11-NOR-4,9-ANHYDROTETRODOTOXIN-6,6-DIOL: A SYNTHON FOR TETRODOTOXIN ANALOGS

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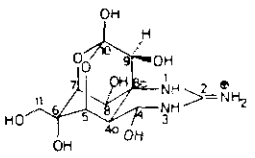
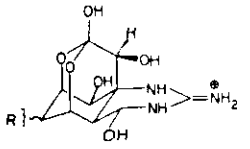
Abstract - Tetrodotoxin (1), the sodium channel-blocking agent, is converted to non-toxic 4,9-anhydrotetrodotoxin (4), which is oxidized to 11-nor-4,9-anhydrotetrodotoxin-6,6-diol (5). This inactive derivative can be hydrolyzed to pharmacologically active 11-nortetrodotoxin-6,6-diol (3). Preliminary studies have been made with the title compound to determine its suitability for the preparation of tetrodotoxin analogs.

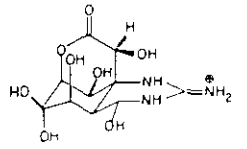
Chemical modifications of tetrodotoxin (1) that retain the sodium channel-blocking activity of tetrodotoxin itself could be of considerable value in studies on the nature of axon sodium channels and the nerve conducting process.[2,3,4] Early studies[5,6] showed that the few modifications of the tetrodotoxin structure which had been prepared resulted in products which were either inactive or almost inactive. The possibility of obtaining active derivatives was established by the isolation of chiriquitoxin[7,8] (2), which, while retaining activity, differs structurally from tetrodotoxin (1) by a modification (of a still unknown nature) of the C-6 substituent. Consequently, interest was renewed in the pharmacology of nortetrodotoxin,[9,10,11,12] the periodate oxidation product of tetrodotoxin. The activity of nortetrodotoxin was originally either not reported[10,11] or it was described as non-toxic.[9] It was later shown that nortetrodotoxin possessed 20-40% of the sodium channel-blocking activity[12,13,14] of tetrodotoxin. Lazdunsky and coworkers[14,15] pioneered



1. Tetrodotoxin



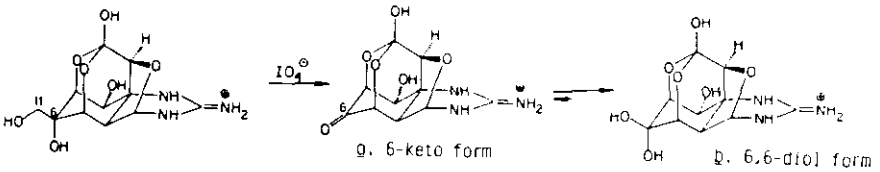
2. Chiriquitoxin
R = 121 mass units



3. Nortetrodotoxin
6,6-diol-7-lactone

chemical modifications of tetrodotoxin at C-6 and C-11 which led to active and useful derivatives. However, the yields of derivatives from nortetrodotoxin have been very low; thus it has major disadvantages as a synthon for elaboration of tetrodotoxin analogs.[12]

In an attempt to circumvent some of these difficulties, we investigated an alternate synthon, 11-nor-4,9-anhydrotetrodotoxin-6,6-diol. Tsuda et al.,[10] using the periodate oxidation of 4,9-anhydrotetrodotoxin[9,10,16] (4) detected the formation of formaldehyde and established the presence of the terminal glycol structure in 4; however, the major product was neither isolated nor characterized. We have found that periodic acid oxidation of 4 under the same conditions[8,14] as those used in converting 1 to 3 produced 5 as a white hygroscopic powder in 85% yield. Since the hydroxyls at



4. 4,9-Anhydrotetrodotoxin

5. 11-Nor-4,9-anhydrotetrodotoxin

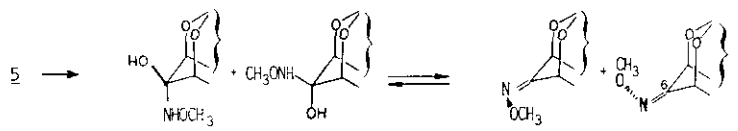
g. 6-keto form

h. 6,6-diol form

C-4 and C-9 are protected as an anhydro bridge, 4 and 5 are more stable to base and more soluble in organic solvents than tetrodotoxin or nortetrodotoxin. In contrast to nortetrodotoxin (3), the nor-anhydro compound 5 exists entirely in the hemilactal form giving clear and reproducible ^{1}H and ^{13}C NMR spectra. The IR and NMR spectra show that 5 exists in the hydrate form 5b; no evidence for a free carbonyl group could be detected. The 4,9-anhydro bridge renders 5 pharmacologically inactive as compared to nortetrodotoxin in 3, just as 4 is relatively inactive with respect to 1. The anhydro compounds 4 and 5 are hydrolyzed in dilute acid to the active parent compounds 1 and 3; thus keto derivatives of 5 should be convertible to the corresponding analogs of 3.

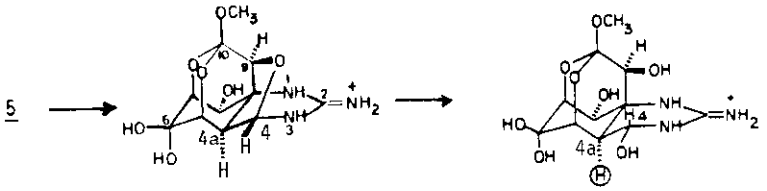
Reaction of 5 with excess methoxyamine[11,18] (CH_3ONH_2) at pH 5 gave two products; unfortunately, limited quantities precluded their individual characterization. The ^{1}H NMR spectrum (Table I) of this mixture is compatible with the presence of both syn and anti forms of an O-methyloxime 6c and 6d; however, the spectrum may also indicate two stereoisomeric 6-OH, 6-$NHOCH_3$ adducts, 6a and 6b. A very large OH signal at 3400 cm^{-1} and a broad adsorption in the 1600-1700 cm^{-1} region of the FT-IR spectrum of this mixture were not diminished by prolonged exposure to high vacuum. The former signal may mask any N-H adsorption and the latter signal may be either an OH harmonic or C=N adsorption of an oxime. Therefore, there is ambiguity concerning the interpretation of these results and those reported[11] for the reaction of 3 with CH_3ONH_2. The deduction of isomeric oximes in hydrated forms 6a-6b is consistent with the stable 6,6-diol structures for both.

Treatment of 5 with anhydrous, methanolic hydrogen chloride in an attempt to prepare the 6,6-dimethoxy ketal of 5, gave a product whose ^{1}H NMR spectrum revealed that only one methoxy group (δ 3.50 ppm) had been introduced and that the 4,9-anhydro bridge remained intact (coupling of C-4a:C-4 protons by less than 1 Hz by virtue of their 90° dihedral angle). Treatment of this product with



6a 6b 6c 6d

DCl in D_2O at 20°C did not remove the methoxy group but opened the anhydro bridge (C-4a proton signal at 2.40 ppm, doublet, J_{4a-4} = 9 Hz). The chemical shift of the OCH_3 signal (δ3.48 ppm) for this hydrolyzed product was essentially the same as that reported (3.45 ppm) by Woodward[16] for the only other known 10-methoxy derivative of tetrodotoxin. We tentatively interpret these reactions as shown in 5 → 7 → 8.



7 8

These studies have been greatly restricted because of the severely limited supply of 5.

EXPERIMENTAL

11-Nor-4,9-anhydrotetrodotoxin 6,6-diol, 5b. *Anhydrotetrodotoxin*[10] (4, 10.4 mg, 34.6 μmole, ^1H and ^{13}C NMR Table I) was dissolved in 3 ml of water containing 40 μl of acetic acid and the solution treated with 8.13 mg of H_4IO_6 (35.3 μmole) at 20°C. After 4 h the mixture was cooled to 0°C and a total of 24 μl of freshly distilled HI was added in portions. Each incremental addition of HI was immediately followed by CCl_4 extraction to remove I_2. This procedure was repeated until addition of HI no longer generated the yellow I_2 color. The clear aqueous layer was frozen and lyophilized, redissolved and relyophilized from 99.7% D_2O to give 9.0 mg (85% yield) of 5b as a white hygroscopic solid. The ^1H and ^{13}C NMR data are given in Table I. This sample was non-toxic.[17]

Hydrolysis of 5. The NMR spectrum of 5b (1 mg in 5% DCl in 99.7% D_2O) after 6 h showed an equilibrium mixture of 65% nortetrodotoxin 3 and 35% noranhydrotetrodotoxin 5. A bioassay of this solution showed a total of 2600 mouse units[17] corresponding to approximately 0.70 mg of 3. Relyophilization of this whole sample and redetermination of the NMR indicated a shift in equilibrium to 44% 3 and 56% 5. This equilibration of nortetrodotoxin (3) and noranhydrotetrodotoxin (5) parallels that between tetrodotoxin (1) and anhydrotetrodotoxin (4) (72:25) under similar

TABLE I

NMR SPECTRAL ASSIGNMENTS FOR 4,9-ANHYDROTETRODOTOXIN (4), 11-NOR-4,9-ANHYDROTETRODOTOXIN (5), METHOXYAMINE PRODUCT 6, AND 8.

Signal Assignment[b]	CHEMICAL SHIFTS,[a-b] δ, ppm					
	Proton				Carbon-13	
	4	5[d]	6	8	4	5
4	5.52 s	5.49 s	5.87 s	5.68 s	41.4	41.6
8	4.62 d[e]	4.48 d[e]	4.68[f]	4.70 d[e]	70.7[f]	67.3
9	4.58 s	4.54 s	4.63[f]	4.58 d	82.5	82.5[f]
5	4.33 d[g]	4.21 d[g]	4.42[g]	4.40 t[h]	66.3	74.0[f]
7	4.17 d[e]	4.05 d[e]	4.10[e]	4.26 t[i]	70.1[f]	80.1[f]
11	3.98 q[j]	---	---	---	63.9	---
4_a	2.89 d[g]	2.85 d[g]	3.08[g]	3.03 d[g]	85.2	85.4
2					155.6	155.7
10					109.8	108.2
6					76.7	89.0
8a					61.2	60.8
OCH_3			4.22[k]	3.50 s		

a) Spectra taken on Varian XL-100, 100 MHz NMR instrument in FT mode; D_2O, δ ppm downfield relative to CHD_2COOD set at 2.03 ppm relative to TMS = 0. All proton signals had relative area 1 except CH_2 signal in 4 and OCH_3 signals in 6 and 8. b) Signals are for the ^1H and ^{13}C atoms as numbered in formulas 4, 5, and 6. c) Signals are either singlets, s, or doublets, d, with coupling constants as given in footnotes. d) Taken on Bruker 360 MHz instrument in FT mode; complete decoupling study made for proton assignments. e) J_{7-8} or J_{8-7} = 2 Hz. f) Assignment by analogy to those of compounds 1 and 4. g) J_{4a-5} or J_{5-4a} = 3 Hz. h) Triplet, J_{4a-5} = 3 Hz, J_{5-7} = 2 Hz. i) Triplet J_{5-7} = 2 Hz, J_{7-8} = 3 Hz. j) AB quartet, J = 4.5 Hz as revealed at 300 MHz. k) Two peaks at δ 4.20 and 4.24 ppm in approximate ratio of 1.0 to 0.8; combined area of 3.

conditions.[1,9,10]

Reaction of 5 with Methoxyamine. Since it seems possible that methoxyamine could react with the carbinol-amine, lactone or hemilactal structures of 1, 2, 3, or 5, it was first determined that tetrodotoxin itself lost about 5% of its activity when treated with methoxyamine at pH 5 after 5 h but 75% after 25 h. The NMR spectrum of anhydrotetrodotoxin (4) did not change significantly in the presence of methoxyamine at pH 5 over a 24-h period. Noranhydrotetrodotoxin, prepared as described from 1.0 mg (3.3 μmole) of 4, was dissolved in 1.0 ml of water containing 5 μl of trifluoroacetic acid and 1 μl (20 μmole) of distilled methoxyamine[18] at pH 5. After 24 h at 20°C the solution was lyophilized and the resulting white powder relyophilized from 99.7% D_2O to give the product 6. The NMR spectral data are given in Table I.

Attempted Ketalization of 5. A sample of 5 prepared as above from 2.77 mg of 4 was dissolved in a mixture of 0.6 N anhyd. methanolic HCl and tetramethylorthocarbonate (1.5 ml, 1:1) and stirred under N_2 at 60°C for 15 h. The reaction mixture was stored in a desiccator beside a separate container of anhyd. KOH-CH_3OH. The solution was concentrated and lyophilized with D_2O (3X) to give a white hygroscopic powder which had a ^1H NMR spectrum closely resembling that of starting 5 (Table I) except for the appearance of a single new methoxy signal (δ 3.50, 3 H). There was no coupling between the proton signals at C-4 and C-4a showing that the anhydro bridge was not hydrolyzed. The spectrum is compatible with the orthoester structure 7. This sample was dissolved in 5% DCl in D_2O (350 μl); after 9 h the NMR showed a somewhat broadened doublet at 2.40 ppm, J = 9.0 Hz, characteristic of the signal for the C-4 proton in 1 (δ 2.30 ppm, J_{4a-4} = 9.0 Hz) and 3 (δ 2.21 ppm, J_{4a-4} = 9.5 Hz). The methoxy signal at 3.48 ppm remained and could not be diminished by repeated lyophilizations. These data are consistent with structure 8. This material failed to show signigicant toxicity in the mouse test.[17]

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17. Toxicity was by intraperitoneal injection in female Swiss-Webster mice, measured in mouse units, MU. A mouse unit, MU, is that amount of substance injected intraperitoneally which will kill a 20 g Swiss-Webster female white mouse in 20 min. The lethalities of the pertinent compounds are: tetrodotoxin (1), 7000-8000 MU/mg; nortetrodotoxin (3), 3500 MU/mg;[12,13] noranhydrotetrodotoxin (5), less than 50 MU/mg; anhydrotetrodotoxin (4), about 90 MU/mg.[19] In addition to the differences in lethalities between 1 and 3, it was observed that the death times with the minimum lethal dose for tetrodotoxin was approximately 25 min, while for nortetrodotoxin it was approximately 10 min.[1,12,13] During the hydrolysis studies 4 the observed death times corresponded to that of 3 as expected.

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