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# Insecticidal Activity of Various 3-Acyl and Other Derivatives of Veracevine Relative to the *Veratrum* Alkaloids Veratridine and Cevadine

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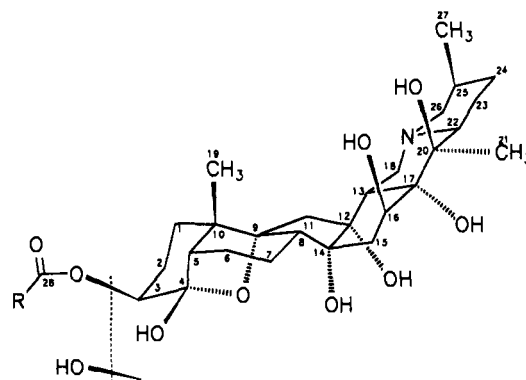
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Sixty-five 3-acyl derivatives of veracevine, including 44 aromatic and 21 aliphatic esters or related derivatives, were isolated or prepared and their insecticidal activities determined by topical application to adult houseflies and milkweed bugs. Of these the naturally occurring veratridine [3-(3,4-dimethoxybenzoyl)veracevine] and cevadine [3-[(Z)-2-methylbut-2-enoyl]veracevine] are near optimal in potency. Piperonyl butoxide synergism indicates that oxidative detoxification limits the toxicity of all the compounds to houseflies. Five other veratridine and cevadine derivatives examined are inactive, but the related protoveratrine A has high insecticidal potency. The most active of the synthetic aliphatic esters, 3-pivaloylveracevine, is equally potent to or less potent than cevadine. In the aromatic ester series, the 2,5- and 3,5-dimethoxy analogues are more potent than veratridine and the 3,5-dimethoxy compound is also improved in selective toxicity, i.e., LD<sub>50</sub> ratio for mice vs the insects tested. These findings lay the background for determining the relative importance of metabolism and receptor site sensitivity in species specificity.

## INTRODUCTION

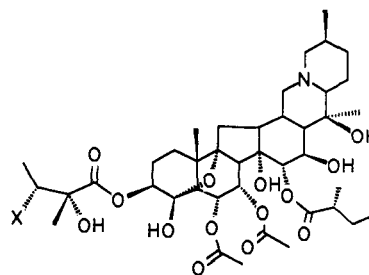
*Veratrum* preparations, from plants of the Liliaceae family, were used for centuries in the treatment of circulatory disorders and the control of insects (Kupchan and Flacke, 1967; Kupchan and By, 1968; Crosby, 1971). However, they were superseded in medicine by other alkaloids and synthetic drugs with fewer side effects and in agriculture by simpler and more effective synthetic insecticides. The major biologically active principles belong to a unique class of C-nor-D-homosteroidal alkaloids whose structure and stereochemistry were assigned in the 1950s after decades of extensive chemical investigations [Barton et al., 1954; Kupchan et al., 1959; reviewed by Narayanan (1962)] and later confirmed by X-ray crystallography (Coddling, 1983).

The alkaloids of greatest biological significance in this series are the ceveratrum esters, which are characterized by a high degree of hydroxylation and a hemiacetal bridge between the A and B rings. Specific examples are veratridine (1) and cevadine (2), i.e., the 3-veratroyl and 3-angeloyl esters, respectively, of veracevine (3), which have been recognized as the insecticidal principles of "sabadilla" preparations from *Schoenocaulon officinale* (also known as *Veratrum sabadilla*) (Ikawa et al., 1945; Crosby, 1971). Some species specificity has been noted; aromatic ester 1 is more toxic than aliphatic ester 2 to houseflies (*Musca domestica*) (Ikawa et al., 1945), whereas the reverse is noted for milkweed bugs (*Oncopeltus fasciatus*) (Allen et al., 1945). The toxicity of sabadilla to houseflies is increased by various pyrethrum synergists, including piperonyl butoxide (PB) (Blum and Kearns, 1956), suggesting



- |                                  |             |
|----------------------------------|-------------|
| 1: R = 3,4-(MeO) <sub>2</sub> Ph | veratridine |
| 2: R = (Z)-MeCH=CMe              | cevadine    |
| 3: C-3 hydroxyl                  | veracevine  |

the involvement of oxidases in the detoxification of these compounds. Related *Veratrum* alkaloids, e.g., germitrine and protoveratrines A and B, are also highly toxic to housefly larvae (Bergmann et al., 1958).



- |        |                  |
|--------|------------------|
| X = H  | protoveratrine A |
| X = OH | protoveratrine B |

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‡ Dedicated to Professor Emeritus Thomas C. Allen for initiating the interest of J.E.C. in sabadilla-based insecticides.

Apart from these observations there are no systematic investigations on the relationship between structure and insecticidal activity of the *Veratrum* alkaloids. The present study, as a first step, focuses on the influence of an acyl group introduced at the C-3 hydroxyl of veracevine, as derived from both aromatic and aliphatic carboxylic acids. A limited number of esters of other hydroxyl groups of the parent compound are also considered, as well as some other derivatives. Specifically, this paper establishes the structure-activity relationships for the compounds in houseflies and milkweed bugs and for houseflies after pretreatment with PB such that oxidative detoxification is minimized. It also investigates their selective toxicity to insects compared with mice.

## MATERIALS AND METHODS

**Chemicals. General Procedures.** Proton and  $^{13}\text{C}$  NMR spectra were recorded at 300 and 75 MHz, respectively, on a Bruker WM-300 spectrometer for samples in deuteriochloroform with tetramethylsilane as the internal standard. Attached proton test (APT) spectra were obtained according to the method of Patt and Shooley (1982). IR spectra were measured in chloroform on a Perkin-Elmer 1600 series FTIR spectrophotometer. Electron impact mass spectrometry (MS) at 70 eV involved the direct insertion probe at 200 °C with a Hewlett-Packard 5985B system. Fast atom bombardment (FAB) spectra were obtained by using a Kratos MS-50 instrument (Department of Chemistry, University of California, Berkeley, CA). Melting points were determined on a Fisher-Jones apparatus and are uncorrected. Analytical and preparative TLC utilized silica gel plates (0.25 and 1 mm, respectively) and cyclohexane/ethyl acetate/triethylamine (7:2:1) for development. The compounds were visualized first under UV, then by exposure to iodine vapor, and finally by dipping the plate into an ethanolic solution of 3% vanillin containing 1%  $\text{H}_2\text{SO}_4$  and heating briefly at 110 °C. Preparative column and flash column chromatographies, the latter effected by using Pasteur pipets, were carried out on silica gel 60 (0.063–0.20 and 0.040–0.063 mm, respectively) with cyclohexane/ethyl acetate/triethylamine (100:5–30:1) for elution.

**Veracevine and Other Intermediates.** Alkaloids 1 and 2 originally present in commercial veratrine (Sigma Chemical Co., St. Louis, MO) in a 2:1 ratio were separated by column chromatography, as above. Veracevine (3) was obtained from veratrine by mild alkaline hydrolysis according to the method of Pelletier and Jacobs (1953). Cevine, the 3 $\alpha$ -epimer of 3, was prepared by alkaline isomerization of 3 (Kupchan et al., 1953; Pelletier and Jacobs, 1953). 4-Ethynylbenzoic acid, 3,4-dimethoxyphenyl isocyanate, and 2,3,3-trichloroacryloyl chloride were prepared as described by Havens and Hergenrother (1984), Brunner and Wöhr (1934), and Bergmann and Haskelberg (1941), respectively. Other acylating agents were obtained from commercial sources or prepared according to conventional procedures. Methylene chloride was distilled from  $\text{CaH}_2$ , and pyridine and triethylamine were dried over KOH pellets. CAUTIONARY NOTE: Appropriate care should be exercised in the handling of isocyanates and acid chlorides.

**3-Acyl Derivatives (Methods A–E) (See Tables I and II for the Reaction Conditions Used and Selected Characteristic Physical Properties of the Products).** Various 3-acyl derivatives were prepared from 3 either with the appropriate acid chloride in the presence of an acid scavenger or with the acid, directly, by dicyclohexylcarbodiimide (DCC) mediated coupling. Thus, 3 (45 mg, 0.088 mmol) was treated with the desired acid chloride (1.5–4 molar excess) under one of two conditions: method A, in pyridine; method B, in methylene chloride with pyridine or triethylamine as acid scavenger. Alternatively, 3 was reacted with the free acid (2.5–4 molar excess), DCC (2–4 molar excess), and a catalytic amount of 4-(dimethylamino)pyridine (4-DMAP) in pyridine (method C) or in methylene chloride and pyridine (method D). The reagents were mixed at 0 °C, and then the reaction mixture was stirred at room temperature until TLC monitoring indicated that most of the alcohol was consumed (8–48 h). In some cases an additional equivalent of acid chloride or acid plus DCC was needed for sufficient conversion of the

starting alcohol. For workup, the reaction mixture was poured into ice-cold aqueous  $\text{NH}_4\text{OH}$  solution (5 mL, pH 8), and then the product was extracted into chloroform ( $5 \times 15$  mL). The organic extracts were combined, washed with brine, dried ( $\text{MgSO}_4$ ), and concentrated, and the residue was purified by preparative TLC. After drying at 0.1 mmHg/100 °C for 1 h, the products were obtained as amorphous solids, >95% pure as indicated by TLC and  $^1\text{H}$  NMR. In particularly efficient acylations the initial workup steps were omitted and the reaction mixtures were separated directly by preparative TLC. For non-UV-visualizable aliphatic derivatives, the crude products were purified by flash pipet column chromatography, except for the chloroacetyl derivative (60) which was obtained directly by crystallization from ethyl ether/acetone. Special conditions (method E) were needed for some of the experiments. The 2,2-dimethylbutyryl ester (57) was only obtained via the anhydride, which had been prepared by a phase-transfer reaction (Plusquellec et al., 1988). Acylation with *N*-methylisatoic anhydride (Brown and Bradley, 1985) in benzene/tetrahydrofuran gave a mixture of the fluorescent C-3 acyl derivative (26) and its C-16 isomer, which were subsequently separated by repeated chromatography. The *N,N*-diethylaminoacetyl derivative (61) was synthesized by treating a benzene solution of the 2-chloroacetyl derivative (60) with excess diethylamine. Carbamates 45, 46, and 65 were prepared from the corresponding isocyanates in pyridine by using a catalytic amount of 4-DMAP.

**Other Acyl Derivatives (Method F) (Tables III).** 1 was treated with excess acetic anhydride in pyridine according to the method of Vojdšek et al. (1957) to give the 4,16-diacyl derivative (68). Cevadine *D*-orthoacetate (71) was synthesized as described previously (Stoll and Seebeck, 1952). Thus, intramolecular base-acid-catalyzed methanolysis (Kupchan et al., 1966) of cevadine 4,16-diacetate, prepared by acetylation of 2 with acetic anhydride/perchloric acid, yielded cevadine *D*-orthoacetate 4-monoacetate which, in turn, was subjected to mild alkaline hydrolysis at C-4 to give cevadine *D*-orthoacetate (71).

**Other Derivatizations (Method G) (Table III).**  $\text{OsO}_4$ -mediated Lemieux dihydroxylation of 2 with *N*-methylmorpholine *N*-oxide in aqueous acetonitrile in the presence of pyridine (Ray and Matson, 1980) afforded two diastereomers of the *vic*-dihydroxy derivative (62) which were not separated. Silylation of 3 with *tert*-butyldimethylsilyl chloride in methylene chloride containing triethylamine and a catalytic amount of 4-DMAP yielded the corresponding silyl ether derivative (66). Treatment of 1 with excess methyl iodide in benzene in a capped vial for 24 h at 60 °C afforded the *N*-methiodide (69), which was recrystallized from ether/acetone. Veratridine *N*-oxide (70) was prepared by briefly heating 1 with excess 30% aqueous hydrogen peroxide in methanol, as described previously for 3 (Pelletier and Jacobs, 1953), and purified by TLC.

**Bioassays.** Insecticidal activity was determined with adult female houseflies and adult milkweed bugs by topical application of the test compounds in acetone (0.5 and 1.0  $\mu\text{L}$  for the flies and bugs, respectively) to the ventrum of the abdomen. Synergized toxicity in flies was assayed by topical pretreatment on the abdomen with PB at 250  $\mu\text{g/g}$  1 h before application of the candidate insecticide. Treated flies in batches of 10 were held with sugar and water and treated bugs in groups of 2–4 with water for 24 h at 25 °C. The experiments were repeated three or four times with a 2-fold dose differential. The reproducibility of the assays was verified by using 1 as the internal standard in each series. The toxicity to mammals was determined with male albino Swiss-Webster mice (18–22 g) 24 h after intraperitoneal (ip) administration of a methoxytriglycol solution of the test compound.  $\text{LD}_{50}$  values were determined from log dose-probit mortality plots.

## RESULTS

**Structural Assignments and NMR Spectroscopy (Tables I–III).** In the assignment of structures to the new esters and related derivatives, the key issues were to confirm the introduction of the modifying group and to verify its position of attachment.  $^{13}\text{C}$  NMR and IR spectroscopy were particularly suited to the first point; specifically, the formation of the ester is indicated by the

**Table I. Physical Properties and Selected Spectroscopic Data for Veracevine Derivatives Containing a 3-Aromatic Ester or Related Substituent**

compd		preparation			IR $\nu_{\text{C=O}}$ , $\text{cm}^{-1}$	NMR ( $\text{CDCl}_3$ ), ppm		
no.	R at C-28	method <sup>a</sup>	yield, %	mp, $^{\circ}\text{C}$		H-3 <sup>b</sup>	C-3	C-28
Veratridine								
1	3,4-(MeO) <sub>2</sub> Ph <sup>c</sup>			170–175 <sup>d</sup>	1695	5.14	75.2	166.3
Unsubstituted Aroyl and Heteroaroyl								
4	phenyl	A	92	174–175	1710	5.12	75.7	166.9
5	1-naphthyl	C	73	170–171	1702	5.27	75.8	168.0
6	2-naphthyl	C	37	173–175	1710	5.24	75.9	167.4
7	2-thienyl	C	54	185–187	1701	5.11	75.9	162.7
8	2-pyrrolyl	D	21	188–190	1684	5.14	75.0	161.9
9	3-nicotinyl	C	28	225–227 dec	1718	5.21	76.1	165.6
Alkoxy- and Aryloxyaroyl								
10	2-MeOPh	B	38	146–147	1710	5.15	75.6	166.5
11	3-MeOPh	D	60	160–161	1706	5.16	75.8	166.9
12	4-MeOPh	C	40	168–169	1700	5.13	75.6	167.1
13	2,3-(MeO) <sub>2</sub> Ph	B	49	162–163	1710	5.16	75.9	167.0
14	2,4-(MeO) <sub>2</sub> Ph	B	12	140–142	1699	5.11	75.4	166.3
15	2,5-(MeO) <sub>2</sub> Ph	D	42	154–156	1700, 1716	5.14	75.8	166.5
16	3,5-(MeO) <sub>2</sub> Ph	D	50	164–166	1700	5.15	75.9	166.9
17	3,4,5-(MeO) <sub>3</sub> Ph <sup>c</sup>	A	21	233–235	1700	5.16	76.0	167.0
18	4-EtOPh	C	46	147–148	1698	5.12	75.5	167.5
19	4- <i>i</i> -PrOPh	C	60	170–172	1700	5.12	75.5	167.1
20	3-MeO,4-EtOPh	D	50	140–141	1698	5.14	75.6	167.1
21	3,5-(EtO) <sub>2</sub> Ph	D	64	140–141	1700	5.15	75.8	166.9
22	3-MeO,4-(HC $\equiv$ CCH <sub>2</sub> O)Ph	C	30	143–146	1700	5.15	75.7	166.9
23	3,4-(OCH <sub>2</sub> O)Ph	C	36	168–170	1710	5.11	75.7	166.6
24	3-PhOPh	C	52	166–171	1710	5.15	75.8	166.4
25	4-MeO-1-naphthyl	D	63	166–168	1700, 1716	5.22	75.4	167.8
Aminobenzoyl								
26	2-MeNHPh	E	35	168–169	1673	5.09	75.0	169.3
27	4-Me <sub>2</sub> NPh	C	18	156–158	1678	5.09	75.3	168.2
28	4-MeC(O)NHPh	B	50	201–202	1700	5.11	75.5	166.4
Other Substituted Benzoyl								
29	4-MePh	C	63	154–155	1703	5.14	75.7	167.3
30	3,5-Me <sub>2</sub> Ph	D	35	156–157	1700	5.15	75.7	167.7
31	4- <i>t</i> -BuPh	C	68	186–187	1702	5.15	75.6	167.2
32	2-ClPh	A	77	183–185	1722, 1711	5.18	76.4	166.2
33	4-ClPh	B	59	175–176	1711	5.16	75.9	166.1
34	3,4-Cl <sub>2</sub> Ph	A	32	175–176	1718	5.13	76.3	165.0
35	2,6-F <sub>2</sub> Ph	A	52	158–160	1720	5.18	obsc	161.6
36	F <sub>3</sub> Ph	A	29	150–151	1727	5.20	77.3	obsc
37	4-MeSPh	C	60	163–165	1709	5.14	76.6	166.9
38	4-HC $\equiv$ CPh	D	14	140–141	1711	5.16	76.0	166.4
39	4-N $\equiv$ CPh	A	45	235–236	1717	5.20	76.4	165.1
40	4-N <sub>3</sub> Ph	D	54	157–158	1700	5.15	75.8	166.3
Related Aromatic and Heterocyclic								
41	PhCH=CH	A	38	168–170	1698	5.05	75.4	167.8
42	3,4-(MeO) <sub>2</sub> PhCH=CH	C	38	153–154	1697	5.04	75.4	168.1
43	3,4-(MeO) <sub>2</sub> PhOCH <sub>2</sub>	C	10	153–154	1753	5.03	75.9	169.7
44	3,4-(MeO) <sub>2</sub> PhCH <sub>2</sub>	C	21	128–130	1725	4.92	75.5	172.5
Carbamates								
45	PhNH <sup>c</sup>	E	20	185–186	1723	4.88	76.1	154.1
46	3,4-(MeO) <sub>2</sub> PhNH	E	15	160–161	1716	4.86	76.2	154.5

<sup>a</sup> See Materials and Methods. <sup>b</sup>  $d, J = \sim 3.5\text{--}4.4$  Hz. <sup>c</sup> MS  $[M^+]$  673 for 1, 703 for 17, and 628 for 45. <sup>d</sup> Lit. 160–180 °C (Kupchan et al., 1953).

<sup>13</sup>C resonances for the newly introduced carbonyl group at 162–168 ppm for the aromatic and at 170–178 ppm for the aliphatic carbonyl carbons (C-28) and by an absorption at  $\sim 1680\text{--}1730$   $\text{cm}^{-1}$  in the IR spectra. In selected cases CI-MS or FAB-MS spectra were also recorded to further characterize the compounds. Relative to the position of derivatization, only three of the seven hydroxyl groups in the starting material (3) are readily accessible to acylating agents, and these have a distinct order of reactivity (C-3  $\gg$  C-16  $\gg$  C-4) (Narayanan, 1962). In addition, earlier <sup>1</sup>H NMR studies on *Veratrum* alkaloids (Itô et al., 1964) and related compounds (Möhrle et al., 1968), and more importantly the recent complete <sup>13</sup>C and <sup>1</sup>H assignments of 1 and 2 (Krishnamurthy and Casida, 1988), provide the

necessary information for assigning the key resonances in the present compounds.

For acyl or other derivatives 4–68, changes with regard to the spectra of starting materials 1, 2, or 3 are only seen in the chemical shifts of those protons and carbon atoms and their neighbors, which are affected by the particular reaction. The chemical shifts of the other protons and carbons show differences of only  $\pm 0.01$  and  $\pm 0.2$  ppm, respectively. For example, in the parent alkaloid 3, protons at C-3 and C-16 resonate as a doublet at 3.74 ppm ( $J = 4$  Hz) and as an apparent triplet at 4.15 ppm ( $J = 3$  Hz), respectively. Acylation at C-3 is characterized by a downfield shift of the C-3 proton to  $\sim 5.1$  and  $\sim 4.9$  ppm for aromatic and aliphatic esters, respectively. Acylation at

**Table II. Physical Properties and Selected Spectroscopic Data for Veracevine Derivatives Containing a 3-Aliphatic Ester or Related Substituent**

compd		preparation			IR $\nu_{C=O}$ , $\text{cm}^{-1}$	NMR ( $\text{CDCl}_3$ ), ppm		
no.	R at C-28	method <sup>a</sup>	yield, %	mp, $^{\circ}\text{C}$		H-3 <sup>b</sup>	C-3	C-28
Cevadine								
2 <sup>c</sup>	( <i>Z</i> )-MeCH=CMe			209–210 <sup>d</sup>	1698	5.00	74.5	167.8
2,3-Alkenoyl								
47	( <i>E</i> )-MeCH=CMe	C	60	230–232 <sup>e</sup> dec	1697	4.97	75.2	168.6
48	Me <sub>2</sub> C=CH	C	40	234–245 dec	1692	4.93	74.5	167.5
49	Cl <sub>2</sub> C=CCl	B	14	177–179	1725	5.05	77.5	160.5
Alkanoyl and Cycloalkanoyl								
50	Me	A	69	196–200 <sup>f</sup>	1724	4.91	75.1	171.9
51	<i>n</i> -Pr	A	68	234–235 dec	1719	4.90	74.9	174.5
52	<i>i</i> -Pr	A	71	153–154	1720	4.92	74.8	177.8
53	<i>t</i> -Bu	A	32	144–146	1716	4.90	74.9	176.0
54	<i>c</i> -Pr	B	38	170–171	1710	4.90	75.3	176.0
55	<i>c</i> -Pen	A	24	245–246 dec	1716	4.91	74.8	177.7
56	( $\pm$ )- <i>s</i> -Bu <sup>g</sup>	A	23	196–198 <sup>h</sup>	1716	4.94	74.8	177.6, 177.8
57	CH <sub>3</sub> CH <sub>2</sub> CMe <sub>2</sub>	E	30	153–155	1710, 1722	4.92	74.7	178.9
58	Me <sub>3</sub> CCH <sub>2</sub>	A <sup>i</sup>	50	201–202	1711, 1721	4.94	74.8	173.4
59	( <i>Z</i> )-Me(CH <sub>2</sub> ) <sub>7</sub> CH=CH(CH <sub>2</sub> ) <sub>7</sub>	C	51	68–70	1722	4.92	75.0	174.9
Related Alkanoyl Derivatives								
60	ClCH <sub>2</sub>	B	80	214–215 dec	1726, 1754	5.00	76.6	167.5
61	Et <sub>2</sub> NCH <sub>2</sub>	E	53	124–125	1727	4.96	75.0	172.2
62	( $\pm$ )-MeCH(OH)C(OH)Me <sup>g</sup>	G	12	178–180	1726	5.01	76.2, 76.4	175.1, 175.6
63	(1 <i>R</i> , <i>cis</i> )-3-(Br <sub>2</sub> C=CH)-2,2-Me <sub>2</sub> - <i>c</i> -Pr	A	56	151–152	1710	4.85	75.3	171.1
64	<i>i</i> -PrO	B	64	154–155, 224 dec	1726	4.75	78.2	155.2
65	<i>t</i> -BuNH	E	16	166–167	1700	4.69	75.3	156.8
3- <i>O</i> -Silyl Ether								
66	<i>t</i> -BuMe <sub>2</sub> Si <sup>j</sup>	G	24	169–171		3.68	74.2	

<sup>a</sup> See Materials and Methods. <sup>b</sup> d,  $J = \sim 3.5$ –4.4 Hz. <sup>c</sup> MS [ $M^+$ ] 591. <sup>d</sup> Lit. 209–211 °C (Kupchan et al., 1953). <sup>e</sup> Changed crystal form at  $\sim 160$  °C. <sup>f</sup> Lit. 205–207 °C (Kupchan et al., 1953). <sup>g</sup> Mixture of two stereoisomers. <sup>h</sup> Lit. 198–200 °C (Vejdšek et al., 1957). <sup>i</sup> With catalytic 4-DMAP. <sup>j</sup> IR 838  $\text{cm}^{-1}$  (SiOC), <sup>13</sup>C NMR 18.0 ppm (SiCC).

**Table III. Physical Properties and Selected Spectroscopic Data for Other Veratridine and Cevadine Derivatives**

no.	compd	preparation			IR $\nu_{C=O}$ , $\text{cm}^{-1}$	MS [ $M^+$ ]	NMR ( $\text{CDCl}_3$ ), ppm		
		method <sup>a</sup>	yield, %	mp, °C			H-3 <sup>b</sup>	C-3	C-28
67	3 $\alpha$ -epiveratridine	A <sup>b</sup>	41	148–149 <sup>c</sup>	1710	673	4.95 <sup>d</sup>	76.5	165.8
68	4,16-diacetylveratridine	F	50	163–165	1716, 1735, 1748	757	5.95 <sup>e</sup>	70.5	165.1
69	veratridine <i>N</i> -methiodide	G	58	208–210	1700	<i>f</i>	5.06 <sup>g</sup>	76.1 <sup>g</sup>	166.1 <sup>g</sup>
70	veratridine <i>N</i> -oxide	G	82	192–194	1692	<i>h</i>	5.14	75.1	167.0
71	cevadine 12,14,17-orthoacetate	F	81	220–221 <sup>i</sup>	1700	615	5.08	75.1	168.3

<sup>a</sup> See Materials and Methods. <sup>b</sup> From cevine. <sup>c</sup> Lit. 147–149 °C (Vejdšek and Trčka, 1955). <sup>d</sup> dd,  $J_{2e,3} = 5.7$  Hz;  $J_{2a,3} = 9.2$  Hz. <sup>e</sup> d,  $J = 3.5$  Hz. <sup>f</sup> [ $M - 1$ ]<sup>+</sup> 689 (FAB). <sup>g</sup> Acetone-*d*<sub>6</sub>. <sup>h</sup> [ $M + 1$ ]<sup>+</sup> 690 (FAB). <sup>i</sup> Lit. 241–245 °C dec (Stoll and Seebeck, 1952).

C-16 involves a shift of the C-16 proton to  $\sim 5.3$  ppm. In the diacylated derivative of 1, the C-3 proton appears at  $\sim 5.9$  ppm as a doublet ( $J = 3.5$  Hz). The coupling constants for each of the <sup>1</sup>H resonances in question are not affected substantially, indicating that the conformation of the parent compound is maintained. Less significant but still characteristic shifts are seen in the corresponding <sup>13</sup>C data for the C-3 carbon, which is deshielded by 0.6–3.6 ppm depending on the acyl moiety. The <sup>13</sup>C shifts at C-16 and C-4 are less useful in this context. It is noteworthy that the NMR studies can be used to substantiate the earlier results on the relative reactivities of the various hydroxyl groups; reactions with even 3-fold excess of the acylating agent gave products regioselectively acylated at the C-3 hydroxyl with only traces of the C-4- or C-16-monoacyl products.

In the two esters derived from chiral acylating agents, the <sup>13</sup>C resonances of the acyl moiety are distinct. Thus, for the diastereomers of ester 56, the resonances of the acyl moiety appear at 177.6 and 177.8 ppm for C-28, 41.1 and 41.2 ppm for  $\alpha$ -C, 26.8 and 26.9 ppm for  $\beta$ -C, 16.4 and 16.5 ppm for  $\beta'$ -C, and 11.4 and 11.5 ppm for  $\gamma$ -C. Moreover, in the dihydroxy ester 62, obtained by cis-dihydroxylation of the double bond of 2, in addition to the

various carbon atoms of the acyl group appearing as distinct resonances, the diastereomeric C-3 carbons are also observed (76.2 and 76.4 ppm).

**Other Derivatives.** In the <sup>1</sup>H spectrum of 3 $\alpha$ -epi-veratridine (67) the axial C-3 proton appears as a characteristic double doublet ( $J_{2e,3} = 5.7$  Hz,  $J_{2a,3} = 9.2$  Hz) at 4.95 ppm. Relative to 1, the <sup>13</sup>C spectrum of its epimer 67 shows several distinct features; i.e., C-3 is deshielded by 1.3 ppm (from 75.2 to 76.5 ppm), C-4 is shielded by 1.3 ppm (from 77.8 to 76.5 ppm), and C-1 and C-5 are deshielded by 1.9 and 4.4 ppm, respectively, presumably due to the  $\gamma_{\text{gauche}}$  effect.

4,16-Diacetylveratridine (68) formation is indicated by the appearance of two methyl singlets at 2.01 and 2.09 ppm in the <sup>1</sup>H spectrum and signals at 21.6, 21.8, 165.1, and 168.8 ppm in the <sup>13</sup>C spectrum. For compounds 69, 70, and 71, conventional one-dimensional NMR techniques were not sufficient for unambiguous assignments of the resonances of interest in the proton and carbon NMR spectra. However, on the basis of the results of earlier studies with related systems or model compounds (Itô et al., 1964; Möhrle et al., 1968) as well as APT, <sup>1</sup>H–<sup>1</sup>H COSY, and one-bond <sup>1</sup>H–<sup>13</sup>C COSY experiments, the relevant <sup>1</sup>H and <sup>13</sup>C NMR signals of these derivatives could be



identified. Supporting evidence for the structural assignments is provided by the IR and MS spectra.

Two derivatives were prepared involving modifications at nitrogen. In methiodide 69, the quaternary *N*-methyl resonance is observed as a singlet at 3.54 and 54.2 ppm in the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra, respectively. In addition, the C-18, C-22, and C-26 resonances are all shifted downfield by 9.6, 3.5, and 10.2 ppm with regard to those of 1 (51.2, 63.3, and 61.0 ppm, respectively). In agreement with results of  $^1\text{H}$  NMR studies with quinolizidine model systems related to cevine (Möhrle et al., 1968), both the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of 69 suggest that quaternization of the nitrogen atom of 1 proceeds with inversion at nitrogen. Veratridine *N*-oxide (70) is characterized by a band at 947  $\text{cm}^{-1}$  in its IR spectrum and a deshielding of the resonances at C-18, C-22, and C-26 by 15.6, 1.4, and 11.5 ppm, respectively, relative to 1 (see above).

The last compound considered here is cevadine 12,14,17-orthoacetate (71). The orthoacetate functionality is indicated by a three-proton singlet at 1.57 ppm in its  $^1\text{H}$  spectrum and the characteristic COC bands at  $\sim 1140\text{ cm}^{-1}$  in the IR spectrum. Formation of the cage structure requires conformational changes in the D-ring from chair to twist-boat, as noted earlier (Itô et al., 1964), which are reflected in the  $^1\text{H}$  spectrum of 71 relative to that of 2, e.g., by downfield shifts of the singlet of the C-21 protons (to 1.21 from 1.11 ppm) and of the triplet of the C-16 proton (to 4.27 from 4.12 ppm) as well as a change in its coupling constant (to 7.5 from 3.1 Hz). The  $^{13}\text{C}$  spectrum shows characteristic changes of the chemical shifts of the C-12–C-22 carbons. For example, the chemical shifts for C-12 and C-14 in 71 are 89.3 and 85.7 ppm (81.5 and 80.5 ppm, respectively, for the corresponding carbon atoms in 2) while C-16 resonates at 71.2 ppm (70.7 ppm for 2). The signals appearing at 74.8 and 75.6 ppm are assigned to quaternary carbons C-17 and C-20.

**Insecticidal Activity. General.** Forty-four of the 73 compounds examined show significant insecticidal activity to either houseflies or milkweed bugs or to both. The most active veracevine esters with aromatic acids and related acyl groups are shown in Table IV and with aliphatic acyl groups and related substituents are given in Table V. The parent alcohol (3) shows no toxicity to either houseflies or milkweed bugs (no kill at 50 and 17  $\mu\text{g/g}$ , respectively), indicating that the 3-hydroxyl group must be esterified for insecticidal activity (Table VI). In general, the toxicity to houseflies of both the natural esters 1 and 2 as well as the active synthetic analogues is synergized 2- and 15-fold with PB.

**3-Aromatic Ester and Related Derivatives of Veracevine (Tables I and IV).** The natural product 1 is among the most active compounds with  $\text{LD}_{50}$ s of 6.5 and 2.6  $\mu\text{g/g}$  for houseflies with PB and milkweed bugs, respectively. The most active analogues are the 2,5- and particularly the 3,5-dimethoxybenzoates 15 and 16, respectively. The potency of 13 other compounds to one or more frequently to both species is within 2-fold that of 1, i.e., 1-naphthoate 5, 2- and 3-methoxybenzoates 10 and 11, various di- and trialkoxybenzoates 13, 14, 17, and 20–22, methoxynaphthoate 25, 3,5-dimethylbenzoate 30, dimethoxyphenyl acetate 44, and phenyl carbamate 45. Many other 3-aromatic esters and related derivatives of each type are less active insecticides.

**3-Aliphatic Esters and Related Derivatives of Veracevine and Protoveratrine A and B (Tables II and V).** The natural product 2 is overall the most active aliphatic ester derivative of 3 examined ( $\text{LD}_{50}$  of 10  $\mu\text{g/g}$  for houseflies with PB and 0.51  $\mu\text{g/g}$  with milkweed bugs).

**Table IV. Insecticidal Activity of Veracevine Derivatives Containing a 3-Aromatic Ester or Related Substituent**

compd <sup>a</sup>		LD <sub>50</sub> , μg/g		
		housefly		
no.	R at C-28	no PB	+PB	milkweed bug
Veratridine				
1	3,4-(MeO) <sub>2</sub> Ph	18	6.6	2.6
Unsubstituted Aryol				
4	phenyl	>50	18	>17
5	1-naphthyl	50	3.4	5.8
6	2-naphthyl	50	24	>17
Alkoxy and Aryloxyaryl				
10	2-MeOPh	>50	7.6	2.2
11	3-MeOPh	30	4.0	5.8
12	4-MeOPh	33	13	>17
13	2,3-(MeO) <sub>2</sub> Ph	>50	14	5.1
14	2,4-(MeO) <sub>2</sub> Ph	32	7.5	11
15	2,5-(MeO) <sub>2</sub> Ph	13	2.3	1.5
16	3,5-(MeO) <sub>2</sub> Ph	9.4	1.4	0.51
17	3,4,5-(MeO) <sub>3</sub> Ph	34	17	4.3
18	4-EtOPh	>50	27	>17
19	4- <i>i</i> -PrOPh	>50	29	>17
20	3-MeO-4-EtOPh	27	11	5.1
21	3,5-(EtO) <sub>2</sub> Ph	10	3.0	5.8
22	3-MeO-4-(HC≡CCH <sub>2</sub> O)Ph	50	7.0	5.9
23	3,4-(OCH <sub>2</sub> O)Ph	45	17	>17
24	3-PhOPh	>50	50	>17
25	4-MeO-1-naphthyl	50	9.0	4.1
Aminobenzoyl				
26	2-MeNHPPh	>50	23	>17
27	4-Me <sub>2</sub> NPh	23	13	8.8
Other Substituted Benzoyl				
29	4-MePh	>50	15	>17
30	3,5-Me <sub>2</sub> Ph	50	5.5	3.2
32	2-ClPh	>50	33	>17
35	2,6-F <sub>2</sub> Ph	>50	20	>17
36	F <sub>3</sub> Ph	23	23	>17
40	4-N <sub>3</sub> Ph	>50	14	17
Related Aromatic and Heterocyclic				
42	( <i>E</i> )-3,4-(MeO) <sub>2</sub> PhCH=CH	>50	>50	9.7
44	3,4-(MeO) <sub>2</sub> PhCH <sub>2</sub>	>50	>50	3.6
Carbamates				
45	PhNH	45	>50	4.3

<sup>a</sup> Analogues with  $\text{LD}_{50} > 50\text{ }\mu\text{g/g}$  with PB for housefly and  $\text{LD}_{50} > 17\text{ }\mu\text{g/g}$  for milkweed bug are 7–9, 28, 31, 33, 34, 37–39, 41, 43, and 46.

Comparison of 2, 47, and 48 indicates that the configuration of the double bond in the acyl group is more important in the toxicity to milkweed bugs than to houseflies. The *tert*-butyl or equivalent moieties appear to be important in conferring activity since they occur in the other more active aliphatic esters (53, 57, and 58) and a carbamate (65) of 3.

Protoveratrine A with the monohydroxymethylbutyryl substituent is the most potent aliphatic ester examined. The protoveratrine has the same steroid skeleton as the veracevine series but differ in their hydroxylation pattern. However, the importance of the acyl moiety is again illustrated since protoveratrine B with the dihydroxymethylbutyryl substituent is much less active.

**Other Derivatives (Table III).** The 3-epimer (67), 4,16-diacetyl derivative (68), *N*-methyl derivative (69), and *N*-oxide derivative (70) of 1 and the orthoester derivative (71) of 2 were not active at the maximum dose tested (i.e., no kill at 50  $\mu\text{g/g}$  for houseflies alone or with PB or 17  $\mu\text{g/g}$  for milkweed bugs).

**Selective Toxicity of Veratrum Alkaloids and Analogues (Table VI).** The natural esters 1 and 2 are both highly toxic to insects and mice relative to their hy-

**Table V. Insecticidal Activity of Veracevine Derivatives Containing a 3-Aliphatic Ester or Related Substituent and of Protoveratrines A and B**

no.	compd <sup>a</sup> R at C-28	LD <sub>50</sub> , µg/g		
		housefly		milkweed bug
		no PB	+PB	
2	(Z)-MeCH=CMe	Cevadine >50	10	0.51
		2,3-Alkenoyl		
47	(E)-MeCH=CMe	>50	13	1.7
48	Me <sub>2</sub> C=CH	>50	14	3.1
		Alkanoyl and Cycloalkanoyl		
50	Me	>50	16	>17
52	i-Pr	>50	25	>17
53	t-Bu	>50	9.4	4.1
45	(±)-s-Bu	>50	15	17
57	CH <sub>3</sub> CH <sub>2</sub> CMe <sub>2</sub>	>50	13	5.8
58	Me <sub>3</sub> CCH <sub>2</sub>	>50	22	3.2
		Others		
64	i-PrO	>50	30	>17
65	t-BuNH	>50	23	4.9
		Related <i>Veratrum</i> Alkaloids		
	protoveratrine A	33	1.6	2.2
	protoveratrine B	>50	10	

<sup>a</sup> Analogues with LD<sub>50</sub> > 50 µg/g with PB for housefly and LD<sub>50</sub> > 17 µg/g for milkweed bug are 49, 51, 54, 55, 59–63, and 66.

**Table VI. Selective Toxicity of *Veratrum* Alkaloids and Analogues**

compd <sup>a</sup>	mouse ip LD <sub>50</sub> , mg/kg	LD <sub>50</sub> ratio, mouse/insect	
		housefly with PB	milkweed bug
veratridine (1)	9.0	1.4	3.5
cevadine (2)	5.8	0.6	11
3-(3,5-dimethoxy- benzoyl)veracevine (16)	7.5	5.4	15
3-pivaloylveracevine (53)	8.3	0.9	2.0
protoveratrine A	0.20	0.1	0.09

<sup>a</sup> Veracevine (3) is not toxic at the discriminating levels in this study, i.e., mouse ip LD<sub>50</sub> > 100 mg/kg, housefly LD<sub>50</sub> > 50 µg/g alone or with PB, and milkweed bug LD<sub>50</sub> > 17 µg/g.

drolisis product 3. In a comparison of potent aliphatic esters, the synthetic analogue 53 is not improved relative to the natural 2 in their selective toxicity to PB-treated houseflies and to milkweed bugs vs ip-treated mice. Interestingly, in the aromatic esters, synthetic 16 (the most potent insecticide prepared) is greatly enhanced in selective toxicity relative to natural 1. Protoveratrine A is favorable in its insecticidal activity but not in its selective toxicity.

## DISCUSSION

The neurotoxicity of the *Veratrum* alkaloids and related compounds is attributable to their modification of the properties of the Na<sup>+</sup> channel. Alkaloid 1 and the structurally related batrachotoxin, aconitine, and grayanotoxin activate receptor site 2 of the voltage-dependent Na<sup>+</sup> channel in excitable membranes by prolonging its open state (Catterall, 1980, 1988; Coddington, 1983; Kosower, 1983). Compounds 1 and 2 and 3-(4-ethoxybenzoyl)veracevine (compound 18 in the present study) modify Na<sup>+</sup> channels in the same way (Leibowitz et al., 1987). On the basis of the effects of these highly hydroxylated neurotoxins on <sup>22</sup>Na<sup>+</sup> influx, [<sup>3</sup>H]saxitoxin and [<sup>3</sup>H]batrachotoxin A 20α-benzoate binding, and electrophysiological properties, the receptor binding site differs from that of

the pyrethroids (Jacques et al., 1980; Pauron et al., 1989). Cross-resistance relationships support these differences; i.e., resistance to pyrethroids in Super *kdr* houseflies extends to aconitine (Bloomquist and Miller, 1986) but not to batrachotoxin (Pauron et al., 1989) or 1 (Bloomquist and Soderlund, 1988).

As for the pyrethrins (Elliott, 1989), 1 and 2 are botanical insecticides containing an ester functionality and in which the acyl group is a significant determinant of the biological activity (Bergmann et al., 1958; Leibowitz et al., 1987; Vejdělek and Trčka, 1955). A similar situation extends to batrachotoxin, the arrow poison from frogs (Warnick et al., 1975). The ryanoid-type esters, however, provide an interesting contrast since in ryanodine the pyrrolecarboxylate group is necessary for mammalian toxicity and binding at the Ca<sup>2+</sup>-activated Ca<sup>2+</sup> channel but is not a prerequisite for insecticidal activity (Waterhouse et al., 1987). These considerations provided the impetus to evaluate in a systematic way the influence of the acyl group in derivatives of the *Veratrum* alkaloids and to determine if the naturally occurring esters confer optimal insecticidal activity and selective toxicity. The present study establishes that aromatic esters of 3 with electron-rich substituents at the ortho or meta positions and especially methoxy group(s) result in both more potent and selective insecticides. Results with two related polyester steroid alkaloids, protoveratrines A and B, show that the 3-(dihydroxymethyl)butyryl compound A is about 6 times more toxic to houseflies than its (monohydroxymethyl)butyryl analogue B. In contrast, a similar dihydroxy ester of veracevine, synthetic derivative 62, is inactive, indicating that the substituents on the steroid skeleton play a decisive role in activity. Investigations focusing on the steroid nucleus are needed if the minimal structural requirements for selective insecticidal activity are to be established.

Although the *veratrum* alkaloids played an early role in medicine, they were known to be acutely toxic in mammals (Swiss and Bauer, 1951; Vejdělek and Trčka, 1955). In addition, they have an unfavorable therapeutic ratio (antihypertensive vs emetic effect). Attempts to improve the hypotensive activity and safety led to detail structure-activity investigations (Kupchan and Flacke, 1967; Green et al., 1985) but did not yield practically useful analogues. In the present study, which emphasizes insecticidal activity, the observed variations in structure-activity relationships among houseflies, milkweed bugs, and mice suggest species differences in their metabolism or pharmacokinetics or in their Na<sup>+</sup> channel receptor sites. A portion of the species variations in sensitivity is probably due to oxidative detoxification as evident by the PB synergism ratio of 2–15-fold for different compounds with houseflies.

The discovery of more potent insecticidal analogues with improved selectivity (e.g., 16 of this study) is a prelude to and provides background information for the differentiation of the relative importance of metabolism and receptor site sensitivity as determinants of species specificity.

## ABBREVIATIONS USED

APT, attached proton test; DCC, dicyclohexylcarbodiimide; 4-DMAP, 4-(dimethylamino)pyridine; FAB, fast atom bombardment; ip, intraperitoneal; MS, mass spectrometry; PB, piperonyl butoxide; Me, methyl; Et, ethyl; Pr, propyl; Bu, butyl; Pen, pentyl; Ph, phenyl or substituted phenyl; *n*, normal; *i*, iso; *s*, secondary; *t*, tertiary; *c*, cyclo.

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## LITERATURE CITED

- Allen, T. C.; Link, K. P.; Ikawa, M.; Brunn, L. K. The Relative Effectiveness of the Principal Alkaloids of Sabadilla Seed. *J. Econ. Entomol.* 1945, 38, 293-296.
- Barton, D. H. R.; Jeger, O.; Prelog, V.; Woodward, R. B. The Constitutions of Cevine and Some Related Alkaloids. *Experientia* 1954, 10, 81-90.
- Bergmann, E. D.; Levinson, Z. H.; Mechoulam, R. The Toxicity of *Veratrum* and *Solanum* Alkaloids to Housefly Larvae. *J. Insect Physiol.* 1958, 2, 162-177.
- Bergmann, F.; Haskelberg, L. Synthesis of Lipophilic Chemotherapeutics. III. Remarks on the Properties of Halogenacylureas, Amides and Related Compounds. *J. Am. Chem. Soc.* 1941, 63, 1437-1439.
- Bloomquist, J. R.; Miller, T. A. Sodium Channel Neurotoxins as Probes of the Knockdown Resistance Mechanism. *Neurotoxicology* 1986, 7, 217-224.
- Bloomquist, J. R.; Soderlund, D. M. Pyrethroid Insecticides and DDT Modify Alkaloid-Dependent Sodium Channel Activation and Its Enhancement by Sea Anemone Toxin. *Mol. Pharmacol.* 1988, 33, 543-550.
- Blum, M. S.; Kearns, C. W. The Effect of Pyrethrum Activators on the Toxicity of Sabadilla to House Flies. *J. Econ. Entomol.* 1956, 49, 283.
- Brown, G. B.; Bradley, R. J. Batrachotoxinin-A N-Methylanthranilate, a New Fluorescent Ligand for Voltage-Sensitive Sodium Channels. *J. Neurosci. Methods* 1985, 13, 119-129.
- Brunner, O.; Wöhr, R. *Monatsh. Chem.* 1934, 63, 374-384.
- Catterall, W. A. Neurotoxins That Act on Voltage-Sensitive Sodium Channels in Excitable Membranes. *Annu. Rev. Pharmacol. Toxicol.* 1980, 20, 15-43.
- Catterall, W. A. Structure and Function of Voltage-Sensitive Ion Channels. *Science* 1988, 242, 50-61.
- Codding, P. W. Structural Studies of Sodium Channel Neurotoxins. 2. Crystal Structure and Absolute Configuration of Veratridine Perchlorate. *J. Am. Chem. Soc.* 1983, 105, 3172-3176.
- Crosby, D. G. Minor Insecticides of Plant Origin. In *Naturally Occurring Insecticides*; Jacobson, M., Crosby, D. G., Eds.; Dekker: New York, 1971; pp 177-239.
- Elliott, M. The Pyrethroids: Early Discovery, Recent Advances and the Future. *Pestic. Sci.* 1989, 27, 337-351.
- Green, M. J.; Hatch, R. P.; Saskena, A. K. Neogermine 7,9,14-Orthoesters: Synthesis and Use in the Preparation of Hypotensive Germine 2,7,15-Triesters. *Tetrahedron* 1985, 41, 1155-1164.
- Havens, S. J.; Hergenrother, P. M. Ethynyl-Terminated Polyarylates: Synthesis and Characterization. *J. Polym. Sci., Polym. Chem. Ed.* 1984, 22, 3011-3025.
- Ikawa, M.; Dicke, R. J.; Allen, T. C.; Link, K. P. The Principal Alkaloids of Sabadilla Seed and Their Toxicity to *Musca domestica* L. *J. Biol. Chem.* 1945, 159, 517-524.
- Itô, S.; Stothers, J. B.; Kupchan, S. M. Nuclear Magnetic Resonance Spectra of *Veratrum* Alkaloids. *Tetrahedron* 1964, 20, 913-928.
- Jacques, Y.; Romey, G.; Cavey, M. T.; Kartalovski, B.; Lazdunski, M. Interaction of Pyrethroids with the Na<sup>+</sup> Channel in Mammalian Neuronal Cells in Culture. *Biochim. Biophys. Acta* 1980, 600, 882-897.
- Kosower, E. M. A Hypothesis for the Mechanism of Sodium Channel Opening by Batrachotoxin and Related Toxins. *FEBS Lett.* 1983, 163, 161-164.
- Krishnamurthy, V. V.; Casida, J. E. Complete Spectral Assignments of Cevadine and Veratridine by 2D NMR Techniques. *Magn. Reson. Chem.* 1988, 26, 980-989.
- Kupchan, S. M.; By, A. W. Steroid Alkaloids: The *Veratrum* Group. In *The Alkaloids: Chemistry and Physiology*; Manske, R. H. F., Ed.; Academic Press: New York, 1968; Vol. X, pp 193-285.
- Kupchan, S. M.; Flacke, W. E. Hypotensive *Veratrum* Alkaloids. In *Antihypertensive Agents*; Schlittler, E., Ed.; Academic Press: New York, 1967; pp 429-458.
- Kupchan, S. M.; Lavie, D.; Deliwala, C. V.; Andoh, B. Y. A. *Schoenocaulon* Alkaloids. I. Active Principles of *Schoenocaulon officinale*. Cevacine and Protocevane. *J. Am. Chem. Soc.* 1953, 75, 5519-5524.
- Kupchan, S. M.; Johnson, W. S.; Rajagopalan, S. The Configuration of Cevine. *Tetrahedron* 1959, 7, 47-61.
- Kupchan, S. M.; Eriksen, S. P.; Liang, Y.-T. S. Intramolecular Catalysis. IX. Intramolecular General Base-General Acid Catalysis of Ester Solvolysis. *J. Am. Chem. Soc.* 1966, 88, 347-350.
- Leibowitz, M. D.; Schwarz, J. R.; Holan, G.; Hille, B. Electrophysiological Comparison of Insecticide and Alkaloid Agonists of Na Channels. *J. Gen. Physiol.* 1987, 90, 75-93.
- Möhrle, H.; Karl, C.; Scheidegger, U. *Tetrahedron* 1968, 24, 6813-6824.
- Narayanan, C. R. Newer Developments in the Field of *Veratrum* Alkaloids. *Fortschr. Chem. Org. Naturst.* 1962, 20, 298-371.
- Patt, S. L.; Shoolery, J. N. Attached Proton Test for Carbon-13 NMR. *J. Magn. Reson.* 1982, 46, 535-539.
- Pauron, D.; Barhanin, J.; Amichot, M.; Pralavorio, M.; Bergé, J.-B.; Lazdunski, M. Pyrethroid Receptor in the Insect Na<sup>+</sup> Channel: Alteration of Its Properties in Pyrethroid-Resistant Flies. *Biochemistry* 1989, 28, 1673-1677.
- Pelletier, S. W.; Jacobs, W. A. The Veratrine Alkaloids. XXXV. Veracevine, the Alkanolamine of Cevadine and Veratridine. *J. Am. Chem. Soc.* 1953, 75, 3248-3252.
- Plusquellec, D.; Roulleau, F.; Lefevre, M.; Brown, E. A New Synthesis of Carboxylic and Carbonic Acid Anhydrides Using Phase Transfer Reactions. *Tetrahedron* 1988, 44, 2471-2476.
- Ray, R.; Matteson, D. S. Osmium Tetroxide Catalyzed Hydroxylation of Hindered Olefins. *Tetrahedron Lett.* 1980, 21, 449-450.
- Stoll, A.; Seebeck, E. *Helv. Chim. Acta* 1952, 35, 1942-1954.
- Swiss, E. D.; Bauer, R. O. Acute Toxicity of *Veratrum* Derivatives. *Proc. Soc. Exp. Biol. Med.* 1951, 76, 847-849.
- Vejdšek, Z. J.; Trčka, V. *Chem. Listy* 1955, 49, 529-538.
- Vejdšek, Z. J.; Macek, K.; Buděšínský, B. *Collect. Czech. Chem. Commun.* 1957, 22, 98-119.
- Warnick, J. E.; Albuquerque, E. X.; Onur, R.; Jansson, S.-E.; Daly, J.; Tokuyama, T.; Witkop, B. The Pharmacology of Batrachotoxin. VII. Structure-Activity Relationships and the Effects of pH. *J. Pharmacol. Exp. Ther.* 1975, 193, 232-245.
- Waterhouse, A. L.; Pessah, I. N.; Francini, A. O.; Casida, J. E. Structural Aspects of Ryanodine Action and Selectivity. *J. Med. Chem.* 1987, 30, 710-716.

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