* **43**, 1263 (1978); *Tetrahedron Lett.* 4480 (1977).
[21] We are indebted to Dr O. E. Edwards (National Research Council of Canada, Ottawa, Canada) for providing us with the PMR, IR and mass spectra of natural anatoxin-a and N-acetyl anatoxin-a.
[22] E. J. Corey, M. G. Bock, A. P. Kozikowski, A. V. Rama Rao, D. Floyd and B. Lipshutz, *Tetrahedron Lett.* 1051 (1978).
[23] D. S. Tarbell, Y. Yamato and B. M. Pope, *Proc. Natn. Acad. Sci. U.S.A.* **69**, 730 (1972).
[24] N. N. Schwartz and J. H. Blumbergs, *J. Org. Chem.* **29**, 1976 (1964).
[25] G. F. Woods and H. Sanders, *J. Am. Chem. Soc.* **68**, 2483 (1946).
[26] R. K. Crossland and K. L. Servis, *J. Org. Chem.* **35**, 3195 (1970).
[27] C. J. Pouchart (Editor), *The Aldrich Library of Infrared Spectra.* Aldrich Chemical Co., Inc., Milwaukee, Wisconsin (1975).
[28] C. J. Pouchart and J. R. Campbell (Editors), *The Aldrich Library of NMR Spectra.* Aldrich Chemical Co., Inc., Milwaukee, Wisconsin (1974).