Insecticidal and Neuromuscular Activities of Domoic
Acid and Its Related Compounds

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Domoic acid isolated from a seaweed, Chondria armata, showed extremely strong insecti-
cidal activity against cockroaches and houseflies. The minimum effective dose against the
American cockroach by subcutaneous injection was 0.8 µg/g. This compound also induced
significant contraction of the hindgut excised from the American cockroach. The site of
action may be the neuromuscular junction, similar to that of α-kainic acid. The structure-
activity relationships of domoic and α-kainic acids and their analogs were also investigated.
The synthetic analogs were useful tools for delineating the structurally essential requirement
for the insecticidal activity.

INTRODUCTION

Domoic acid was isolated by Takemoto and co-workers from a seaweed, Chondria armata,
as an anthelmintic in the mid 1950’s.1) Its structure and stereochemistry were confirmed
to be (2S, 3S, 4S)-2-carboxy-4-[l-methyl-5(R)-carboxyl-l (Z), 3(E)-hexadienyl]pyrrolidine-3-
acetic acid (I).2-4) Along with α-kainic acid, (2S, 3S, 4S)-2-carboxy-4-isopropenylpyrroli-
dine-3-acetic acid (II),5,6) which was also iso-
lated from a seaweed, Digenea simplex, domoic
cid acid is regarded as an analog of L-glutamic acid where isoprene units are condensed as
the side chain which is cyclized with the amino group. Takemoto observed that flies, which
had been attracted to and contacted, Chondria armata being dried on the seashore, died
shortly afterwards. Curtis and Crawford found that L-glutamic acid (III) and some analogs
were excitants of neurons in various parts of

the mammalian central nervous system,7) while Cook and Holman showed that this class
of compounds were excitatory transmitters at the neuromuscular junction of insects.8) From
the chemical similarities of L-glutamic acid to domoic and α-kainic acids, we expected that
these compounds would have excitatory or inhibitory action on the neuromuscular jun-
cion in insects.

(1) (II) (III)

This paper reports insecticidal activity of
domoic and α-kainic acids against various
insects as well as its contracting effect on the
hindgut of the American cockroach. The
structure-activity relationships of domoic and
α-kainic acids and their derivatives had aided
us in identifying the structure requirements
for the insecticidal and neuromuscular activi-

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MATERIALS AND METHODS

1. Materials

Domoic acid (I), α-kainic acid (II) and α-allo-kainic acid (IV) were generously supplied by Professor Tsunematsu Takemoto, Toku-shima Bunri University. α-Kainic acid di-methyl ester (V) was derived from α-kainic acid from the treatment with CH₂N₂ in MeOH.

N-Methyl (VI), -ethyl (VII), and -isopropyl (VIII) kainic acids were synthesized by the reductive amination of corresponding carbonyl compounds (formaldehyde, acetaldehyde and acetone) with α-kainic acid using sodium cyanoborohydride.⁹ The carbonyl analog of α-kainic acid (IX) was prepared by ozonolysis of α-kainic acid according to the procedure described in a previous paper.¹⁰ The Z, Z (X), E, E (XI) and Z, E (XII) side-chain geometrical isomers of domoic acid “monomethylol” were obtained from the Wittig reaction of the protected (2S, 3S, 4S)-2-carboxy-[1-methyl-2(Z or E)-formyl-ethenyl]pyrrolidine-3-acetic acid and the protected (2S)-methyl-1-bromo-propane.⁵,⁶ L-Glutamic acid and other reagent grade chemicals were obtained from Nakarai Chemicals Co., Kyoto.

2. Insect

German cockroaches, Blattella germanica L., American cockroaches, Periplaneta americana L., and houseflies, Musca domestica L. (Takatsuki strain) were reared on artificial diet at 25°C, 60% relative humidity under short-day photoperiods (12L : 12D).

3. Insecticidal Activity

For the activity test against the American cockroaches, a series of aqueous solution was prepared containing various concentrations of each compound. Each solution (10 µl) was injected subcutaneously into the abdomen (between the third and the fourth segments avoiding the central zone) of an adult male American cockroach with a micrometer syringe. These cockroaches were kept in a plastic cup at 25°C and the mortality was counted after 24 hr. To inhibit the oxidative metabolic activity of the insects, piperonyl butoxide (50 µg per insect) in McOH (1 µl) was injected 1 hr prior to each test compound. The test compound was injected on the opposite side where piperonyl butoxide had been injected. With the houseflies and German cockroaches, the activity was determined by the topical application method. A 90% methanol solution (1 µl) containing various concentrations of each test compound was applied to individual female flies on the dorsal thoracic region. With adult male German cockroaches, an aqueous solution was applied. The mortality was counted after 24 hr.

4. Contractile Activity on the Hindgut from American Cockroach

Adult male American cockroaches were given only water for at least 7 days before dissection. The hindgut tissue was excised according to a procedure similar to that of Cook and Holman.¹¹ Each excised hindgut was perfused with saline solution of pH 6.8 including 156 mM NaCl, 2.7 mM CaCl₂ and 22 mM glucose. The two ends of the hindgut were tightly tied at the pharyngeal and the proventriculus regions with silk threads. The remainder of the thread used to tie the pharyngeal area was looped over the recording beam of a Nihon-Koden Model TD III Isotonic Transducer. The thread tying the proventriculus end was fixed to the bottom of a cell containing 5 ml of a pH 7.2 insect saline solution including 110.4 mM NaCl, 2.9 mM KCl, 1.8 mM CaCl₂, 1.8 mM Na₂HPO₄.
and 0.2 mM KH₂PO₄. With air bubbled from a capillary located at the bottom of the cell, various amounts of stock solution of each compound were added to the cells. Muscle contractions were detected by the transducer and recorded with a conventional pen-recorder for each dose. The hindgut was repeated used for the contraction experiments with various compounds. Before each experiment, the hindgut was washed three times with fresh saline (pH 6.8) and allowed to recover its sensitivity for 15 min. All of the experiments were conducted at 25°C.

5. Neurophysiological Experiments with Central Nerve Cords of American Cockroach

Neuroexcitatory and neuroblocking activities were determined by means of an extracellular technique using excised central nerve cords of adults of the male American cockroach containing all thoracic and abdominal ganglia and a single cercal nerve at 20(±1)°C according to a procedure described recently.¹²

RESULTS

1. Insecticidal Activity

Domoic (I) and α-kainic (II) acids exhibited significant insecticidal activity when they were injected subcutaneously into the abdomen of American cockroaches. As shown in Table 1, domoic acid (I) is highly active, the minimum dose being 0.8 µg/g insect. In addition, the effect is exerted remarkably rapidly. The insecticidal activity is enhanced by simultaneous application of piperonyl butoxide. Compound (IX), the ozonolyzed product of α-kainic acid, has an insecticidal activity similar to that of α-kainic acid (II). N-Methyl-(VI), -ethyl-(VII), and -isopropyl-(VIII) kainic acids and α-kainic acid dimethyl ester (V) do not show insecticidal activity at the dose of 100 µg/g. α-Allo-kainic acid (IV), a stereoisomer of α-kainic acid at C-4 in pyrrolidine ring, is also inactive. The Z, Z (X) and E, E (XI) geometrical isomers of domoic acid “mono-methylol” are inactive, whereas the Z, E isomer (XII) is about 1/10 as active as domoic acid. With piperonyl butoxide treatment against the American cockroach, the minimum lethal dose of domoic acid (I) corresponds to 1.3×10⁻⁷ mol/insect which is comparable with the value of phenothin, 3.2×10⁻¹⁹ mol,¹² and that of DDT, 7.4×10⁻⁹ mol,¹³ while that of α-kainic acid (II), 9×10⁻⁸ mol/insect, is higher than that of DDT.

Domoic acid (I) is a potent topical insecticide against houseflies as well as German cockroaches, as shown in Table 2. The dose of domoic acid (I) required for 50% mortality against German cockroaches corresponds to 1.9×10⁻⁹ mol/insect which is about the same as that of γ-BHC, 6.5×10⁻¹⁰ mol.¹⁴ The dose against houseflies corresponds to 3×10⁻¹⁰ mol/insect which is also about the same as that of γ-BHC, 9.3×10⁻¹¹ mol, as well as that of DDT, 3.1–8.1×10⁻¹⁰ mol.¹⁴

2. Contractile Activity on Cockroach Hindgut

Figure 1 shows the myographic recording in which the peak height corresponds to the extent of contraction. Dose-response relationships of domoic and L-glutamic acids are shown in Fig. 2. The threshold concentration of domoic acid (I) was about 1×10⁻⁵ g/ml and

Table 1  Insecticidal activity against American cockroach by injection.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (µg/g)</th>
<th>Mortality (%)</th>
<th>Without PB</th>
<th>With PB</th>
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<tr>
<td>I</td>
<td>1</td>
<td>100</td>
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<td></td>
<td>0.8</td>
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<td>0.4</td>
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<td>II</td>
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<td>IX</td>
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<td>10</td>
<td>0</td>
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¹³) Four adult male American cockroaches were used and all treatments were replicated at least twice.

¹⁴) PB: piperonyl butoxide.
that of L-glutamic acid about 5 × 10⁻⁴ g/ml. With L-glutamic acid, the contraction reached a plateau at 1 × 10⁻⁵ g/ml. Within this concentration range, the contraction was transitory within 15 sec, followed by rapid desensitization (Fig. 1). At the dose of 4 × 10⁻⁴ g/ml of L-glutamic acid, the muscle was completely desensitized 1 min after application and did not respond to further addition of L-glutamic acid. When the hindgut was rinsed with fresh saline solution of pH 6.8, the sensitivity was almost completely recovered from the desensitized state (Fig. 1).

\[ \alpha-Kainic \ acid \ (II) \ has \ significant \ contractile \ activity, \ as \ shown \ in \ Table \ 3, \ but \ it \ is \ lower \ than \ that \ of \ L-glutamic \ acid \ (III). \ N-Alkyl \ kainic \ acids \ (VI, VII, VIII), \ dimethylster \ of \ \alpha-kainic \ acid \ (V) \ and \ \alpha-allo-kainic \ acid \ (IV) \ show \ no \ contractile \ potency \ at \ 20 \mu g/ml. \ The \ acetyl \ analog \ (IX) \ exhibits \ an \ activity \ approximately \ equal \ to \ that \ of \ \alpha-kainic \ acid \ (II), \ whereas \ the \ side-chain \ analogs \ (X, XI) \ are \ less \ active \ than \ 1/100 \ of \ the \ parent \ domoic \ acid \ (I), \ and \ compound \ (XII) \ is \ as \ active \ as \ \alpha-kainic \ acid \ (II). \]

### 3. Neurophysiological Effect on the Central Nervous System

Domoic acid (I) (100 μg/ml) showed neither excitatory nor blocking effect on the central
nerve cords excised from the American cockroach.

DISCUSSION

Domoic (I) and α-kainic (II) acids show significant insecticidal activities against the American cockroach, as shown in Table 1. Domoic acid (I) is also highly insecticidal against the German cockroach and housefly by topical application. Comparison of the data in Table 3 with those in Table 1 indicates that the contractile potency of the hindgut parallels the insecticidal activity against cockroaches for the set of analogs except for L-glutamic acid.

From this difference in the structure-activity relationship for L-glutamic acid, two possibilities are suggested. The first is that the site of the insecticidal action of the present set of compounds is neither the hindgut neuromuscular junction nor the central nerve cord. The second is that the hindgut is in fact the target site. Being a natural transmitter, L-glutamic acid may be taken up into surrounding cells or organs or may be metabolized to ineffective compounds soon after interacting with the postsynaptic receptor site to prevent prolonged excitation. There are no such regulatory mechanisms for domoic and α-kainic acids, and these excitants maintain the desensitized state of the receptor site by inhibiting the normal response to the natural transmitter, L-glutamic acid, thus leading to paralysis followed by death. Further studies are required to clarify the insecticidal mechanisms.

Sakai has examined the insecticidal activity of domoic acid (I) and found only low toxicity against the houseflies and German cockroaches by topical application and no effect on the dorsal vessel. This is not inconsistent with our result in that the topical activity is significantly lower than that by injection against cockroaches.

The data in Tables 1 and 3 suggest the following structural requirements for the high insecticidal and hindgut-contractile activity found for the compounds examined, except for L-glutamic acid:

1) An active compound must have free carboxyl and imino groups in its structure. This requirement conforms with that delineated by Holman and Cook in which the presence of two acid groups and an amino group was essential for excitatory activity on the hindgut.

2) The geometry of two carbon-carbon double bonds (Δ1 and Δ3) in the lipophilic side-chain of domoic acid (I) must maintain the Z and E configurations.

3) The configuration at C-4 in the pyrrolidine ring must be S.

ACKNOWLEDGMENTS

The authors thank Professor Tsunematsu Takemoto of Tokushima Bunri University for his encouragement, and Professor Shozo Takahashi of the Pesticide Research Institute of Kyoto University for supplying the German cockroaches.

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9) Y. Ohfune, N. Higuchi & M. Saito: unpublished

**要約**

ドウモイ酸および関連化合物の殺虫活性と筋肉収縮活性

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海藻, ハナナナギより抽出して得たドウモイ酸は、ゴキブリおよびイエバエに対して強い殺虫活性を示した。注解法によりワモンゴキブリ雄成虫に対する本物質の最小致死用量は 0.8 μg/g 体重であった。またワモンゴキブリ雄成虫より抽出した後腸に対しても本物質は顕著な収縮活性を示した。以上の事実から, ドウモイ酸の昆虫体内での作用点は神経・筋接合部位であると推論した。本物質の構造活性相関を調べるためにドウモイ酸関連化合物を種々合成し、その殺虫活性および筋肉収縮活性を測定した。その結果、殺虫活性、筋肉収縮活性とともに化学構造と密接な相関関係のあることが判明したもの。