solution of sodium (0.3 mol) in dry methanol (100 mL). The methanol was removed on a rotary evaporator, ether (100 mL) and water (100 mL) were added, and the ether layer was dried (K_2CO_3). After removal of the ether the ortho thiolester was purified by fractional distillation. The product had bp 108-110 °C (0.05 mmHg); NMR ($CDCl_3$) δ 7.47 (d, 2 H, J = 9 Hz), 6.88 (d, 2 H, J = 9 Hz), 4.40 (distorted t, 2 H, J = 6 Hz), 3.83 (s, 3 H), 3.27 (s, + t, 5 H).

Product Analysis. (a) NMR. The ortho thiolester was added to an acid solution and immediately extracted with carbon tetrachloride. After evaporation of this solvent the NMR spectra were recorded in $CDCl_3$. The mercapto ester O had an NMR with triplets at δ 4.42 ($-CH_2OOC-$) and 2.86 ($-CH_2SH$), while the thiol ester S had triplets at δ 3.87 ($-CH_2OH$) and 3.28 ($-CH_2SOC-$). The relative amounts of these two was obtained from the integrations at δ 2.86 and 3.28.

(b) UV. The mercapto ester O has λ_{max} 254 nm, while the thiolester S has λ_{max} 290 nm where O has very little absorbance. A curve was therefore obtained of the absorbance at 290 nm as a function of acidity. The actual proportion of the two products in 1 M $HClO_4$ was determined by using the NMR method, and from the 290-nm absorbance in this acid the extinction coefficient of the thiol ester was calculated. This extinction coefficient was then used to determine the fraction of thiol ester in the other acids. This was checked in a couple of cases by using the NMR method.

(c) 96% H_2SO_4. The ortho thiolester was added slowly to a stirred cooled solution of 96% H_2SO_4, and the NMR spectrum was directly recorded with an external Me_4Si reference. This showed δ 8.07 (d, 2 H, J = 8 Hz), 7.07 (d, 2 H, J = 8 Hz), 5.43 (t, 2 H, J = 8 Hz), 4.07 (s), 4.04 (s), and 4.05 (t?), the latter three peaks integrating to 7 H. This spectrum is consistent with the formation of methanol and the 2-(4-methoxyphenyl)-1,3-oxathiolan-2-ylium ion.

Kinetic Analysis. Procedures were identical with those described previously.[7] The high-pH kinetics were studied by following the appearance of mercapto ester O at 254 nm. Low-pH kinetics were studied by following the appearance and disappearance of 1,3-oxathiolan-2-ylium ion at 350 nm. Appearance rates were determined by using the Guggenheim method. (These were only considered reliable when the rate so obtained was at least 10 times the decay rate.) Decay rates were obtained by using the infinity method, taking points only after a time corresponding to 10 half-lives of the formation process.

Acknowledgment. Continued financial support of the Natural Sciences and Engineering Research Council of Canada is gratefully acknowledged.

Registry No. ROMe, 85168-64-5; 2-hydroxy-2-(4-methoxyphenyl)-1,3-oxathiolane, 85168-65-6; 2-mercaptoethyl ether, 2150-02-9; 4-methoxybenzoyl chloride, 100-07-2; 2-(4-methoxyphenyl)-1,3-oxathiolan-2-ylium ion, 85168-66-7.

Supplementary Material Available: Tables S1 and S2, observed rate constants for the hydrolysis of 2-methoxy-2-(4-methoxyphenyl)-1,3-oxathiolane in perchloric acid solutions and in acetic acid buffers (1 page). Ordering information is given on any current masthead page.

Structural Studies of Sodium Channel Neurotoxins. 2. Crystal Structure and Absolute Configuration of Veratridine Perchlorate[1]

Penelope W. Codding

Contribution from the Department of Chemistry, University of Calgary, Calgary, Alberta, Canada T2N 1N4. *Received August 30, 1982*

Abstract: Crystal structure analysis has been carried out for the perchlorate salt of veratridine, a *Veratrum* alkaloid with neurotoxic and hypotensive properties. The crystals have space group symmetry $P2_12_12_1$ with a = 7.551 (1), b = 10.521 (1), and c = 44.361 (10) Å at -100 (5) °C. The absolute configuration as determined by anomalous dispersion is consistent with that determined chemically for cevine[7] and crystallographically for zygacine.[6] A β-configuration for the hydroxyl group at C20 is also confirmed by this analysis. Comparison of the structures of veratridine, aconitine, and batrachotoxin, all agonists of the sodium channel receptor, yield a model for their interaction with the receptor.

Extracts of *Veratrum* plants have been used for hundreds of years as medicinal compounds. The pharmacology of the *Veratrum* ester alkaloids has been extensively studied, especially the hypotensive action of certain members of this class. Unfortunately, the toxic side effects of these compounds has limited their clinical utility. Recently, however, one toxic aspect of veratridine activity has been utilized as a pharmacological tool in the study of the mechanism of action of ion channels.

Veratridine is one of a group of four types of lipid-soluble polycyclic compounds that have similar action on the sodium ion channels that mediate the electrical excitability of nerve, heart, and skeletal muscle. These lipid-soluble neurotoxins have been shown to bind to a single receptor site associated with the sodium channels but not to the ion pore itself.[2] Their effect is to shift activation of the channels to more negative membrane potentials and to block inactivation, thereby producing a lasting depolarization of the excitable membrane. Experiments with neuroblastoma cells[3] demonstrate competitive binding to the "activating" receptor among all four neurotoxins, and, as well, concentration dependences indicate that binding a single toxin molecule activates a single sodium channel. These ion flux studies prove batrachotoxin (**1**, Chart I) to be a full agonist of the receptor while veratridine (**2**), aconitine (**3**), and grayanotoxin (**4**) are partial agonists. Therefore, these neuorotoxins bind with different efficacies to a chemically sensitive site associated with sodium channels; occupation of this site causes persistent activation of the channels. It has been proposed[3] that the lipid-soluble neurotoxins bind with greater affinity to the active state of the channel so that their effectiveness is dependent on their selectivity for the active conformation of the receptor.

Because of the disparate chemical structures of the four neurotoxins being employed in pharmacological studies, it has proved difficult to ascertain the structural requirements for activity by the usual method of chemical modification. One attempt has been

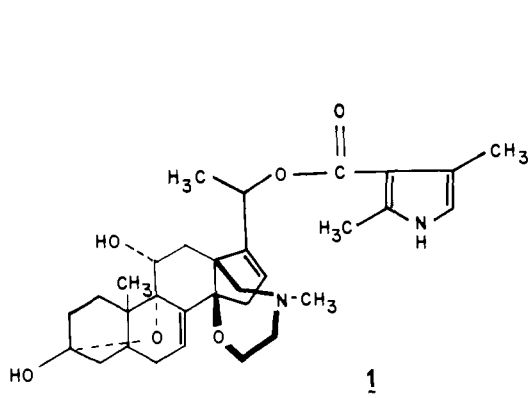
(1) Part 1 of this series: Codding, P. W. *Acta Crystallogr., Sect. B* **1982**, *B38*, 2519-2522.

(2) Three recent reviews of the pharmacology of sodium channels have appeared: (a) Catterall, W. A.; Hartshorne, R. P.; Beneski, D. A. *Toxicon* **1982**, *20*, 27-40. (b) Catterall, W. A. *Ann. Rev. Pharmacol. Toxicol.* **1980**, *20*, 15-43. (c) Narahashi, T. *Adv. Cytopharm.* **1979**, *3*, 293-303.

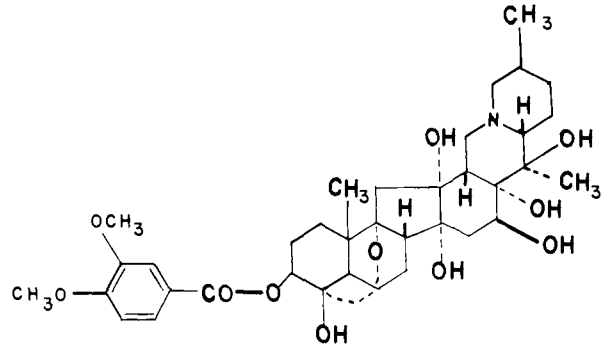
(3) Catterall, W. A. *J. Biol. Chem.* **1977**, *252*, 8669-8676.

0002-7863/83/1505-3172$01.50/0

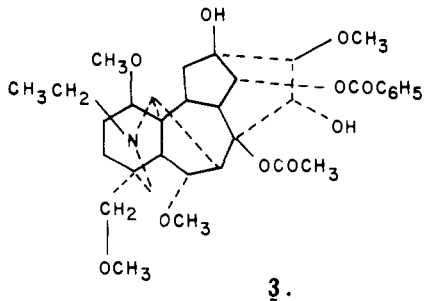
Chart I



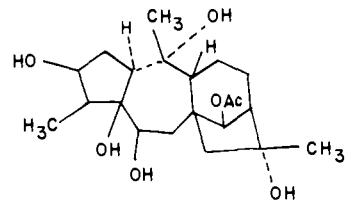
1



2



3



4

made using the grayanotoxin series of compounds,[4] unfortunately, the logical jump to three-dimensional positions of substituent groups has proved difficult without structural data. Determination of the crystal structures of drugs in this series has been undertaken to provide the topochemical information required for the identification of the common structural features that are needed for toxin action. The crystal structure of batrachotoxinin A has been published,[5] the structure of aconitine is part 1 of this series,[1] and this paper reports the determination of the structure of veratridine.

Chemically, the structure of veratridine is interesting because there are relatively few examples of the steroid C_{27} bases of the ceveratrum group available in the crystallographic literature. The structure of zygacine acetonide hydriodide[6] established the absolute configuration of zygadenine, germine, and protoveratrine and the probable relative configuration (β) of the hydroxyl group at C20 for cevine, which had been proposed on chemical grounds.[7] However, definitive confirmation of the stereochemistry awaited an X-ray structure determination. An intramolecular catalysis of ester solvolysis in these molecules has been proposed,[8] which would be facilitated by the hydroxyl group on C20 having a cis-1,3-diaxial relationship to the ester group at C16. Although veratridine has an OH substituent at C16, determination of the hydrogen bonds formed by the hydroxyl groups in this molecule may provide useful information about the interaction of these groups.

Experimental Section

Veratridine free base was purchased from Sigma Chemical Co. and converted to the perchlorate salt by using the acid. Crystals were grown by slow evaporation of an ethanol solution. Crystal data are given in Table I. The crystal was glued to a fiber and mounted in a stream of cold N_2 vapor (−100 (5) °C) on a CAD4F diffractometer. Two octants of data were collected to a maximum θ of 65° by using the $\omega/2\theta$ scan technique and a scan speed between 0.72 and 6.71 deg/min dependent on peak maximum. The scan width was calculated as 1.5(0.54 + 0.14 tan θ); two-thirds of the scan time, at the center of the scan, was taken as peak, and one-sixth of the time at each side was taken as background. Friedel pairs were measured at $\pm 2\theta$ to a maximum θ = 27.6°. In all, 4230 reflections and 1098 Friedel reflections were measured; of the 3466 unique reflections, 2981 had $I > 3\sigma(I)$. Lorentz and polarization corrections were applied. No absorption correction was made.

Table I. Crystal Data for Veratridine Perchlorate

mol form.	$[C_{36}H_{52}NO_{11}]^+ClO_4^-$	mol wt, g mol^{-1}	774.26
cell dimensions	a = 7.551 (1) Å	c = 44.361 (10) Å	
	b = 10.521 (1) Å	V = 3524 (1) Å^3	
space group	$P2_12_12_1$	Z = 4	
calcd density, g cm^{-3}	1.454	linear abs coeff, cm^{-1} (Cu Kα)	16.21
cryst size, mm	0.34 × 0.24 × 0.20	wavelength (Cu Kα; Ni filter), Å	1.5418

The initial model for the structure was determined by using MULTAN 78.[9] All carbon, nitrogen, oxygen, and chlorine atoms were located in the first E map, and all hydrogen atoms were located in subsequent difference electron density maps. The structural model was refined with anisotropic thermal parameters for all but the hydrogen atoms, which had fixed isotropic thermal parameters. The weights were assigned as follows: wt = (sin θ)/0.5 if sin θ < 0.5, wt = 50/F_o if F_o > 50, otherwise wt = 1.0. Weighted full-matrix least-squares refinement gave final residuals of R = 0.037 and R_w = 0.043. The absolute configuration was determined by comparing the ratios of the calculated magnitudes of the Friedel pairs ($F_c(+)/F_c(-)$) for the two enantiomers to the observed ratios. Of the 91 ratios that differed by 5.0%, i.e., $F(+)/F(-)$ > 1.05 or < 0.95, 82 agreed, thus determining the absolute configuration.

Results and Discussion

Figure 1 shows the conformation, absolute configuration, and atomic labeling of veratridine perchlorate. The fractional atomic coordinates and anisotropic thermal parameters are in Table II and Table III,[10] respectively; the atomic parameters for the hydrogen atoms are in Table IV.[10] Tables V–VII[10] contain the bond distances, bond angles, and hydrogen atom bond distances, respectively. Selected torsion angles are in Table VIII.[10]

(4) Masutani, T.; Seyama, I.; Narahaski, T.; Swasa, J. *J. Pharmacol. Exp. Ther.* **1981**, *217*, 812–819.
(5) Karle, I. L.; Karle, J. *Acta Crystallogr., Sect. B* **1969**, *B25*, 428–434.
(6) Bryan, R. F.; Restivo, R. J.; Kupchan, S. M., *J. Chem. Soc., Perkin Trans. 2* **1973**, 386–390.
(7) Kupchan, S. M.; Johnson, W. S., Rajagopalan, S. *Tetrahedron*, **1959**, *7*, 47–61.
(8) Kupchan, S. M.; Eriksen, S. P.; Liang, Y.-T. S. *J. Am. Chem. Soc.* **1966**, *88*, 347–350.
(9) Germain, G.; Main, P.; Woolfson, M. M. *Acta Crystallogr., Sect. A* **1971**, *A27*, 368–376.
(10) See paragraph at the end of the paper regarding supplementary material.
(11) Albuquerque, E. X.; Daly, J. W.; Witkop, B. *Science (Washington, D.C.)* **1971**, *172*, 995–1002.

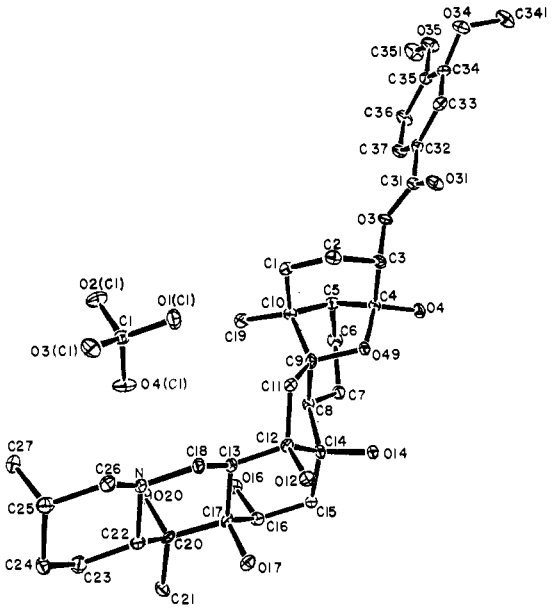


Figure 1. Absolute configuration and atomic labeling for veratridine perchlorate.[18] The thermal ellipsoids of all carbon, nitrogen, oxygen, and chlorine atoms are depicted at the 50% probability level for the refined anisotropic thermal parameters.

Pharmacological activity of these neurotoxins must depend on their common features, namely, the large number of potential hydrogen-bond-donor and -acceptor atoms. All of the polycyclic neurotoxins are composed of fused ring systems which will be relatively inflexible and serve as the scaffolding for the oxygen and nitrogen atoms that presumably interact with the receptor surface. Comparison of veratridine to other lipid-soluble neurotoxins is best approached by studying the simplest member of the series for which crystallographic data are available, namely, batrachotoxin (BTX). Preliminary findings[12] on the similarities between aconitine and BTX can now be expanded and more fully developed with the additional data from veratridine.

Masutani et al.[4] have proposed a model for the interaction of these neurotoxins with the Na channel receptor. In their model each molecule has three reactive oxygen atoms that form a triangle of specific dimension, and in each molecule there is a methyl moiety nearby. In BTX the methyl moiety was the 2′-methyl group on the 20α-pyrrole ring, and in veratridine the methyl moiety came from a methoxy substituent on the 3-benzoate group. Unfortunately, these predictions have not been supported by recent pharmacological studies nor by the crystallographically determined structures.

Batrachotoxin was extensively studied when it was first isolated;[11] the SAR work focused on the most potent methyl-substitution pattern on the 20α-pyrrole-3-carboxylate group. It was thought that the derivative structure, batrachotoxinin A ρ-bromo benzoate (BTX-B), determined by Karle and Karle[5] was inactive. Recent efforts to produce a radioactive ligand for binding studies to voltage-sensitive sodium channels have demonstrated that batrachotoxinin A 20α-benzoate is equally as potent at BTX.[13] Therefore, methyl substitution on the aromatic moiety at C20 is not required for activity; that leads to the conclusion that BTX need not have a methyl group proximate to the three reactive oxygen atoms to be active.

It has been noted[14] that veratridine and BTX are similar in the position of their tertiary nitrogens relative to the general steroidal skeleton. Using the model of three reactive oxygen atoms separated by similar distances and nearly the same positions for the nitrogen atoms, a reasonable fit of the veratridine structure to the BTX-B framework can be obtained. A least-squares[15] fit of the three rigidly fixed oxygen atoms of BTX, the 3α,9α-hemiketal and the 3-OH and 11-OH groups, to three oxygen atoms with similar separations in veratridine was done (see Figure 2). Masutani et al. chose a triangle of oxygen atoms for veratridine that included the hydroxyl groups on C12, C14, and C17; when these groups were fit to the BTX-B structure, the nitrogen atoms were 8.8 Å apart. However, if the hemiketal in veratridine, O49, along with the hydroxyl groups on C4 and C14 are used as the

Table II. Fractional Coordinates (×10⁵) and U_{eq} (×10⁴) for Veratridine Perchlorate[a]

atom	x/a	y/b	z/c	U_{eq}
Cl	27 676 (12)	−31 447 (8)	30 337 (2)	145
O1 (Cl)	44 736 (38)	−35 711 (28)	31 395 (6)	231
O2 (Cl)	14 065 (41)	−39 611 (27)	31 451 (7)	243
O3 (Cl)	27 589 (40)	−31 594 (27)	27 090 (6)	219
O4 (Cl)	24 626 (41)	−18 594 (24)	31 425 (6)	238
C1	70 392 (54)	−44 912 (35)	37 462 (8)	145
C2	89 804 (55)	−48 460 (38)	37 048 (8)	168
C3	100 896 (51)	−45 968 (35)	39 862 (8)	146
C4	95 462 (50)	−33 376 (35)	41 310 (8)	129
C5	76 070 (54)	−32 823 (34)	42 243 (8)	147
C6	71 907 (56)	−21 052 (35)	44 193 (8)	170
C7	76 877 (54)	−8 356 (35)	42 700 (8)	145
C8	73 260 (50)	−8 297 (33)	39 329 (8)	120
C9	78 466 (52)	−21 052 (35)	37 857 (8)	131
C10	67 302 (48)	−32 095 (35)	39 081 (8)	121
C11	79 573 (50)	−18 149 (35)	34 475 (8)	120
C12	78 920 (51)	−3 376 (34)	34 150 (8)	118
C13	60 896 (49)	1 134 (35)	32 955 (8)	110
C14	82 845 (48)	1 516 (36)	37 383 (8)	131
C15	77 715 (52)	15 366 (33)	37 906 (8)	125
C16	59 892 (52)	19 349 (36)	36 668 (8)	140
C17	57 868 (50)	15 526 (33)	33 346 (8)	118
C18	59 159 (49)	−2 507 (35)	29 621 (8)	127
C19	47 353 (53)	−29 564 (37)	39 100 (9)	178
C20	39 449 (49)	19 093 (35)	31 981 (8)	129
C21	35 166 (53)	33 090 (36)	32 513 (8)	158
C22	39 224 (51)	15 973 (34)	28 612 (8)	138
C23	22 383 (56)	20 073 (36)	26 996 (8)	174
C24	22 035 (54)	15 793 (36)	23 695 (8)	161
C25	24 119 (57)	1 410 (36)	23 444 (8)	178
C26	41 113 (52)	−2 628 (37)	25 027 (8)	140
C27	7 745 (56)	−5 787 (40)	2 450 (10)	208
C31	109 594 (52)	−65 655 (35)	42 077 (8)	149
C32	106 038 (52)	−74 995 (36)	44 516 (8)	148
C33	115 471 (53)	−86 501 (37)	44 506 (8)	156
C34	112 656 (52)	−95 411 (34)	46 706 (8)	150
C35	100 321 (53)	−93 087 (37)	49 034 (8)	159
C36	91 058 (56)	−81 650 (40)	49 052 (8)	197
C37	93 792 (56)	−72 796 (36)	46 779 (9)	180
C341	134 292 (62)	−109 329 (43)	48 715 (10)	262
C351	86 050 (57)	−100 644 (41)	53 471 (9)	298
N	41 829 (41)	1 721 (28)	28 272 (6)	120
O4	108 086 (37)	−30 785 (25)	43 552 (6)	171
O49	96 662 (33)	−24 092 (24)	38 919 (5)	124
O12	91 667 (35)	1 551 (24)	32 054 (5)	138
O14	101 758 (34)	1 114 (24)	37 868 (6)	144
O16	45 568 (35)	14 140 (24)	38 450 (5)	150
O17	70 612 (36)	22 838 (24)	31 681 (6)	155
O20	25 875 (34)	11 140 (24)	33 254 (5)	140
O3	98 170 (35)	−55 927 (24)	42 110 (6)	147
O31	121 692 (38)	−66 648 (25)	40 279 (6)	188
O34	120 653 (40)	−107 186 (24)	46 507 (6)	191
O35	98 275 (40)	−102 678 (27)	51 037 (6)	232

[a] The estimated standard deviation is in parentheses. U_{eq} is defined as $\frac{1}{3}\sum_i \tilde{U}_{ii}$, where the U_{ii} are the components of the diagonalized thermal parameter matrix.

(12) Codding, P. W. 12th International Congress of the IUCr, 1981, Ottawa, Ontario, Abstr 03.1–15.
(13) Catterall, W. A.; Morrow, C. S.; Daly, J. W.; Brown, G. B. *J. Biol. Chem.* **1981**, *256*, 8922–8927.
(14) Albuquerque, E. X.; Daly, J. W. In "The Specificity and Action of Animal, Bacterial and Plant Toxins"; Cuatrecasas, P., Ed.; Chapman and Hall: London, 1976; pp 298–338.
(15) The fit was done by using the program PROFIT written by Dr. G. D. Smith of The Medical Foundation of Buffalo, Buffalo, NY.
(16) Kupchan, S. M.; Flacke, W. E. In "Antihypertensive Agents"; Schlitter, E., Ed.; Academic Press: New York 1967; pp 429–458.

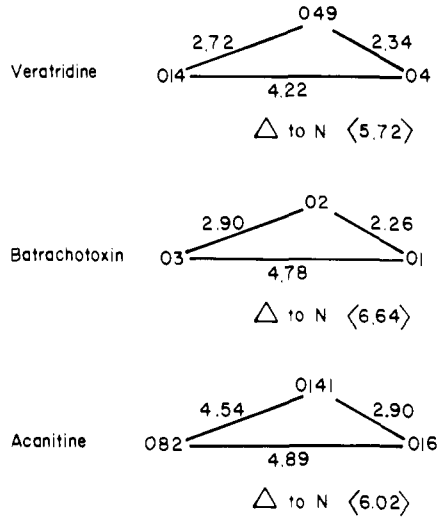


Figure 2. Distances (Å) between the reactive oxygen atoms in batrachotoxin, veratridine, and aconitine. The average separation of these oxygen atoms from the nitrogen atom is depicted as the Δ-N distance.

triangle of oxygen atoms, a least-squares fit of the two structures places the nitrogen atoms 1.8 Å apart. More important, as shown in Figure 3, the nitrogen atoms in the overlapped structures are positioned so that they could hydrogen bond to the same donor atom on the receptor. Thus a model is formulated from this comparison that requires a triangle of oxygen atoms which are approximately 5 Å from a tertiary nitrogen atom. In aconitine,[1] the appropriate triangle of oxygen atoms includes the carbonyl oxygens of the benzoate group and an acetoxy group, so some flexibility in their positions is possible; yet, once again the oxygen atoms are found 6 Å from the nitrogen atom (see Figure 2). For all three molecules the nitrogen atom is found on the other side of the framework from the reactive oxygen functionalities.

It is interesting that the 3-veratroyl group plays no part in the model for Na channel activity. This group may not be required for binding; it is known that what hypotensive activity there is in veratridine is dependent on the group at C3.[16] Perhaps variation of substituents at that position and modification of the groups proposed herein to confer Na channel activity could produce a hypotensive compound with fewer toxic effects.

The conformation of the C_{27} steroid nucleus of veratridine is almost the same as that found in zygacine;[6] a detailed comparison of the relevant torsion angles[10] in the two structures confirms the similarity of the individual ring conformations. In both molecules, rings A, B, and D–F are in chair conformations with slight compensatory opening and closing of torsion angles in rings A and B due to the α-ketol hemiketal system. The largest differences in skeletal torsion angles are in rings C and D, where the loss of the dioxolane ring found in zygacine produces changes in angles from 5 to 15° as shown in Figure 4. Ring C has a different conformation in the two structures; in veratridine it is a half-chair with C8 (0.30 Å) and C14 (0.40 Å) on either side of the plane of the other three atoms, whereas in zygacine, ring C is an envelope with C8 at the flap. In ring D the differences are more subtle, and the overall effect in veratridine is a relaxation toward a strain-free chair conformation from the twist-boat distortion observed in zygacine.

Even with the aforementioned differences, an overall fit of the backbone atoms of veratridine and zygacine using least squares[15] gives an average deviation of 0.179 Å with a maximum deviation (for C24) of 0.367 Å. Thus, in general, the two structures have the same skeletal structures. The conformation of the skeleton

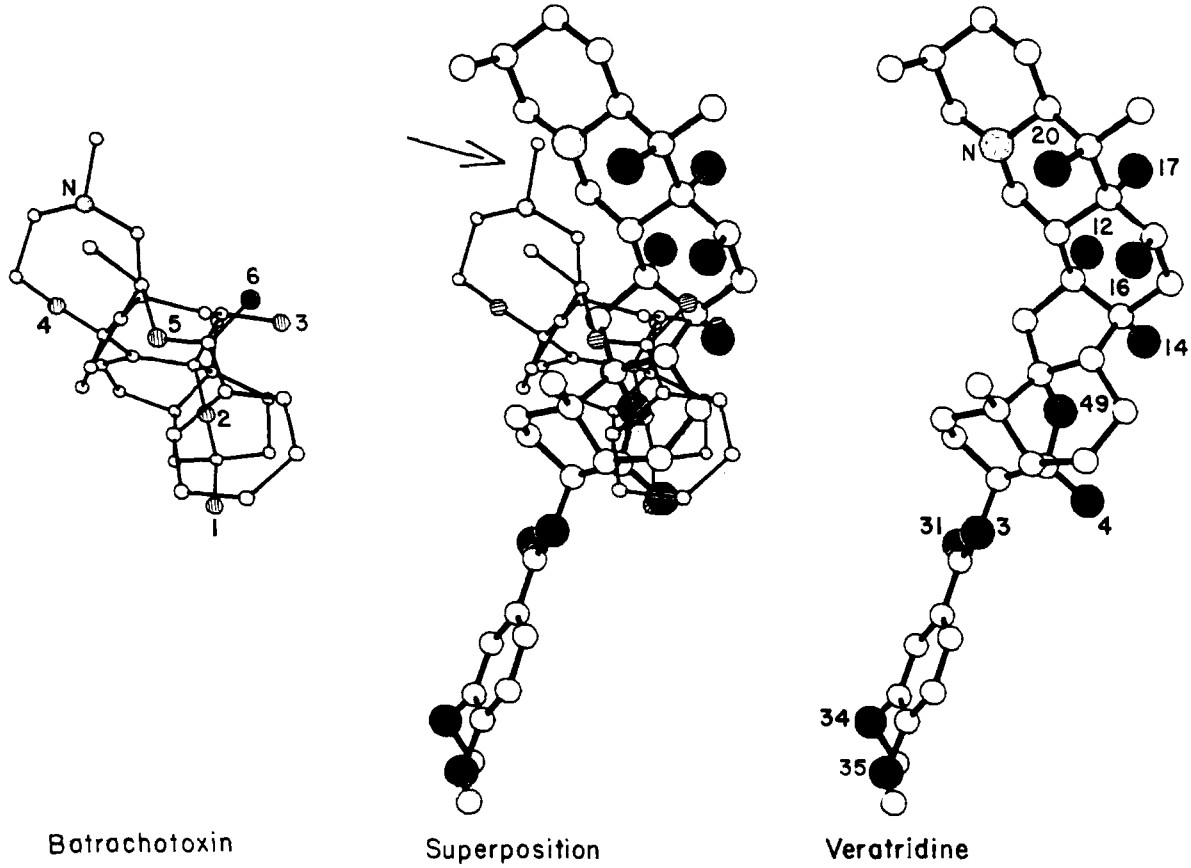


Figure 3. Drawing of batrachotoxinin[5] fit via least squares of the oxygen atom positions to the conformation of veratridine reported herein. On the left is batrachotoxin in the overlap orientation; on the right is veratridine similarly displayed. The center shows the two structures drawn interleaved with an arrow pointing out the position of the nitrogen atoms, which are shaded. The oxygen atoms in veratridine are filled, those of batrachotoxin are hatched. Note that the overlap of the triangle of oxygen atoms brings the nitrogen atoms in close proximity and, in addition, the carbonyl oxygen of the benzoate group, O6, in BTX-B is very near the hydroxyl group, O16, on veratridine.

Table IX. Hydrogen-Bond Parameters for Veratridine Perchlorate

Intramolecular Bonds

atoms	dist, Å O-H	dist, Å H···Y	dist, Å O-Y	angle O-H-Y, deg
O20-H20···O16	0.74 (4)	2.08 (4)	2.761 (4)	152 (4)
O17-H17···O12	0.87 (4)	2.01 (4)	2.752 (4)	143 (4)
O12-H12···O14	0.84 (4)	2.20 (4)	2.690 (4)	117 (3)
O14-H14···O49	0.71 (4)	2.10 (4)	2.720 (4)	147 (4)
N-HN···O4 (Cl)	0.80 (4)	2.14 (4)	2.866 (4)	150 (4)

Intermolecular Bonds

atoms	equiv position	dist, Å O-H	dist, Å H···Y	dist, Å O-Y	angle O-H-Y, deg
O16-H16···O31	$(x-1, 1+y, z)$	0.83 (4)	2.02 (4)	2.827 (4)	165 (4)
O4-H4(O)···O34	$(x, y+1, z)$	0.77 (4)	2.31 (4)	2.964 (4)	144 (4)
O12-H12···O20	$(1+x, y, z)$	0.84 (4)	2.06 (4)	2.824 (4)	151 (4)

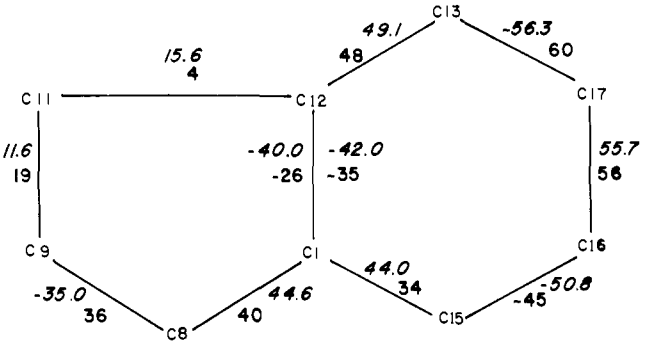


Figure 4. Comparison of the torsion angles in veratridine (italics) and zygacine acetonide[6] for rings C and D.

of another C_{27} steroid alkaloid, protoveratrine C,[17] appears, qualitatively, to be the same as that observed in veratridine and zygacine, thereby reinforcing the conclusion that these molecular backbones are inflexible.

Variations in the pharmacological effects of the *Veratrum* alkaloids appear to be achieved by the interplay of the substituents attached to the essentially rigid molecular framework. Structure–activity studies[16] on the hypotensive effect of various *Veratrum* ester alkaloids support this concept; for example, veratroylzygadine (C12-H, C15-OH, C16-OH, C17-H) is much more potent than veratridine. Thus, variation in the positions of hydroxyl groups determines the hypotensive utility of the drug and, according to this model, the neurotoxic properties as well. It would be useful to test some other *Veratrum* alkaloids for Na channel activity to further delineate the most effective substitution pattern.

Intramolecular hydrogen bonding enhances the stability of the molecular conformation observed for these steroid alkaloids. All of the hydrogen atoms were identified in this structure determination, which makes it possible to describe in detail all of the hydrogen bonds. Table IX contains the parameters for all hydrogen bonds found in the crystal structure. There are five intramolecular bonds, three of which link the α-hydroxyl groups in a hydrogen-bonded chain to the hemiketal oxygen atom, O49; O17–H17···O12–H12···O14–H14···O49. All of these bonds have similar parameters (see Table IX) except the one involving H12; the O12–H12···O14 angle is closed to 117 (3)°. Another close contact to H12 occurs with O20 at $(1+x, y, z)$, which suggests that H12 forms a bifurcated hydrogen bond of mixed intra- and intermolecular types.

The β-hydroxyl groups form one intramolecular hydrogen bond with O20 donating a hydrogen atom to O16. This interaction confirms the possible catalytic potentiation of the C16 ester by an oxygen functionality on C20 as proposed by Kupchan et al.[8] Both hydroxyl groups also form intermolecular bonds, O20 as above, and O16 forms an intermolecular bond by donating H16 to the carbonyl oxygen of the benzoate group, O31 at $(1+x, y-1, z)$. Another intermolecular hydrogen bond is formed between O4 and the ether oxygen atom, O34 at $(x, y+1, z)$; this interaction appears to be weak: H4···O34 is 2.30 (5) Å, as could be expected since an ether oxygen atom is a weak acceptor.

All other intermolecular contacts involve the ClO_4^- ion. One hydrogen bond is formed from N to O4(Cl); other contacts seem to be dependent on van der Waals and electrostatic forces.

In summary, the structural analysis of veratridine indicates that C_{27} steroid alkaloids consist of inflexible molecular frameworks with varying patterns of oxygenated substituents that determine their pharmacological effect. The framework conformation is stabilized by extensive intramolecular hydrogen bonding. Comparison of the veratridine structure to those found for two other neurotoxins, batrachotoxin and aconitine, yields a model for receptor binding that requires a triangle of reactive oxygen atoms of specific dimensions (see Figure 2) and a nitrogen atom 5–6 Å away from the triangle. Pharmacological studies indicate that these bases are not protonated at physiological pH; therefore, the nitrogen atom can be considered to be a hydrogen-bond acceptor. This could explain the activity of the grayanotoxin compounds since they do not contain a nitrogen atom but do contain potential acceptors in the form of hydroxy or acetoxy groups. Structural analysis on members of the grayanotoxin series are in progress and will provide further data to test this model for sodium channel activity.

Acknowledgment. This research was supported by an Establishment Grant and Scholarship from the Alberta Heritage Foundation for Medical Research. I thank Dr. M. H. Benn for assistance with the crystallization, Dr. J. W. Daly for making his results available before publication, and Dr. K. A. Kerr for use of the diffractometer.

Registry No. Veratridine, 71-62-5; veratridine perchlorate, 6183-77-3.

Supplementary Material Available: Fractional atomic coordinates for the hydrogen atoms, all temperature factors, bond distances and bond angles, torsion angles, and observed and calculated structure factor amplitudes (22 pages). Ordering information is given on any current masthead page.

(17) Saksena, A. K.; McPhail, A. T. *Tetrahedron Lett.* **1982**, *23*, 811–814.
(18) Johnson, C. K. "ORTEP-II, A Fortran Thermal Ellipsoid Plot Program for Crystal Structure Illustrations"; Oak Ridge National Laboratory, Oak Ridge, TN, Report No. ORNL-5138.