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Synthetic studies on a stereochemically complex natural product: designs for the total synthesis of (−)-tetrodotoxin

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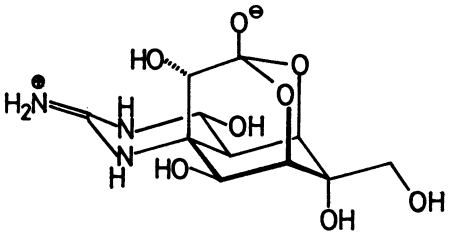
Abstract - Tetrodotoxin is a potent neurotoxin responsible for blocking the sodium channels on nerve cell membranes of mammals. This paper deals with the studies on a chemical synthesis of this natural compound in the optically active form. The synthetic plan toward the toxin is initiated by a Diels-Alder cycloaddition of a chiral diene, levoglucosenone. The adduct serves as the precursor for the cyclohexane ring in the tetradotoxin molecule. The synthetic problems are summarized into the following four essential processes: *i*) cleavage of the 1,6-anhydro bridge, *ii*) regio-selective introduction of a one-carbon unit, *iii*) hydroxylation and amination to the cyclohexane and finally *iv*) the guanidinium and ortho ester group formation. Several model reactions to solve these problems are demonstrated with an indole alkaloid synthesis to indicate the validity of the levoglucosenone strategy. The promising intermediate for tetrodotoxin is described.

INTRODUCTION

Tetrodotoxin (TTX) has been a major food toxin for thousands of years, since people began consuming puffer fish, *Spheroides rubripes*. There are still 40-50 TTX intoxications reported annually, of which *ca.* 15 are fatal. Its structure was reported in 1964 by three research groups at the IUPAC Symposium on Natural Products in Kyoto, Japan (ref. 1-3). Since then, tetrodotoxin has been found not only in the fish but also in Californian newt (*Taricha torosa*) (ref. 4), Costa Rican frogs (*Atelopus sp.*) (ref. 5), Australian octopus (*Hapalochlaena maculosa*). Recently TTX is found in a goby from Taiwan and Amami-Islands (*Gobius criniger*), Japanese shellfishes (*Babylonia japonica*; *Charonia sauliane*), Phylippino crab (*Atergatis floridus*). The origin of this toxin is now identified to be a bacterium (*Pseudomonas sp.*) which was collected and isolated from the toxic organisms (ref. 6). The bio-synthetic studies on TTX, however, are not reported yet. On the other hand, TTX has been of importance for pharmacological interests. On neurocell membranes it exhibits strong neurotoxicity by blocking the sodium channel that should mediate the modulation of the sodium ion permeability of the membrane. The primary structure of the sodium channel protein consisting of 1,820 amino acid residues was deduced through the cloning and sequencing technique of the cDNA of an eel, *Electrophorus electricus* (ref. 7). Specific binding experiments are awaited with this protein using neurotoxins and their analogs to elucidate the gating structure and to understand the molecular mechanisms of this selective ion transport. Our current research interests suggested that a positive contribution to solving these biological problems could be made through a chemical synthesis of optically active TTX. The preparation of some essential intermediates in this synthesis are now described.

A synthesis of tetrodotoxin was achieved by Kishi *et al.* in 1972 in a racemic form (ref. 8). Several other interesting efforts toward TTX synthesis have been reported (refs. 9-11) but so far no synthesis has yielded the optically active form.

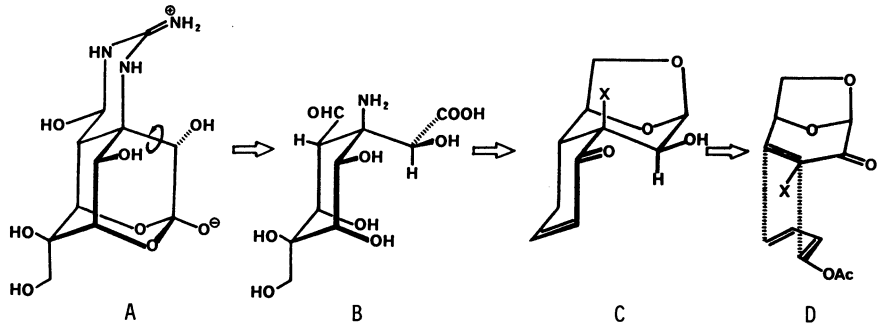


Tetrodotoxin (1)

RETROSYNTHETIC ANALYSIS OF TETRODOTOXIN

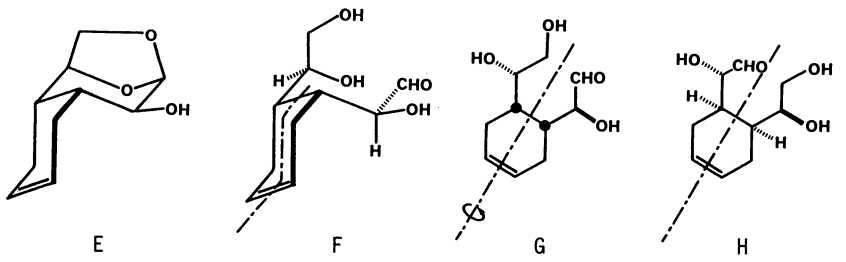
Scheme 1 illustrates a retrosynthesis of TTX, the first process being the removal of the polar functional guanidinium moiety, causing a diminished solubility in any solvent. When the ortho ester in A is hydrolized and the C-C bond is rotated 180° as indicated by the arrow, the hypothetical intermediate B is obtained, this is an equivalent of the real synthetic intermediate, suggesting that the amino or aldehyde groups in the real molecule have to be protected. The precursor of the aldehyde could be the 1,2-glycol, which is present in a masked form. The amino group can be introduced in a step either between B and C or between C and D. A one carbon fragment, equivalent to the hydroxymethyl group, should be introduced perhaps by a conjugate addition to the enone in C. Other hydroxy groups have to be introduced after the construction of the chiral cyclohexane ring in C. The crucial Diels-Alder cycloaddition is shown in D, which may or may not have an amino group equivalent (*e.g.* nitro group) as X. The initial reaction along this retro-synthetic analysis is the Diels-Alder reaction of levoglucosenone, which is obtainable by pyrolysis of cellulose.

Scheme 1



The simple Diels-Alder adduct (below) would show the validity and generality of the above strategy, leading to the hydrolized form F, which is a chiral cyclohexene with two distinguishable side chains, namely, G and H which are considered to be quasi enantiomers of each other.

Scheme 2



DIELS-ALDER CYCLOADDITION WITH LEVOGLUCOSENONE

The Diels-Alder cycloaddition of levoglucosenone is demonstrated with the 1,3-butadiene derivatives as depicted in Table 1. The simplest diene gave the *endo* adduct 3 (X = H) = 6 in quantitative yield (entry 1). After converting its carbonyl group into the corresponding hydrazone (X = NNH_2), 6 was treated with sodium dimsylate in DMSO to yield the vinyl ether 7, which was acetylated and further converted into the lactone 8. Another cycloaddition of levoglucosenone with 1-acetoxy-1,3-butadiene (entry 2) initiated the preparation of an important synthetic intermediate for (-)-reserpine (16) which requires an additional carbon unit. This was utilized as the common intermediate for TTX, although it requires the introduction of the additional carbon at a different position (see Scheme 3) to complete the backbone synthesis. Entries 3-5 were not practical.

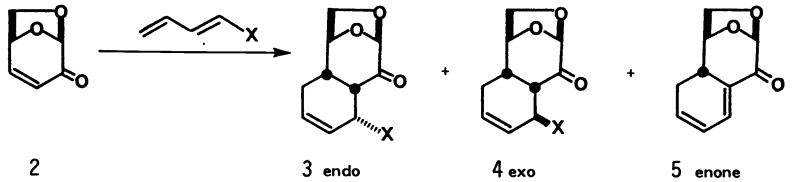
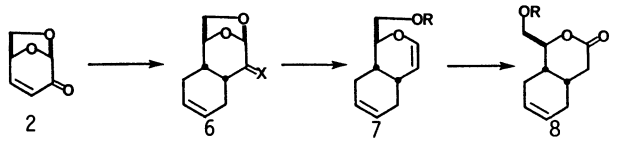


Table 1

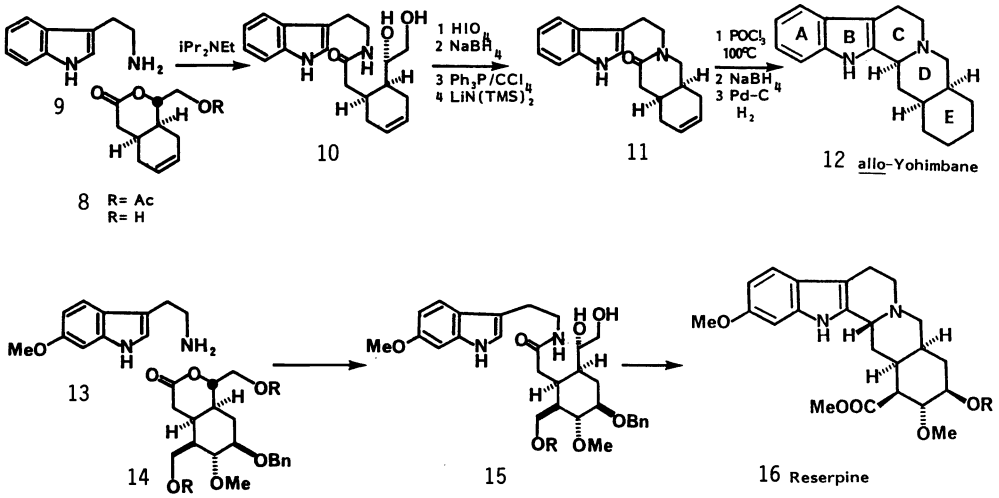
entry	diene	product ratios			yield (%)
	X	*endo*	*exo*	enone	
1	H	1	0	0	98
2	OAc	3	0	1	83
3	OAc	0	0	1	100
4	OTMS	2	1	0	91
5	CH_2OBn	1	1	1	38

*prolonged heating

The lactone 8 was aminated with tryptamine in the presence of a *tert*-amine to afford the amide diol 10. Oxidative cleavage (IO_4^-) of the diol 10 was followed by reduction ($NaBH_4$) and it was further activated by chlorination. The lactam ring D was closed by a base ($LiN(TMS)_2$) and the C-ring was cyclized in two steps ($POCl_3$, $NaBH_4$). A catalytic hydrogenation (Pd-C/H_2) of the olefin in E produced (-)-allo-yohimbane (ref. 12). The validity of this strategy can be extended to another model study directed toward reserpine 16. A hypothetical intermediate lactone 14 would undergo the same chemistry as indicated in the ring closure D (Scheme 1).



Scheme 3

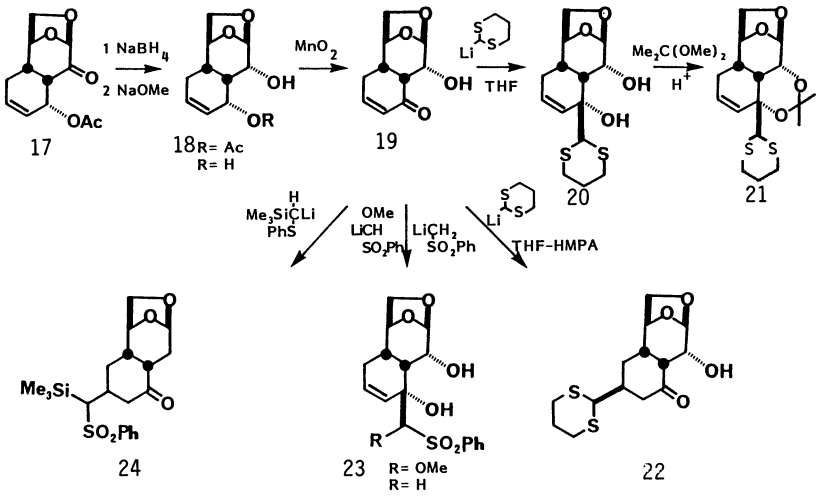


MODEL STUDIES VALIDATING THE STRATEGY TOWARD SYNTHESIS OF RESERPINE

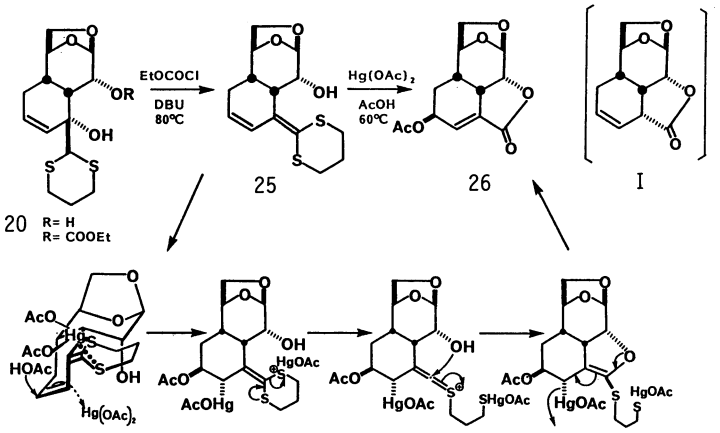
The cycloadduct 17 was converted into the enone 19 *via* 18. Introduction of various one-carbon units was examined with this enone; the lithium carbanion of 1,3-dithiane in THF afforded the 1,2-adduct 20, whose stereochemistry was proven to be *cis* by formation of the corresponding acetonide 21. The same nucleophile added with opposite regioselectivity to 19, when the reaction was carried out in a mixture of THF-HMPA. The resulting 1,4-adduct 22 was utilized for TTX, whilst 20 was utilized for the reserpine synthesis (Scheme 4).

The formation of the lactone 26 is summarized in Scheme 5, although we attempted to obtain the other lactone I by hydrolysis of the dithioketene acetal 25. When 25 was heated with Hg(II), it was, however, oxidized to the acetate lactone 26 *via* oxymercuration.

Scheme 4

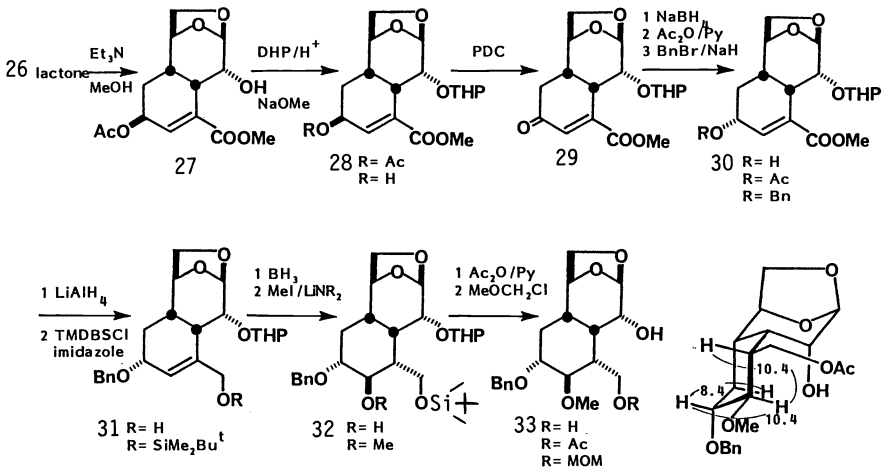


Scheme 5



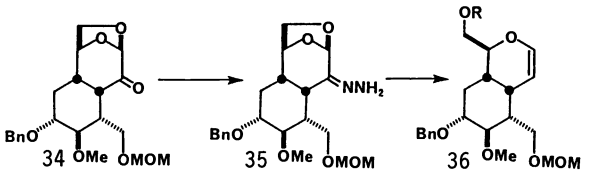
After the lactone ring in 26 was opened by methanolysis to 27, the acetoxy group was inverted to the configuration shown in 30 *via* the following 4 steps: protection of OH (THP), methanolysis of the acetyl group (NaOMe), oxidation (PDC) to the enone 29 and then reduction ($NaBH_4$). The reduction of the methoxycarbonyl in 30 and subsequent protection of the hydroxy group (DMRBSC1) yielded the non-conjugated hydroxy compound 32, and two of the hydroxy groups were differentiated as in 33 bearing a free hydroxy group.

Scheme 6



Wolff-Kishner eliminative reduction was performed with the corresponding ketone 34 *via* the hydrazone 35 to produce 36. This product can potentially in the same vinyl-ether step be coupled with the tryptamine derivative to produce reserpine, as was the case in allo-yohimbane.

Scheme 7



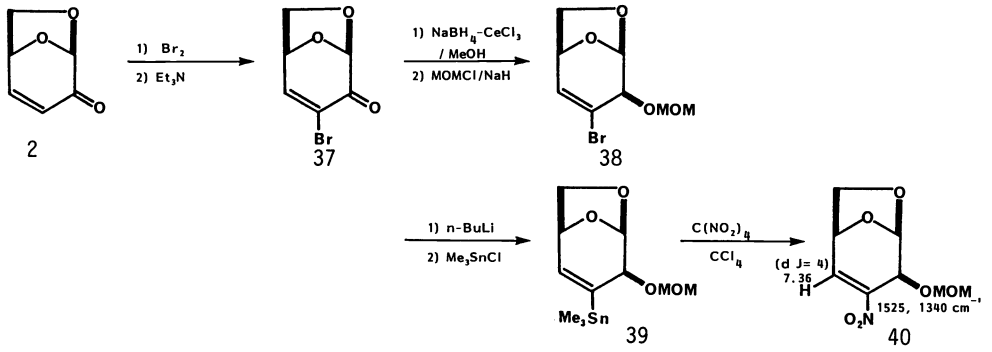
These model studies led us to conclude that we in principle can manage the synthesis of tetrodotoxin along this line, although the position of the carbon side chain and the stereochemistry of the hydroxyl groups are different.

NITROGEN ATOM(GROUP) INTRODUCTION

The guanidinium group in TTX will be introduced in the final step in this synthesis, but it requires the prior introduction of a C-N bond on the chiral cyclohexane ring. Of the several possibilities available, the following example demonstrates a nitrogen atom (group) introduction in an early stage of the total synthesis. The bromination and subsequent dehydrobromination of levoglucosenone produced the α-bromo-enone 37. Reduction of the enone in 1,2-manner ($NaBH_4$-$CeCl_3$) followed by protection, gave 38. It should be noted that the hydroxy group is oriented in the correct configuration for the synthesis of TTX.

The bromine atom was successively converted first to Li, then to $SnMe_3$ (39) and finally into NO_2 as in 40. Generally, nitro-olefins are good dienophiles in the Diels-Alder cycloaddition as desired in the retro-synthesis.

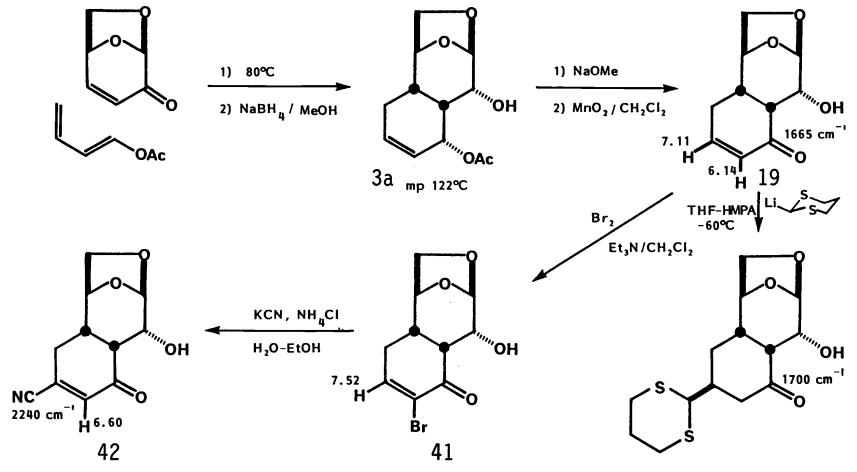
Scheme 8



FUNCTIONALIZATION AND STEREOCONTROL OF CYCLOHEXANE UNIT

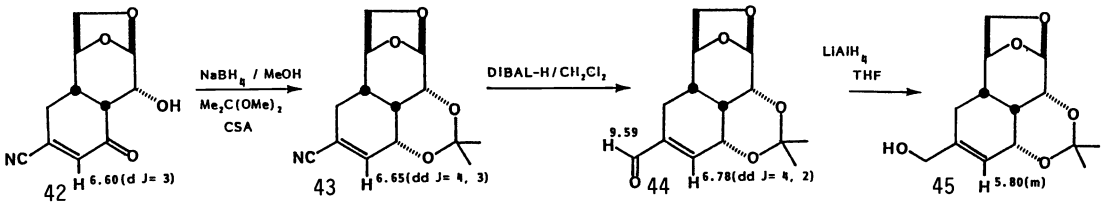
The common intermediate 19 was brominated to 41 to which one carbon unit was introduced in the form of unsaturated nitrile 42 (Scheme 9).

Scheme 9



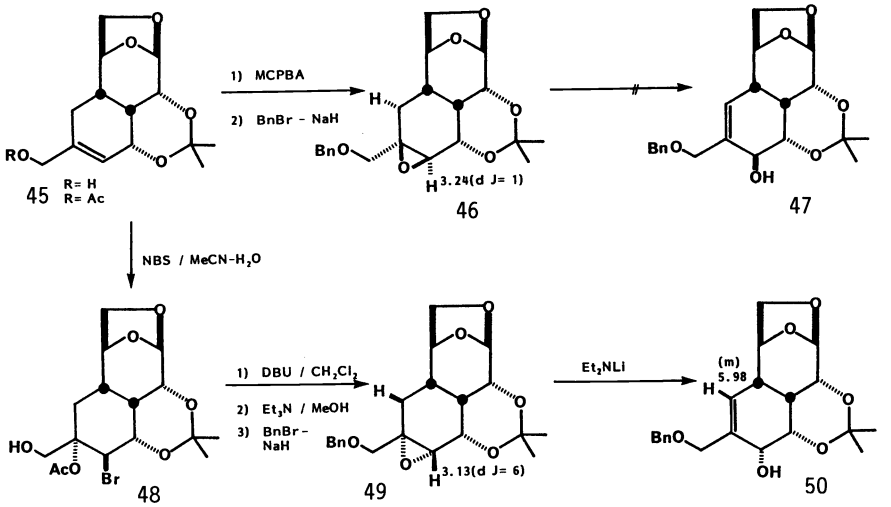
Reduction of the ketone 42 afforded the diol, both hydroxyl groups of which were protected in the form of corresponding acetonide 43. The nitrile group was reduced to the aldehyde 44 (DIBAL-H) and subsequently to the alcohol 45 ($LiAlH_4$).

Scheme 10



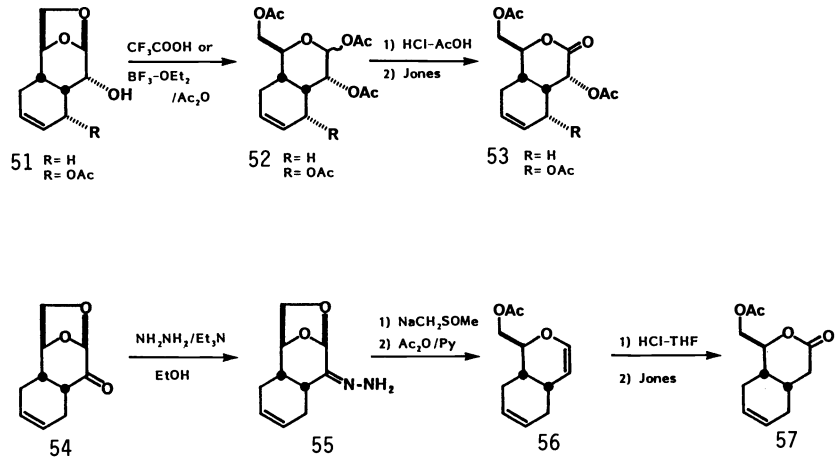
In order to examine the nature of the ring system of 45, it was epoxidized selectively to 46 and 49, respectively, and then an attempt was made to open the oxiran ring. Treatment of these epoxides with strong bases such as Et_2NLi yielded the allylic alcohol 50 from 49 only in a low yield. The formation of the double bond in 47 or 50 may not be favoured with the 1,6-anhydro bridge system because of some torsional strain around the olefin.

Scheme 11



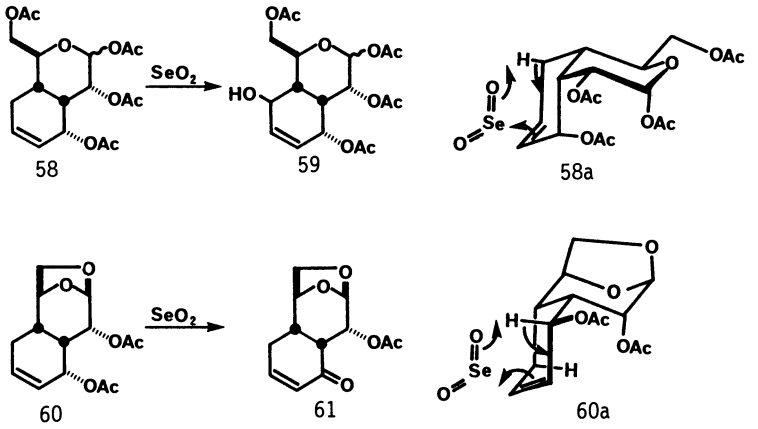
Cleavage of the 1,6-anhydro bridge was then studied under two different conditions. The first method was an acid catalyzed one with *in situ* trapping of the hydroxyl groups as acetates. The second method was the eliminative Wolff-Kishner reduction of the starting ketone 54 to give 56 *via* the hydrazone 55. Both methods resulted in lactone formation 53 or 57.

Scheme 12



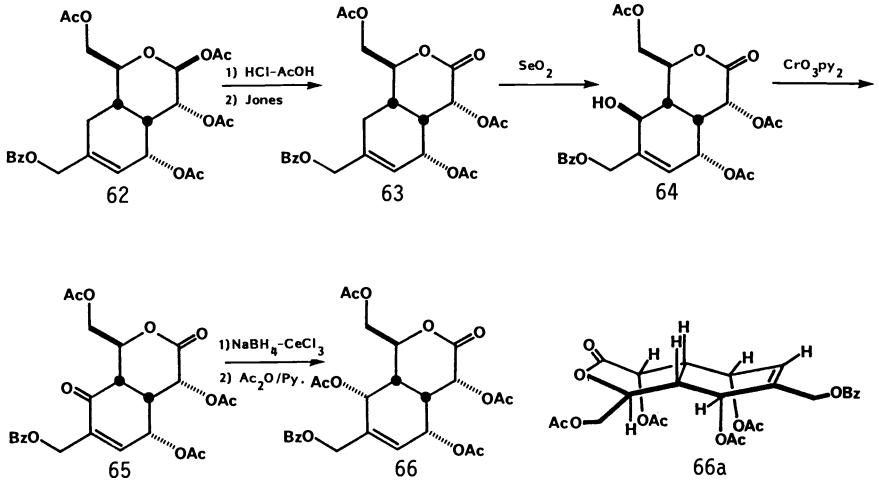
The torsional strain energy around the olefin in 47 or 50 might explain the following two different results in the allylic oxidation (SeO_2). Thus, the initial ene reaction as shown in 58a produces an allylic seleno intermediate involving the double bond which then rearranges to yield the allyl alcohol 59. The oxidation with 60 afforded the enone 61 instead of the corresponding allyl alcohol. This can be due to the different conformation of the cyclohexanes, 58 and 60 which allow the reaction to occur only on the convex face.

Scheme 13



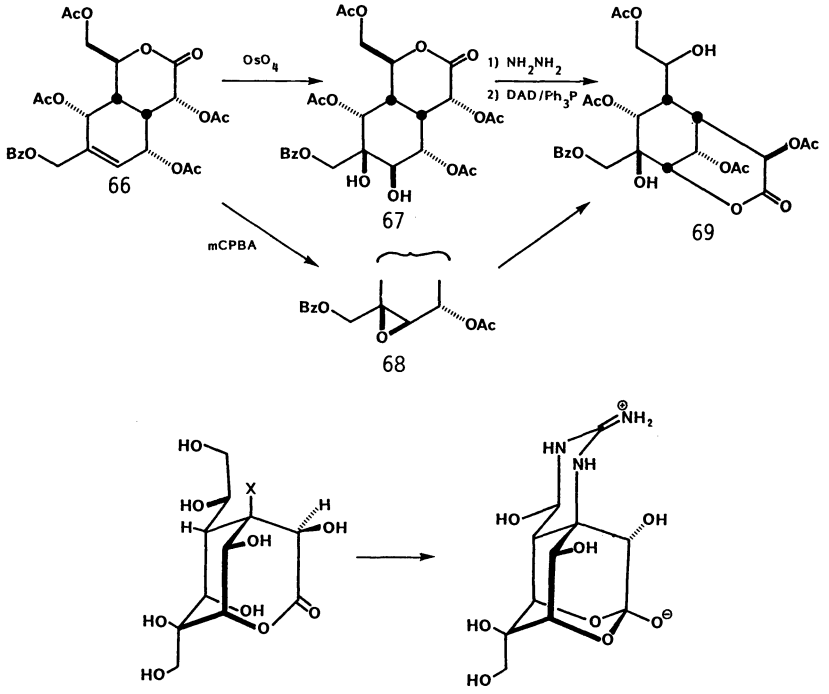
In Scheme 14 the reactions directed toward TTX and based upon the above examinations are summarized. The alcohol 45, the best precursor, was benzoylated, and the two acetal groups were hydrolyzed by acid as indicated in Scheme 12 to produce compound 62. Selective hydrolysis of its acetal acetate was followed by Jones oxidation to yield the lactone 63. Judicious combination of the allylic oxidation (SeO_2), oxidation (CrO_3-2Py) and reduction ($NaBH_4$) afforded the hydroxyl group in a concave orientation (66). Its acetate exhibited a new signal at 5.83 ppm as br.d (J = 5 Hz).

Scheme 14



Further oxidation of the double bond in 66 was achieved with OsO_4 to give diol 67 and then with MCPBA to the epoxide 68. The epoxide proton appeared at 3.63 ppm (d, J = 2 Hz). Inversion of the configuration of the secondary β-O-C bonds in 67 and 68 should give 69, which implies that all the problems for the cyclohexane ring of TTX then will be solved.

Scheme 15



Final steps to be developed are the introduction of the nitrogen group and the completion of the guanidinium group to conclude the total synthesis of tetrodotoxin. Further studies are in progress.

Acknowledgements

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