Exploring the Structural Basis of Neurotoxicity in C_{17}-Polyacetylenes Isolated from Water Hemlock

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Received April 27, 2000

Water hemlock, *Cicuta virosa*, belonging to the Umbelliferae, is well-known as a toxic plant responsible for lethal poisonings in humans as well as animals, causing tonic and clonic convulsions and respiratory paralysis. Cicutoxin (**1**), being a major violent toxin of the plant, is a chemical in the class of C_{17}-polyacetylenes bearing a long π-bond conjugation system, a terminal hydroxyl, and an allylic hydroxyl in its structure, and a variety of its analogues have been isolated from the plant. In the present study, various derivatives of these toxins were synthesized through acetylation, methylation, and oxidation of cicutoxin (**1**) and virol A (**3**) and B (**4**). 1-Dehydroxyvirol A (**28**) was prepared through the coupling of (7*S*)-dodeca-3,5-dien-1-yn-7-ol and 1-iodopentyne under Sonogashira's conditions. A monoacetylenic compound (**29**) was also prepared through the coupling of (5*S*)-1-chlorodeca-1,3-dien-5-ol and 1-iodopentyn-5-ol. The structure−activity relationships involved in the acute toxicity of cicutoxin derivatives in mice were investigated, and the length and geometry of π-bond conjugation and the *O*-functional groups were found to be important for activity. The potency in inhibition of the specific binding of the noncompetitive GABA antagonist, [^{3}H]EBOB, to GABA-gated Cl^- channels of GABA receptors in rat brain cortex was found to be correlated with acute toxicity, indicating that the ability to bind to these channels plays an important role in the acute toxicity of these compounds.

Introduction

Neurotoxins previously isolated from animals are mainly peptides of considerable length, consisting of 60−125 amino acid residues, whereas those from plants and marine organisms are small molecules exhibiting a wide variety of different chemical features such as alkaloids, steroids, and compounds with more complex structures.[1] As has occurred in the past with tetrodotoxin from the Japanese fugu,[2] batrachotoxin from the Colombian arrow poison frog,[3] and kainic acid from red alga,[4] neurotoxins have progressively emerged as tools applied in neurochemistry with the aim of acquiring a better understanding of biological mechanisms.

Cicutoxin (**1**), a major toxic principle of water hemlock (*Cicuta virosa*), a famous poisonous Umbelliferous plant, is a chemical belonging to the class of conjugated polyacetylenes, and it is thought to be produced from oleic acid in a biotransformation pathway involving oxidation and decarboxylation reactions.[5] The cicutoxin (**1**) molecule bears the following functional groups: (i) a hydroxyl group and an allylic hydroxyl group at C-1 and C-14, respectively, (ii) a diacetylene group, and (iii) an *all-E*-triene group conjugated with the diacetylene. Regarding the pharmacological properties of cicutoxin (**1**), it is well-known to act directly on the central nervous system, causing tonic and clonic convulsions and respiratory paralysis.[6] Even though cicutoxin (**1**) is well-known to be a violent convulsant, its precise mode of action and structure−activity relationships (SARs) have not been studied because of its chemical instability. Investigation of the structural features essential for the toxicity of polyacetylenes such as cicutoxin (**1**) is quite necessary in order to understand the properties of this type of compounds. Also, such investigations might lead to further understanding of the mode of action and provide information valuable for development of a novel anticonvulsant drug.

Our phytochemical studies on water hemlock have led to the isolation of novel polyacetylenes named virol A (**3**), B (**4**), and C (**5**) in addition to the major toxic principle cicutoxin (**1**).[7] The compounds **2** and **6**−**11** were also isolated from the plant (Chart 1). After the isolation of these polyacetylenes, we synthesized virol A (**3**), B (**4**), and C (**5**) in amounts sufficient for chemical transformations to explore the SARs involved in the acute toxicity of these compounds.[8]

γ-Aminobutyric acid type A ($GABA_A$) receptors mediate synaptic inhibition in the mammalian brain, and a variety of medicinal compounds such as barbiturates and benzodiazepines, which are typical anticonvulsant drugs, modulate $GABA_A$ receptor functions, producing pharmacological effects. Assays of the binding of cicutoxin (**1**) and virol A (**3**) to GABA agonist sites, benzodiazepine sites, and GABA-gated Cl^- channels of $GABA_A$

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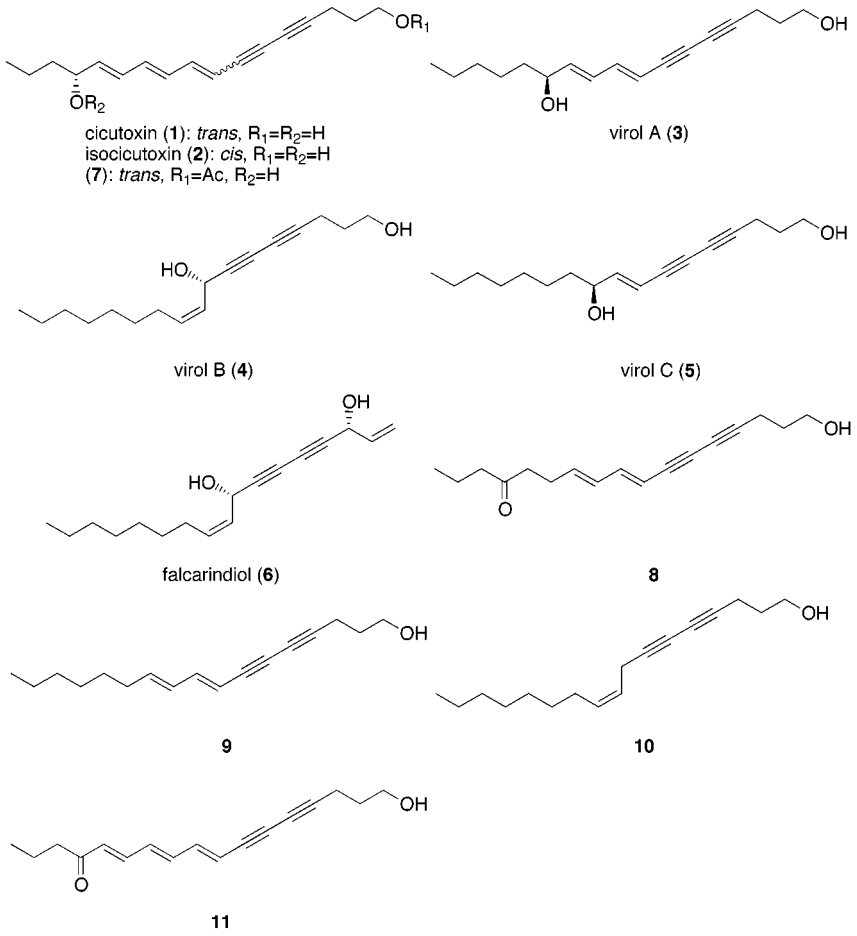
10.1021/jm000185k CCC: $19.00
Published on Web 10/26/2000

Chart 1. Polyacetylenes from *C. virosa*



receptors have been carried out, demonstrating that both of these compounds (**1** and **3**) clearly inhibit the specific binding of [^{3}H]4′-ethynyl-4-*n*-propylbicycloorthobenzoate ([^{3}H]EBOB) to GABA-gated Cl^- channels in rat brain cortex.

In the present study, therefore, we focused on the chemistry of cicutoxin (**1**) and its analogues and determined the 50% lethal dose (LD_{50}) of each in mice and their potency in inhibiting the specific binding of [^{3}H]-EBOB to GABA-gated Cl^- channels.

Chemistry

Phytochemical studies on the subterranean part of water hemlock led to the isolation of several C_{17}-polyacetylenes such as cicutoxin (**1**), isocicutoxin (**2**), virol A (**3**), B (**4**), and C (**5**), and falcarindiol (**6**), as well as compounds **7**−**11**.

To obtain a variety of C_{17}-polyacetylenic compounds, cicutoxin (**1**) and its congeners were chemically transformed. Acetylation of cicutoxin (**1**) resulted in two monoacetylated products (**7** and **12**) and a diacetylated product (**13**). Methylation of cicutoxin (**1**) with CH_3I and K_2CO_3 in DMF yielded two monomethyl ethers (**14** and **15**) and one di-*O*-methyl ether (**16**) (Scheme 1).

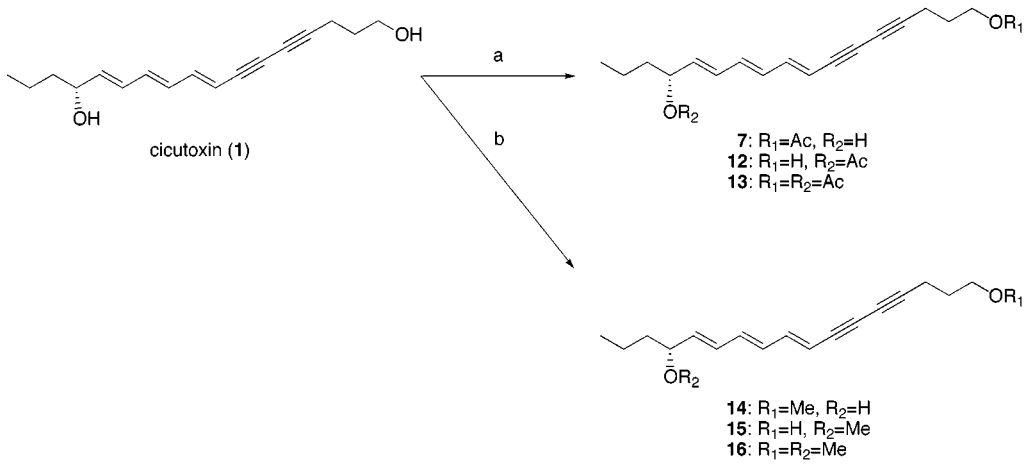
The oxidative transformation of the primary alcohol and the secondary allylic alcohol of cicutoxin (**1**) with SO_3−pyridine complex and Et_3N resulted in three products, an aldehyde (**17**), an enone (**11**), and an enoaldehyde (**18**), the latter two (**11** and **18**) of which were isomerized to **19** and **20** possessing 8*Z*,10*E*,12*E*-stereochemistry under daylight in solution (Scheme 2). For example, 0.05 M **11** in $CDCl_3$ reached equilibration with **19** after 2 h at room temperature under daylight, whereas no isomerization of **11** to **19** occurred in the dark in the same solvent. These geometrical isomers were thus kept in the dark in order to avoid equilibration after separation by silica gel chromatography.

Oxidation of virol A (**3**) with MnO_2 was next carried out and resulted in a conjugated enone (**21**) which also showed isomerization to its geometrical isomer at the position of the double bond, but the rate of its photoisomerization was much slower than that in the case of compound **11** derived from cicutoxin (**1**). The conjugated enone **21** was then converted to the racemic virol A (**22**) by reduction with $NaBH_4$, and **22** is a suitable model compound for use in studies examining the role of chirality at the secondary allylic hydroxyl group in terms of toxicity (Scheme 3).

The aldehyde **17** was transformed to a methyl ester (**23**) which was hydrolyzed to a carboxylic acid (**24**). Amidation of **24** yielded two amides (**25** and **26**) (Scheme 4).

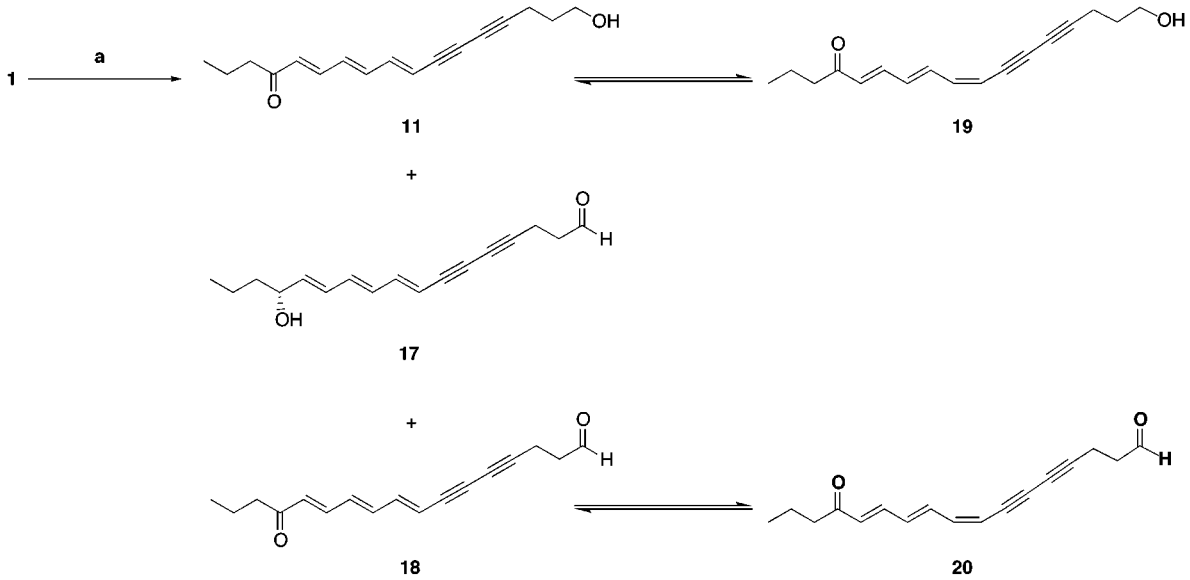
Oxidation of virol B (**4**) with MnO_2 yielded an enone (**27**) having a cross-conjugated system within the molecule. 1-Dehydroxyvirol A (**28**) was prepared by a synthetic route similar to that previously developed for the synthesis of virol A (**3**).[8] Thus, the coupling of (7*S*)-dodeca-3,5-dien-1-yn-7-ol and 1-iodopentyne under Sonogashira's conditions yielded **28**. The monoacetylenic compound **29** was also prepared through the coupling

Scheme 1[a]



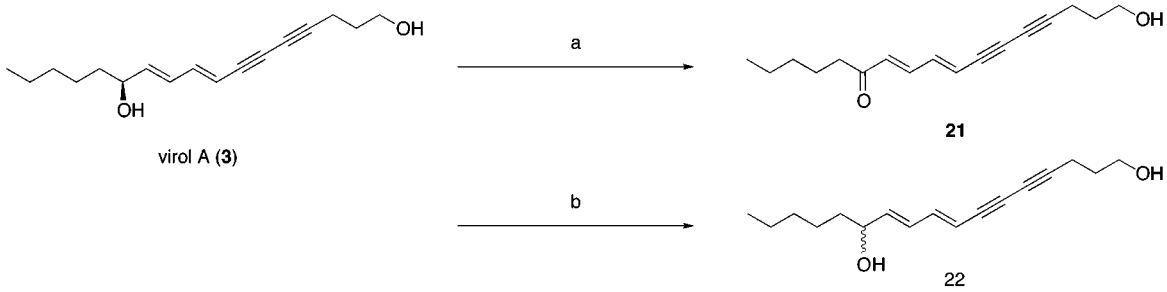
[a] Reagents and conditions: (a) Ac_2O (13 equiv), pyridine (31 equiv), 0 °C, 4 h (**7** 45%, **12** 5%, **13** 21%); (b) MeI (4 equiv), powdered KOH (8 equiv), DMF, rt, 15 min (**14** 24%, **15** 5%, **16** 27%).

Scheme 2[a]



[a] Reagents and conditions: (a) SO_3−pyridine (6 equiv), Et_3N (29 equiv), CH_2Cl_2, DMSO, rt, 4 h (**11** 8%, **17** 16%, **18** 13%).

Scheme 3[a]



[a] Reagents and conditions: (a) MnO_2 (20 equiv), CH_2Cl_2, rt, 8 h (91%); (b) $NaBH_4$ (0.5 equiv), MeOH, 0 °C, 15 min (80%).

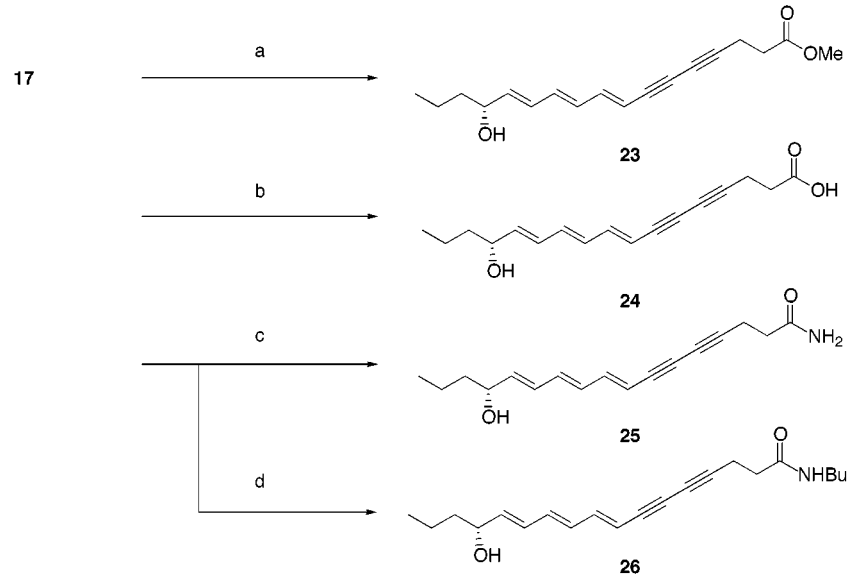
of (5*S*)-1-chlorodeca-1,3-dien-5-ol and 1-iodopentyn-5-ol (Scheme 5).

Analytical HPLC experiments using two systems ((1) column: YMC Pack SIL A003 (4.6 × 250 mm), solvent: *n*-hexane−AcOEt and (2) column: TOSOH TSK-GEL ODS-120A (4.6 × 250 mm), solvent: CH_3CN-H_2O) confirmed the >95% purity of the compounds (see Supporting Information).

Biological Activity

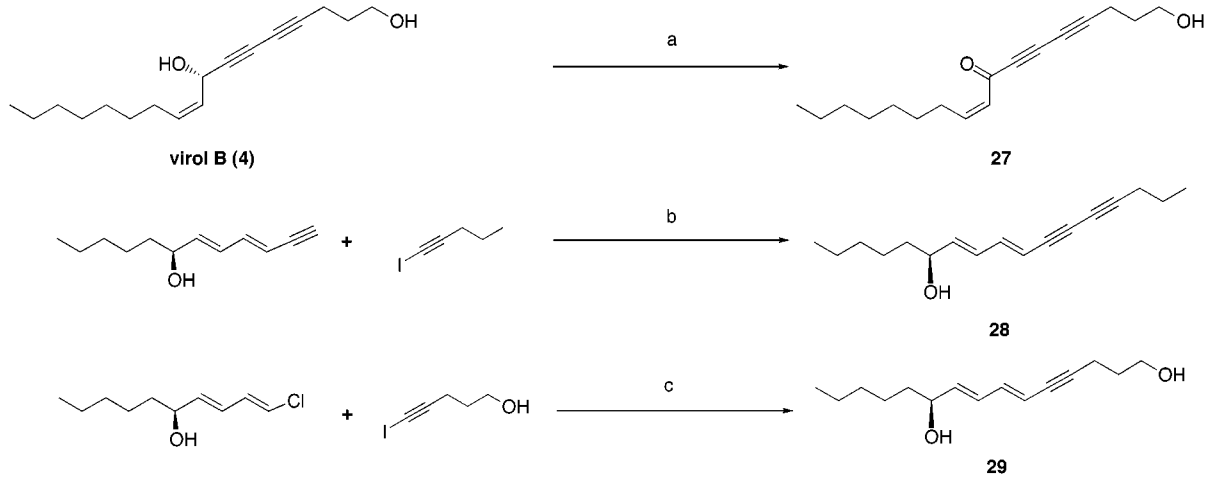
Summarized in Table 1 are the 50% lethal dose (LD_{50}) values obtained for natural C_{17}-polyacetylenes such as cicutoxin (**1**) and their derivatives prepared through chemical transformation reactions. The LD_{50} of virol A (**3**) with a conjugated diendiyne system in the molecule was 9.5 mg/kg indicating that it is ca. 3 times less toxic than cicutoxin (**1**, LD_{50}: 2.8 mg/kg). Virol C, a conju-

Scheme 4[a]



[a] Reagents and conditions: (a) I_2 (1.3 equiv), KOH (2.6 equiv), MeOH, rt, 15 min (71%); (b) 1 N NaOH, MeOH, rt, 9 h (75%); (c) NH_4Cl (2.4 equiv), DPPA (2.4 equiv), Et_3N (5 equiv), DMF, 0 °C, 8.5 h (63%); (d) $BuNH_3Cl$ (2.4 equiv), DPPA (2.4 equiv), Et_3N (5 equiv), DMF, 0 °C, 9.5 h (70%).

Scheme 5[a]



[a] Reagents and conditions: (a) MnO_2 (20 equiv), CH_2Cl_2, rt, 7.5 h (84%); (b) CuI (10 mol %), pyrrolidine (100 equiv), rt, 2 h (83%); (c) $(PhCN)_2PdCl_2$ (5 mol %), CuI (10 mol %), piperidine (110 equiv), rt, 3 h (85%).

gated endiyne compound showed surprisingly lower toxicity (**5**, LD_{50}: >105 mg/kg), being over 40 times less potent than cicutoxin (**1**). The conjugated dienyne compound **29**, which resembles virol C (**5**) in terms of having three π-bond conjugation, was not highly toxic (LD_{50}: >70.8 mg/kg). The LD_{50} values of virol B (**4**) and falcarindiol (**6**) (>393 and >200 mg/kg, respectively), in which only two acetylene bonds are conjugated, were also markedly lower than that of cicutoxin (**1**). With regard to the toxicity of compound **10**, the LD_{50} could not be accurately assessed because of an insufficient amount of sample material; however, it is assumed not to show high toxicity (LD_{50}: >24.6 mg/kg). Compound **27** bearing a cross-conjugation system did not display substantial toxicity (LD_{50}: >26.0 mg/kg). The present data suggest the importance of a π-bond conjugation system consisting of at least two olefins and two acetylenes for relatively high toxicity. Moreover, isocicutoxin (**2**), a 8*Z*-isomer of cicutoxin (**1**), was found to be much less potent (LD_{50}: 38.6 mg/kg) than cicutoxin (**1**), suggesting that the *E*-geometry of the conjugated double bonds is critical for the activity.

Concerning the role of the terminal primary hydroxyl group in the molecule, 1-*O*-methylcicutoxin (**14**) (LD_{50}: 10.6 mg/kg) was found to be ca. 4 times less toxic than cicutoxin (**1**). In addition, compound **28**, a virol A derivative differing structurally from virol A (**3**) in that the terminal hydroxymethyl group in virol A (**3**) is replaced with a methyl group, showed markedly lower toxicity (LD_{50}: >97.6 mg/kg). 1-Acetylcicutoxin (**7**) with an LD_{50} of 1.9 mg/kg was found to have toxicity equal to or even greater than that of the parent compound, cicutoxin (**1**), probably due to rapid hydrolysis of the acetyl group to a hydroxyl group. These results could possibly indicate that the terminal primary hydroxyl group is important for the toxic activity.

The importance of the allylic hydroxyl group in terms of the acute toxicity was next assessed. 14-Acetylcicutoxin (**12**) was found to have an LD_{50} of 2.8 mg/kg, showing toxicity equal to that of cicutoxin (**1**). In

Table 1. 50% Lethal Doses of Polyacetylenic Compounds in Mice and Potency (IC_{50}) in Inhibiting the Specific Binding of [^{3}H]EBOB to GABA-Gated Cl^- Channels of $GABA_A$ Receptors in Rat Brain Cortex

compd	LD_{50} (mg/kg ip)[a]	IC_{50} (μM)[c]
1	2.8 (2.4−3.2)[b]	0.54 ± 0.03
2	38.6 (35.1−42.3)[b]	2.01 ± 0.09
3	9.5 (6.6−13.8)[b]	1.15 ± 0.09
4	>393	6.01 ± 0.29
5	>105	7.87 ± 0.83
6	>200	>10.0
7	1.9 (0.7−4.4)[b]	1.10 ± 0.04
8	1.1 (0.3−3.5)[b]	3.64 ± 0.60
9	>37.8	>10.0
10	>24.6	>10.0
11	7.6 (5.0−11.5)[b]	3.05 ± 0.07
12	2.8 (1.4−5.6)[b]	>10.0
13	15.7 (13.8−18.0)[b]	>10.0
14	10.6 (7.1−15.8)[b]	6.79 ± 0.04
16	>57.2	>10.0
17	4.9 (3.6−6.5)[b]	2.22 ± 0.23
21	>25.8	>10.0
22	7.7 (6.3−9.6)[b]	1.39 ± 0.17
23	1.6 (1.4−1.8)[b]	0.42 ± 0.01
24	0.76 (0.65−0.87)[b]	0.30 ± 0.04
25	1.1 (0.9−1.4)[b]	1.56 ± 0.11
26	4.9 (2.8−8.4)[b]	>10.0
27	>26.0	>10.0
28	>97.6	>10.0
29	>70.8	>10.0
picrotoxin	9.8	1.31 ± 0.06

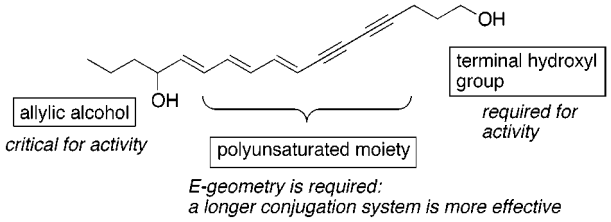
[a] Compounds tested in one experiment only. [b] 95% confidence interval. [c] Variation of IC_{50} values was calculated from the results of duplicate assays during preparation of dose−response curves.

contrast, the conjugated ketone **11**, produced from cicutoxin (**1**) by oxidation at C-14, was found to have an LD_{50} of 7.6 mg/kg, being less toxic than cicutoxin (**1**). Similarly, the LD_{50} of compound **21**, an oxidized product of virol A (**3**), was found to be >25.8 mg/kg, which is lower than that of the parent compound. Moreover, the LD_{50} of compound **9**, a congener of virol A (**3**) not bearing the allylic hydroxyl group at C-12, was >37.8 mg/kg, indicating that it is much less toxic than virol A (**3**). These toxicological findings indicated that the allylic hydroxyl group is also critical for the toxic activity. The results obtained by comparing the activity of natural virol A (**3**) and that of synthetic racemic virol A (**22**) indicated that both the *R*- and *S*-configurations of the allylic hydroxyl are effective.

Derivatives prepared through oxidation of the terminal primary alcohol of cicutoxin (**1**), an aldehyde **17**, a carboxylic acid **23**, an ester **24**, and two amides **25** and **26**, showed potent toxicity, and the LD_{50} values were 4.9, 1.7, 0.76, 1.1, and 4.9 mg/kg, respectively. Three of them, **23**−**25**, showed more potent toxicity than the parent compound, cicutoxin (**1**). Here, we could assume that chemical instability in the case of **17** and the steric effect of the *n*-butyl group connected to the amide nitrogen in the case of **26** affected the toxicity. Thus, the terminal carbonyl group was shown to be an important element for toxicity.

Compound **8** (LD_{50}: 1.1 mg/kg) was found to be more toxic than cicutoxin (**1**). We noticed in the present study that cicutoxin (**1**) and its derivatives elicited signs of toxicity within 10−20 min after administration of the compounds by intraperitoneal injection, whereas, in the case of compound **8**, the signs of toxicity appeared ca. 50−60 min after injection. In view of these findings, we speculate that compound **8** is metabolized within the body to yield metabolites which have potent toxic activity.

Chart 2. Structural Requirements for Toxicity



We next examined the binding of the polyacetylenic compounds to GABA-gated Cl^- channels of $GABA_A$ receptors in rat brain cortex. Cicutoxin (**1**) and its oxidated products, **23** and **24**, strongly inhibited the specific binding of [^{3}H]EBOB to these channels. In addition, we observed inhibition of [^{3}H]EBOB binding by 11 of the polyacetylenic compounds tested (**2**−**5**, **7**, **8**, **11**, **14**, **17**, **22**, **25**) with the IC_{50} values being in the 10^{-6} M order. Except for compounds **4** and **5**, a correlation was observed between the potency of the compounds in inhibiting the specific binding of [^{3}H]EBOB in rat brain cortex and the LD_{50} values (Table 1).

Conclusion

Analogues of C_{17}-polyacetylenes were synthesized to evaluate their acute toxicity through comparison of their LD_{50} values and their potency in inhibiting the specific binding of [^{3}H]EBOB, a noncompetitive GABA antagonist, to GABA-gated Cl^- channels of $GABA_A$ receptors in rat brain cortex. Our findings demonstrate that the length of the π-bond conjugation system and the geometry of the double bonds are critical for toxicity. Moreover, the terminal *O*-functional group and the allylic alcohol are essential for toxicity (Chart 2). Our findings concerning inhibition of the specific binding of [^{3}H]-EBOB to GABA-gated Cl^- channels in rat brain cortex suggested that such activity plays an important role in the pharmacological mode of action of the polyacetylenes.

Although the SARs involved in the antimicrobial activity of the polyacetylenes have been examined in previous studies, little is known about the chemistry and pharmacological activity of the polyacetylenes. The present study sheds new light on the properties of this interesting group of compounds. Further studies are now underway in our laboratory, including a search for more effective and more stable analogues and studies exploring the mechanism of action of these compounds.

Experimental Section

Optical rotations were recorded on JASCO DIP-370 and DIP-340 polarimeters. Infrared and ultraviolet spectra were recorded on JASCO A-100-S-IR and HITACHI U-3000 spectrophotometers, respectively. ^{1}H and ^{13}C NMR spectra were measured by a Varian Gemini 2000 (300 and 75 MHz) spectrometer. Chemical shifts (δ) are reported as ppm downfield from tetramethylsilane (TMS), and coupling constants are given in Hz. Multiplicity is indicated as follows: s (singlet); d (doublet); dd (doublet of doublets); ddd (doublet of double doublets); t (triplet); m (multiplet); br (broad). Mass spectra were recorded on JEOL JMS-DX303 and JMS-AX-500 spectrometers. HPLC analyses were performed using a HITACHI

L-6000 system (column: YMC Pack SIL A003 (4.6 × 250 mm) and TOSOH TSK-GEL ODS-120A (4.6 × 250 mm)).

(4*R*)-(5*E*,7*E*,9*E*)-17-Acetoxyheptadecatriene-11,13-diyn-4-ol (7), (14*R*)-(8*E*,10*E*,12*E*)-14-Acetoxyheptadecatriene-4,6-diyn-1-ol (12), and (4*R*)-(5*E*,7*E*,9*E*)-4,17-Diacetoxyheptadecatriene-11,13-diyne (13). A mixture of cicutoxin (**1**; 8.5 mg, 32.9 μmol), pyridine (16.4 mL, 1.02 mmol) and $(CH_3CO)_2O$ (4.03 mg, 39.5 mmol) was stirred at 0 °C for 8.3 h. AcOEt was added to the reaction mixture and then the resulting solution was washed with H_2O and brine, successively, dried over $MgSO_4$, and concentrated in vacuo. Purification by column chromatography on silica gel (*n*-hexane−AcOEt, 5:1 v/v, 4:1 v/v and 3:1 v/v) gave the compound **13** (2.6 mg, 23%) as a colorless oil, **7** (5.4 mg, 55%) as a colorless oil and **12** (1.0 mg, 10%) as a colorless oil. Compound **7**: $[\alpha]_D$ −15.7° (*c* 0.24, EtOH). UV (EtOH) λ_{max} (log ϵ): 334 (4.65), 317 (4.66), 302 (4.43), 251 (4.17), 241 (3.97) nm. IR ν_{max} ($CHCl_3$): 3400, 2330, 2220, 2120, 1730, 1600, 1240, 990 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ: 0.93 (t, 3H, J = 7.1 Hz), 1.25−1.60 (m, 4H), 1.88 (quint, 2H, J = 6.9 Hz), 2.06 (s, 3H), 2.46 (t, 2H, J = 6.9 Hz), 4.16 (t, 2H, J = 6.3 Hz), 4.18−4.20 (m, 1H), 5.60 (d, 1H, J = 15.4 Hz), 5.83 (dd, 1H, J = 15.1, 6.9 Hz), 6.18−6.38 (m, 3H), 6.71 (dd, 1H, J = 15.4, 10.2 Hz). MS *m*/*z*: 300 (M^+), 257 (M^+ − 43). High-resolution MS calcd for $C_{19}H_{24}O_3$: 300.1725. Found: 300.1716. Compound **12**: $[\alpha]_D$ +36.3° (*c* 0.281, EtOH). UV (EtOH) λ_{max} (log ϵ): 336 (4.45), 317 (4.59), 252 (4.21), 242 (4.04), 205 (4.15) nm. IR ν_{max} ($CHCl_3$): 3626, 3454, 2361, 2226, 1728, 1602 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ: 0.92 (t, 3H, J = 7.1 Hz), 1.23−1.68 (m, 4H), 1.81 (quint, 2H, J = 6.9 Hz), 2.05 (s, 3H), 2.49 (t, 2H, J = 7.1 Hz), 3.76 (br t, 2H), 5.29 (q, 1H, J = 6.9 Hz), 5.62 (d, 1H, J = 15.1 Hz), 5.71 (dd, 1H, J = 15.1, 6.9 Hz), 6.18−6.38 (m, 3H), 6.71 (dd, 1H, J = 15.1, 10.3 Hz). MS *m*/*z*: 300 (M^+), 258 (M^+ − 42), 240, 198, 141, 71, 43. High-resolution MS calcd for $C_{19}H_{24}O_3$ (M^+): 300.1725. Found: 300.1735. Compound **13**: $[\alpha]_D$ +37.0° (*c* 2.00, MeOH). UV (EtOH) λ_{max} (log ϵ): 334 (4.72), 316 (4.72), 303 (4.48), 251 (4.24), 242 (4.00) nm. IR ν_{max} ($CHCl_3$): 2330, 2230, 2140, 1730, 1600, 1230, 990 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ: 0.92 (t, 3H, J = 7.1 Hz), 1.23−1.72 (m, 4H), 1.88 (quint, 2H, J = 6.9 Hz), 2.05 (s, 3H), 2.06 (s, 3H), 2.46 (t, 2H, J = 6.9 Hz), 4.16 (t, 2H, J = 6.3 Hz), 5.29 (q, 1H, J = 6.9 Hz), 5.61 (d, 1H, J = 15.4 Hz), 5.71 (dd, 1H, J = 15.1, 6.9 Hz), 6.20−6.40 (m, 3H), 6.70 (dd, 1H, J = 15.4, 10.2 Hz). High-resolution MS calcd for $C_{21}H_{26}O_4$ (M^+): 342.1830. Found: 342.1795.

(4*R*)-(5*E*,7*E*,9*E*)-17-Methoxyheptadecatriene-11,13-diyn-4-ol (14), (14*R*)-(8*E*,10*E*,12*E*)-14-Methoxyheptadecatriene-4,6-diyn-1-ol (15), and (4*R*)-(5*E*,7*E*,9*E*)-4,17-Dimethoxyheptadecatriene-11,13-diyne (16). A mixture of cicutoxin (**1**; 102.9 mg, 0.399 mmol), MeI (99.3 mL, 1.60 mmol) and KOH (179 mg, 3.19 mmol) in DMF (1 mL) was stirred at 0 °C for 10 min. Et_2O was added to the reaction mixture and then the resulting solution was washed with a 1 N solution of aqueous HCl and brine, successively, dried over $MgSO_4$, and concentrated in vacuo. Purification by column chromatography on silica gel (*n*-hexane−AcOEt, 100:3 v/v, 6:1 v/v and 4:1 v/v) gave the compound **16** (30.8 mg, 27%) as a colorless oil, compound **15** (5.4 mg, 5%) as a colorless oil and compound **14** (26.0 mg, 24%) as a colorless oil. Compound **14**: $[\alpha]_D$ −15.1° (*c* 0.57, EtOH). UV (EtOH) λ_{max} (log ϵ): 334 (4.23), 318 (4.24), 302 (sh), 252 (3.84), 242 (3.68), 204 (3.80) nm. IR ν_{max} ($CHCl_3$): 3603, 2361, 2227, 1603 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ: 0.92 (t, 3H, J = 7.1 Hz), 1.20−1.64 (m, 4H), 1.92 (quint, 2H, J = 6.8 Hz), 2.48 (t, 2H, J = 6.8 Hz), 3.80 (s, 3H), 4.24 (t, 2H, J = 6.2 Hz), 4.15−4.29 (m, 1H), 5.60 (d, 1H, J = 15.4 Hz), 5.83 (dd, 1H, J = 15.0, 6.7 Hz), 6.18−6.42 (m, 3H), 6.71 (dd, 1H, J = 15.4, 9.9 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 13.9, 16.3, 18.6, 27.5, 39.4, 54.9, 66.4, 66.5, 72.3, 75.4, 77.4, 84.1, 110.0, 129.9, 131.7, 135.6, 139.5, 144.6. MS *m*/*z*: 272 (M^+), 258 (M^+ − 14). High-resolution MS calcd for $C_{18}H_{20}O_2$ (M^+): 272.1776. Found: 272.1764. Compound **15**: $[\alpha]_D$ +34.2° (*c* 0.13, EtOH). UV (EtOH) λ_{max} (log ϵ): 334 (4.32), 318 (4.33), 302 (sh), 252 (3.91), 242 (3.74), 204 (3.88) nm. IR ν_{max} ($CHCl_3$): 3600, 2361, 2226, 1602 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ: 0.92 (t, 3H, J = 7.1 Hz), 1.26−1.44 (m, 2H), 1.51−1.65 (m, 1H), 1.66−1.78 (m, 1H), 1.81 (quint, 2H, J = 7.1 Hz), 2.49 (dt, 2H, J = 1.1, 7.1 Hz), 3.76 (t, 2H, J = 7.1 Hz), 3.77 (s 3H), 5.16 (q, 1H, J = 7.1 Hz), 5.63 (d, 1H, J = 15.4 Hz), 5.72 (dd, 1H, J = 15.1, 7.1 Hz), 6.24−6.42 (m, 3H), 6.70 (dd, 1H, J = 15.4, 10.4 Hz). MS *m*/*z*: 272 (M^+), 258 (M^+ − 14). High-resolution MS calcd for $C_{18}H_{20}O_2$ (M^+): 300.1725. Found: 300.1711. Compound **16**: $[\alpha]_D$ +23.8° (*c* 0.21, EtOH). UV (EtOH) λ_{max} (log ϵ): 334 (4.41), 317 (4.42), 302 (sh), 251 (3.98), 242 (3.81), 205 (3.95) nm. IR ν_{max} ($CHCl_3$): 2361, 2337, 1602 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ: 0.92 (t, 3H, J = 7.4 Hz), 1.28−1.42 (m, 4H), 1.50−1.65 (m, 1H), 1.65−1.80 (m, 1H), 1.92 (quint, 2H, J = 7.0 Hz), 2.48 (t, 2H, J = 7.0 Hz), 3.77 (s, 3H), 3.79 (s, 3H), 4.24 (t, 2H, J = 7.0 Hz), 5.12 (q, 1H, J = 7.0 Hz), 5.61 (d, 1H, J = 15.1 Hz), 5.71 (dd, 1H, J = 14.8, 7.0 Hz), 6.24−6.38 (m, 3H), 6.71 (dd, 1H, J = 15.1, 9.9 Hz). MS *m*/*z*: 286 (M^+), 271 (M^+ − 15), 254 (M^+ − 32), 45 (100%). High-resolution MS calcd for $C_{19}H_{26}O_2$ (M^+): 286.1933. Found: 286.1917.

(8*E*,10*E*,12*E*)-14-Oxoheptadecatriene-4,6-diyn-1-al (18), (5*E*,7*E*,9*E*)-17-Hydroxyheptadecatriene-11,13-diyn-4-one (11), and (14*R*)-(8*E*,10*E*,12*E*)-14-Hydroxyheptadecatriene-4,6-diyn-1-al (17). A mixture of cicutoxin (**1**; 637 mg, 2.47 mmol), DMSO (7.5 mL, 106 mmol), Et_3N (10 mL, 71.2 mmol) and SO_3−pyridine complex (4.72 g, 29.6 mmol) in CH_2Cl_2 (7.5 mL) was stirred at 0 °C for 4 h. Et_2O was added and the resulting solution was washed with a saturated solution of aqueous NH_4Cl, saturated solution of aqueous $NaHCO_3$ and brine, successively, dried over $MgSO_4$, and concentrated in vacuo. Purification by column chromatography on silica gel (*n*-hexane−AcOEt, 10:1 v/v, 4:1 v/v, 2:1 v/v) gave the compound **18** (100 mg, 16%) as a colorless oil, compound **17** (82.2 mg, 13%) as a colorless oil and compound **11** (50.5 mg, 8%) as a colorless oil. Compound **18**: UV (EtOH) λ_{max} (log ϵ): 363 (sh), 348 (4.67), 269 (3.95), 243 (4.06), 204 (4.18) nm. IR ν_{max} ($CHCl_3$): 2226, 1730, 1654, 1603, 997 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ: 0.94 (t, 3H, J = 7.1 Hz), 1.68 (m, 2H), 2.54 (t, 2H, J = 7.2 Hz), 2.61−2.83 (m, 4H), 5.78 (d, 1H, J = 15.1 Hz), 6.23 (d, 1H, J = 15.7 Hz), 6.39 (dd, 1H, J = 14.6, 11.0 Hz), 6.58 (dd, 1H, J = 14.6, 11.0 Hz), 6.73 (dd, 1H, J = 15.7, 11.0 Hz), 7.20 (dd, 1H, J = 15.4, 11.0 Hz), 9.91 (s, 1H). MS *m*/*z*: 254 (M^+), 43 (100%). High-resolution MS calcd for $C_{17}H_{18}O_2$ (M^+): 254.1307. Found: 254.1276. Compound **11**: UV (EtOH) λ_{max} (log ϵ): 362 (sh), 347 (4.54), 269 (3.88), 237 (3.69), 215 (3.97) nm. IR ν_{max} ($CHCl_3$): 3626, 3481, 2226, 1655, 1599 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ: 0.94 (t, 3H, J = 7.4 Hz), 1.66 (sex, 2H, J = 7.4 Hz), 1.82 (quint, 2H, J = 6.9 Hz), 2.51 (t, 2H, J = 6.9 Hz), 2.54 (t, 2H, J = 7.4 Hz), 3.76 (t, 2H, J = 7.4 Hz), 5.80 (d, 1H, J = 15.1 Hz), 6.24 (d, 1H, J = 15.7 Hz), 6.41 (dd, 1H, J = 14.6, 11.0 Hz), 6.61 (dd, 1H, J = 14.6, 11.0 Hz), 6.75 (dd, 1H, J = 15.7, 11.0 Hz), 7.16 (dd, 1H, J = 15.4, 11.0 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 13.8, 16.3, 17.8, 30.9, 42.9, 61.4, 65.8, 74.5, 80.0, 86.8, 114.4, 131.2, 133.5, 139.6, 141.2, 143.3, 200.8. MS *m*/*z*: 256 (M^+), 255 (M^+ − 1), 43 (100%). High-resolution MS calcd for $C_{17}H_{20}O_2$ (M^+): 256.1463. Found: 256.1475. Compound **17**: $[\alpha]_D$ +51.4° (*c* 0.14, EtOH). UV (EtOH) λ_{max} (log ϵ): 336 (4.49), 318 (4.55), 302 (sh), 252 (4.10), 243 (3.93), 204 (4.08) nm. IR ν_{max} ($CHCl_3$): 3597, 2357, 1728, 1603, 997 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ: 0.93 (t, 3H, J = 7.1 Hz), 1.30−1.64 (m, 4H), 2.63−2.79 (m, 4H), 4.20 (q, 1H, J = 6.7 Hz), 5.60 (d, 1H, J = 15.4 Hz), 5.83 (d, 1H, J = 15.1, 6.6 Hz), 6.17−6.44 (m, 3H), 6.71 (dd, 1H, J = 15.4, 10.2 Hz), 9.80 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 13.8, 16.3, 17.8, 30.9, 42.9, 61.4, 65.8, 74.5, 80.0, 86.8, 114.4, 131.2, 133.5, 139.6, 141.2, 143.3, 200.8. MS *m*/*z*: 256 (M^+), 238, 228, 213, 43 (100%). High-resolution MS calcd for $C_{17}H_{20}O_2$ (M^+): 256.1463. Found: 256.1448.

(7*E*,9*E*)-17-Hydroxyheptadecatriene-11,13-diyn-6-one (21). A mixture of virol A (**3**; 8.6 mg, 33 μmol) and MnO_2 (57.4 mg, 0.66 mmol) in CH_2Cl_2 (0.1 mL) was stirred at room temperature for 7 h. The reaction mixture was filtrated on Celite pad and concentrated in vacuo. Purification by column chromatography on silica gel (*n*-hexane−AcOEt, 3:1 v/v) gave the title compound **21** (7.7 mg, 91%). A part of the compound was recrystallized with *n*-hexanes−Et_2O to give a colorless needle: mp 65.0−65.5 °C. UV (EtOH) λ_{max} (log ϵ): 336 (4.54),

320 (4.60), 252 (3.69), 203 (3.97) nm. IR ν_{max} ($CHCl_3$): 3626, 3477, 2361, 2227, 1680, 1595 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ: 0.89 (t, 3H, J = 8.2 Hz), 1.22−1.38 (m, 4H), 1.62 (quint, 2H, J = 7.4 Hz), 1.82 (quint, 2H, J = 6.9 Hz), 2.51 (t, 2H, J = 6.9 Hz), 2.55 (t, 2H, J = 7.4 Hz), 3.77 (t, 2H, J = 7.4 Hz), 6.01 (d, 1H, J = 15.4 Hz), 6.24 (d, 1H, J = 15.5 Hz), 6.75 (dd, 1H, J = 15.5, 11.1 Hz), 7.13 (dd, 1H, J = 15.4, 11.1 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 13.9, 16.3, 22.4, 23.9, 30.8, 31.4, 41.2, 61.4, 65.6, 73.8, 81.0, 87.4, 118.9, 131.7, 140.2, 142.1, 200.7. MS m/z: 258 (M^+), 202, 145, 43 (100%). High-resolution MS calcd for $C_{17}H_{22}O_2$ (M^+): 258.1620. Found: 258.1600.

(8*E*,10*E*)-Heptadecadiene-4,6-diyne-1,12-diol (22). To a solution of compound **21** (20 mg, 76.9 μmol) in MeOH (1.0 mL) was added $NaBH_4$ (1.4 mg, 38.5 μmol) at −20 °C. After 30 min, acetone was added to the reaction mixture and concentrated in vacuo. The residue was diluted with Et_2O and then washed with brine, successively, dried over $MgSO_4$ and concentrated in vacuo. Purification by column chromatography on silica gel (*n*-hexane−AcOEt, 3:1 v/v) gave the title compound (16 mg, 80%) as a colorless oil: $[\alpha]_D$ ±0° (*c* 0.32, MeOH). All spectral data excepted optical rotation were agreed with natural virol A (**3**).

Methyl (14*R*)-(8*E*,10*E*,12*E*)-14-Hydroxyheptadecatriene-4,6-diynate (23). To a solution of compound **17** (102 mg, 0.397 mmol) in MeOH (5.3 mL) was added a solution of KOH (57.9 mg, 1.03 mmol) in MeOH (1.3 mL) and a solution of I_2 (131 mg, 0.515 mmol) in MeOH (1.3 mL) at 0 °C. The reaction mixture was stirred for 15 min and quenched with a saturated solution of aqueous $Na_2S_2O_3$. Et_2O was added to the resulting mixture and then washed with brine, successively, dried over $MgSO_4$ and concentrated in vacuo. Purification by column chromatography on silica gel (*n*-hexane−AcOEt, 6:1 v/v) gave the title compound **23** (80.6 mg, 71%) as a colorless oil: $[\alpha]_D$ −15.0° (*c* 0.25, EtOH). UV (EtOH) λ_{max} (log ϵ): 335 (4.57), 318 (4.58), 302 (sh), 252 (4.12), 242 (3.95), 204 (4.01) nm. IR ν_{max} ($CHCl_3$): 3609, 3460, 2229, 2135, 1736, 1602, 997 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ: 0.93 (t, 3H, J = 7.1 Hz), 1.29−1.64 (m, 4H), 2.58 (dt, 2H, J = 2.2, 6.6 Hz), 2.67 (br t, 3H), 3.71 (s, 3H), 4.19 (br q, 1H), 5.60 (d, 1H, J = 15.4 Hz), 5.82 (dd, 1H, J = 14.8, 6.6 Hz), 6.18−6.41 (m, 3H), 6.71 (dd, 1H, J = 15.4, 10.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 13.8, 15.5, 18.5, 32.7, 39.3, 51.9, 66.1, 72.2, 75.4, 77.2, 83.5, 109.8, 129.8, 131.6, 135.6, 139.4, 144.6, 172.0. MS m/z: 286 (M^+), 268, 71(100%). High-resolution MS calcd for $C_{18}H_{22}O_3$ (M^+): 286.1569. Found: 286.1560.

(14*R*)-(8*E*,10*E*,12*E*)-14-Hydroxyheptadecatriene-4,6-diynic Acid (24). A mixture of compound **23** (57.6 mg, 0.201 mmol) and 1 N NaOH in H_2O (22.5 μL) in MeOH (3.6 mL) was stirred at room temperature for 9 h. H_2O was added to the reaction mixture and then 1 N HCl in H_2O was added dropwise to neutrality. AcOEt was added to the resulting mixture and then washed with brine, successively, dried over $MgSO_4$ and concentrated in vacuo. Purification by column chromatography on silica gel (*n*-hexane−AcOEt, 1:2 v/v) gave the title compound **24** (41.0 mg, 75%) as a colorless oil: $[\alpha]_D$ −15.0° (*c* 0.40, EtOH). UV (EtOH) λ_{max} (log ϵ): 336 (4.16), 318 (4.17), 302 (sh), 251 (3.73), 242 (3.57), 207 (4.06) nm. IR ν_{max} ($CHCl_3$): 3603, 3501, 2690, 2361, 2229, 2135, 1716, 1602, 997 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ: 0.94 (t, 3H, J = 5.2 Hz), 1.22−1.65 (m, 4H), 2.58−2.74 (m, 4H), 3.16 (br s, 1H), 4.20 (q, 1H, J = 6.5 Hz), 5.60 (d, 1H, J = 15.7 Hz), 5.82 (dd, 1H, J = 15.0, 6.7 Hz), 6.18−6.38 (m, 3H), 6.72 (dd, 1H, J = 15.7, 10.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 13.9, 15.4, 18.6, 32.6, 39.4, 66.3, 72.4, 75.7, 77.3, 83.3, 109.9, 129.9, 131.7, 135.7, 139.5, 144.8, 175.9. MS m/z: 272 (M^+), 254, 43 (100%). High-resolution MS calcd for $C_{17}H_{20}O_3$ (M^+): 272.1412. Found: 272.1431.

(14*R*)-(8*E*,10*E*,12*E*)-14-Hydroxyheptadecatriene-4,6-diynamide (25). A mixture of compound **24** (10.9 mg, 40 μmol), NH_4Cl (5.1 mg, 96 μmol), DPPA (20.6 μL, 96 μmol), and Et_3N (27.8 μL, 0.20 mmol) in DMF (0.5 mL) was stirred at 0 °C for 8.5 h. AcOEt was added to the resulting mixture and then washed with a saturated solution of aqueous NH_4Cl, saturated solution of aqueous $NaHCO_3$, and brine, successively, dried over $MgSO_4$ and concentrated in vacuo. Purification by column chromatography on silica gel (*n*-hexane−AcOEt, 2:3 v/v) gave the title compound **25** (6.5 mg, 63%) as a colorless oil: $[\alpha]_D$ −16.7° (*c* 0.084, MeOH). UV (MeOH) λ_{max} (log ϵ): 334 (4.43), 317 (4.46), 302 (sh), 252 (4.02), 242 (3.92), 202 (3.98) nm. IR ν_{max} ($CHCl_3$): 3600, 3501, 3405, 2361, 2229, 1653, 1602, 997 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ: 0.91 (t, 3H, J = 5.0 Hz), 1.26−1.62 (m, 4H), 2.45 (t, 3H, J = 7.2 Hz), 2.68 (t, 3H, J = 7.2 Hz), 4.13−4.22 (m, 1H), 5.30−5.60 (m, 2H), 5.57 (d, 1H, J = 14.8 Hz), 5.81 (dd, 1H, J = 14.8, 6.9 Hz), 6.15−6.36 (m, 3H), 6.69 (dd, 1H, J = 14.8, 10.3 Hz). MS m/z: 271 (M^+), 253, 141, 71, 43 (100%). High-resolution MS calcd for $C_{17}H_{21}O_2N$ (M^+): 271.1572. Found: 271.1466.

(14*R*)-(8*E*,10*E*,12*E*)-*N*-Butyl-14-hydroxyheptadecatriene-4,6-diynic Amide (26). A mixture of compound **24** (9.1 mg, 34 μmol), $BuNH_3Cl$ (8.8 mg, 81 μmol), DPPA (17.4 μL, 81 μmol), and Et_3N (23.4 μL, 0.17 μmol) in DMF (0.5 mL) was stirred at 0 °C for 9.5 h. AcOEt was added and resulting mixture was washed with a saturated solution of aqueous NH_4Cl, saturated solution of aqueous $NaHCO_3$, and brine, successively, dried over $MgSO_4$ and concentrated in vacuo. Purification by column chromatography on silica gel (*n*-hexane−AcOEt, 2:1 v/v) gave the title compound **26** (7.8 mg, 70%) as a colorless oil: $[\alpha]_D$ −4.1° (*c* 0.049, MeOH). UV (MeOH) λ_{max} (log ϵ): 333 (3.95), 317 (3.99), 302 (sh), 254 (3.61), 242 (3.56), 205 (3.68) nm. IR ν_{max} ($CHCl_3$): 3602, 3310, 2228, 1658, 1602, 997 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ: 0.88 (t, 3H, J = 6.8 Hz), 0.93 (t, 3H, J = 7.3 Hz), 1.24−1.58 (m, 8H), 2.41 (t, 3H, J = 7.1 Hz), 2.71 (t, 3H, J = 7.1 Hz), 3.29 (dt, 2H, J = 5.5, 7.1 Hz), 4.17−4.24 (m, 1H), 5.50 (br s, 1H), 5.60 (d, 1H, J = 15.4 Hz), 5.84 (dd, 1H, J = 14.6, 6.0 Hz), 6.17−6.37 (m, 3H), 6.71 (dd, 1H, J = 15.4, 11.4 Hz). MS m/z: 327 (M^+), 309, 280, 57 (100%), 43. High-resolution MS calcd for $C_{21}H_{29}O_2N$ (M^+): 327.2198. Found: 327.2208

(9*Z*)-1-Hydroxyheptadecene-4,6-diyn-8-one (27). A mixture of virol B (**4**; 16.7 mg, 63.7 μmol) and MnO_2 (110 mg, 1.28 mmol) in CH_2Cl_2 (0.2 mL) was stirred at room temperature for 6.5 h. The reaction mixture was filtrated on Celite pad and concetrated in vacuo. Purification by column chromatography on silica gel (*n*-hexane−AcOEt, 3:1 v/v) gave the title compound **27** (14.0 mg, 84%) as a colorless oil: UV (EtOH) λ_{max} (log ϵ): 295 (4.00), 279 (4.08), 265 (4.02), 253 (sh), 208 (4.15) nm. IR ν_{max} ($CHCl_3$): 3620, 3439, 2234, 1645, 1604 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ: 0.88 (t, 3H, J = 6.9 Hz), 1.18−1.39 (m, 8H), 1.40−1.55 (m, 2H), 1.83 (quint, 2H, J = 6.9 Hz), 2.53 (t, 2H, J = 6.9 Hz), 2.69 (dq, 2H, J = 1.4, 7.4 Hz), 3.76 (t, 2H, J = 6.9 Hz), 6.17 (dt, 1H, J = 11.5, 1.4 Hz), 6.30 (dt, 1H, J = 15.5, 7.4 Hz), 6.75 (dd, 1H, J = 15.5, 11.1 Hz), 7.13 (dd, 1H, J = 15.4, 11.1 Hz). MS m/z: 260 (M^+), 229, 189, 176, 55 (100%). High-resolution MS calcd for $C_{17}H_{24}O_2$ (M^+): 260.1776. Found: 260.1783.

(6*S*)-(7*E*,9*E*)-Heptadecadiene-11,13-diyn-4-ol (28). A mixture of (7*S*)-(7*E*,9*E*)-dodeca-7,9-dien-11-yn-6-ol (18.4 mg, 0.103 mmol), 1-iodopentyne (30.2 mg, 0.155 mmol) and CuI (2.0 mg, 10 μmol) in pyrrolidine (1 mL) was stirred at room temperature for 1 h. Et_2O was added and resulting mixture was washed with a saturated solution of aqueous NH_4Cl and brine, successively, dried over $MgSO_4$ and concentrated in vacuo. Purification by column chromatography on silica gel (*n*-hexane−AcOEt, 6:1 v/v) gave the title compound **28** (20.8 mg, 83%) as a colorless oil: $[\alpha]_D$+15.4° (*c* 0.67, MeOH). UV (EtOH) λ_{max} (log ϵ): 311 (4.20), 294 (4.25), 236 (4.25), 226 (4.12) nm. IR ν_{max} ($CHCl_3$): 3603, 3443, 2361, 2229, 1591 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ: 0.89 (t, 3H, J = 6.6 Hz), 1.00 (t, 3H, J = 4.1 Hz), 1.24−1.43 (m, 8H), 1.48−1.65 (m, 2H), 2.32 (t, 2H, J = 6.9 Hz), 4.17 (q, 1H, J = 6.3 Hz), 5.61 (d, 1H, J = 15.7 Hz), 5.83 (dd, 1H, J = 15.4, 6.3 Hz), 6.27 (dd, 1H, J = 15.4, 10.9 Hz), 6.68 (dd, 1H, J = 15.7, 10.9 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 13.3, 13.9, 21.5, 21.7, 22.5, 24.9, 31.6, 37.1, 65.4, 72.3, 74.2, 85.7, 119.4, 127.6, 129.1, 140.0, 143.7. MS m/z: 244 (M^+), 215, 173, 145, 71, 43 (100%). High-resolution MS calcd for $C_{17}H_{24}O$ (M^+): 244.1827. Found: 244.1824.

(*S*)-(6*E*,8*E*)-Pentadeca-6,8-dien-4-yne-1,10-diol (29). A mixture of (5*S*)-(1*E*,3*E*)-1-chlorodeca-1,3-dien-5-ol (91.9 mg,

488 μmol), 4-pentyn-1-ol (42.1 mg, 500 μmol), $PdCl_2(PhCN)_2$ (9.4 mg, 25 μmol) and CuI (9.8 mg, 49 μmol) in piperidine (5 mL) was stirred at the room temperature for 3 h. Et_2O was added to the reaction mixture and then the resulting solution was washed with a saturated solution of aqueous NH_4Cl and brine, successively, dried over $MgSO_4$, and concentrated in vacuo. Purification by column chromatography on silica gel (*n*-hexane−AcOEt, 2:1 v/v) gave the title compound **29** (98.0 mg, 85%) as a colorless oil: $[\alpha]_D$ +19.4° (*c* 0.13, MeOH). IR ν_{max} ($CHCl_3$): 3609, 3452, 2210 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ: 0.88 (br t, J = 6.7 Hz, 3H), 1.20−1.44 (m, 6H), 1.46−1.64 (m, 2H), 1.78 (quint, J = 6.2 Hz, 2H), 2.46 (dt, J = 1.9, 6.2 Hz, 2H), 3.74 (t, J = 6.7 Hz, 2H), 4.13 (m, 1H), 5.58 (d, J = 15.5 Hz, 1H), 5.74 (dd, J = 15.2, 6.7 Hz, 1H), 6.22 (dd, J = 15.2, 10.9 Hz, 1H), 6.50 (dd, J = 15.5, 10.9 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 13.9, 16.1, 22.5, 25.0, 31.3, 31.7, 37.1, 61.6, 72.4, 80.2, 92.4, 111.9, 130.0, 138.2, 140.3. MS *m*/*z*: 236 (M^+), 218 (M^+ − 18), 137, 71. High-resolution MS calcd for $C_{15}H_{24}O_2$ (M^+): 236.1776. Found: 236.1772.

Acute Toxicity Test. Male mice of ddY strain (21−25 g) were purchased from Nihon SLC Co. (Hamamatsu, Japan). The mice were housed in groups of 10/cage (30 × 30 × 16 cm), kept in an air-conditioned room (ambient temperature 22 ± 2 °C and 55 ± 5% relative humidity) with 12-h light cycle, and allowed to take food (F-2 obtained from Funabashi Farm Co., Funabashi, Japan) and water ad libitum. Samples were suspended in physiological saline containing 2% arabia gum and administered intraperitoneally (10 mL/kg body weight) to the mice. 10 mice were used per group in the same dose. The LD_{50} values were estimated according to the Litchfield−Wilcoxon method.[14]

Receptor-Binding Assay. Cerebral cortex from rats (SD: IGS, 7w, Charles River Japan Inc.) were removed after decapitation, weighed (wet weight), homogenized in 10-fold volume of 50 mM Tris-HCl (pH 7.4) on an ice bath and centrifuged (50000*g*, 4 °C, 20 min). The membrane fraction thus obtained was washed with a 10-fold volume of buffer by the same centrifugation procedure and strirred at −80 °C until use as receptor standards.

[^{3}H]EBOB binding assays were carried out according to the procedure reported by Lewin et al.[15] Briefly, polyacetylenic alcohols (0.1 mL) were incubated with membranes of rat brain cerebral cortex (5 mg tissue, 0.5 mL), 3 nM [^{3}H]EBOB (0.1 mL) and 50 mM Tris-HCl buffer (pH 7.4, 0.3 mL) at 25 °C for 1 h. After incubation, the mixture were filtrated through GF/B filters which were treated with 0.3% ethylenimine, and then the filters were mixed with liquid scintillator (5 mL). Radioactivity retained on the filters was counted in a PACKARD 1500 liquid scintillation counter. Nonspecific binding was defined in the presence of picrotoxin (1×10^{-4} M).

Supporting Information Available: Purity criteria for new polyacetylenes. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM000185K