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Studies on Crude Drugs Effective on Visceral Larva Migrans. IV.[1] Isolation and Identification of Larvicidal Principles in Pepper

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Eight new amide constituents together with seven known amides were isolated from pepper and their structures were determined by spectroscopic means. They are piperamide-C5 : 1(2*E*) (**2**), -C7 : 1(6*E*) (**3**), -C7 : 2(2*E*,6*E*) (**4**), -C9 : 1(8*E*) (**5**), -C9 : 2(2*E*,8*E*) (**6**), -C9 : 3(2*E*,4*E*,8*E*) (**7**), 1-[(2*E*,4*E*)-2,4-decadienoyl]pyrrolidine (**8**), and 1-[(2*E*,4*E*)-2,4-dodecadienoyl]pyrrolidine (**9**). Among all of the amides isolated, seven (**3**, **4**, **5**, **6**, **8**, **9**, and **15**) showed larvicidal activity against second-stage larva of *Toxocara canis* and the others were inactive. The assay results suggested that the strongest activity was exhibited by the tertiary amides (piperidin- and pyrrolidin-amides) possessing a nine-carbon chain between the aromatic group and the amine moiety [for example, piperamide-C9 : 1(8*E*), MLC: 0.05 mM after 24 h of incubation]. The aliphatic amides **8** and **9** were also larvicidal. Mass spectra of piperamides are also discussed.

Keywords——pepper; *Piper nigrum*; Piperaceous amide; piperamide; pyrrolidin-amide; larvicidal activity; visceral larva migrans; *Toxocara canis*; mass spectrum; structure–activity relationship

Pepper, fruits of *Piper nigrum* L. (Piperaceae), is the most popular spice in the world, and is also used as a stomachic in Chinese medicine.[2] The constituents of pepper have often been investigated, and the fruits contain terpenes[2] and amides,[3,4] among which the latter compounds are particularly interesting from the viewpoint of biological activities. The pungency of pepper is due to the major amide constituent, piperine. Other constituents include the pyrrolidine analog, piperylin,[3a] longer chain congeners such as piperettine,[3b] piperolein-A,[3a] piperolein-B,[3a] pipercide,[3c] dihydropipercide,[4] guineensine,[3d] and other phenolic and non-phenolic amides.[3d-f] Recently, the *N*-isobutyl amides carrying relatively long chains, such as pipercide and guineensine, were shown to possess insecticidal activity.[4]

In the course of our continuing screening work on crude drugs, plant materials, and spices effective against diseases caused by nematodes such as visceral larva migrans, we have observed that the hot water extract of pepper showed strong larvicidal activity against the second-stage larva of dog roundworm, *Toxocara canis*, which is a common pathogenic parasite in visceral larva migrans.[5] Here we report the isolation and identification of active principles, most of which were found to be new amide constituents.

Isolation of Active Principles

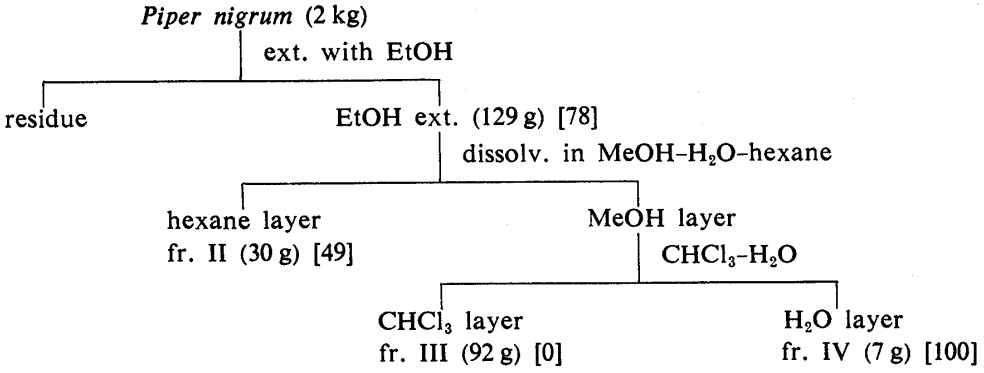
In a preliminary experiment for the isolation of larvicidal principles, ground pepper was successively extracted with hexane, chloroform, methanol, and water under reflux. The larvicidal activity of each fraction (Table I) showed that the activity was concentrated in the organic extracts.

Therefore, pepper was newly extracted with ethanol, and the extract, after concentration, was divided into hexane-soluble fraction (fr. II), chloroform-soluble (fr. III), and aqueous

TABLE I. Preliminary Test of Larvicidal Activity of Pepper Extracts against *T. canis*

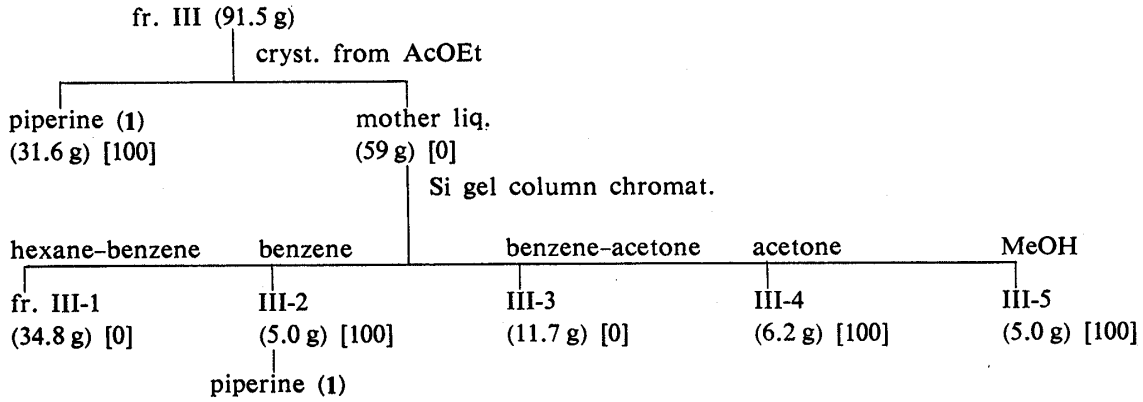
Extract[a]	Yield (%)	RM value[b]			
		1 h	3 h	6 h	24 h
Hexane	15.6	100	99	65	0
Chloroform	10.6	100	95	65	0
Methanol	16.7	100	97	70	0
Water	16.3	100	100	100	100

a) Extraction was done in the order given (from the top). *b*) RM value at 0.1 mg/ml.



( ): yield.
[ ]: RM value at 0.1 mg/ml after 24 h incubation.

Chart 1. Extraction of *Piper nigrum*

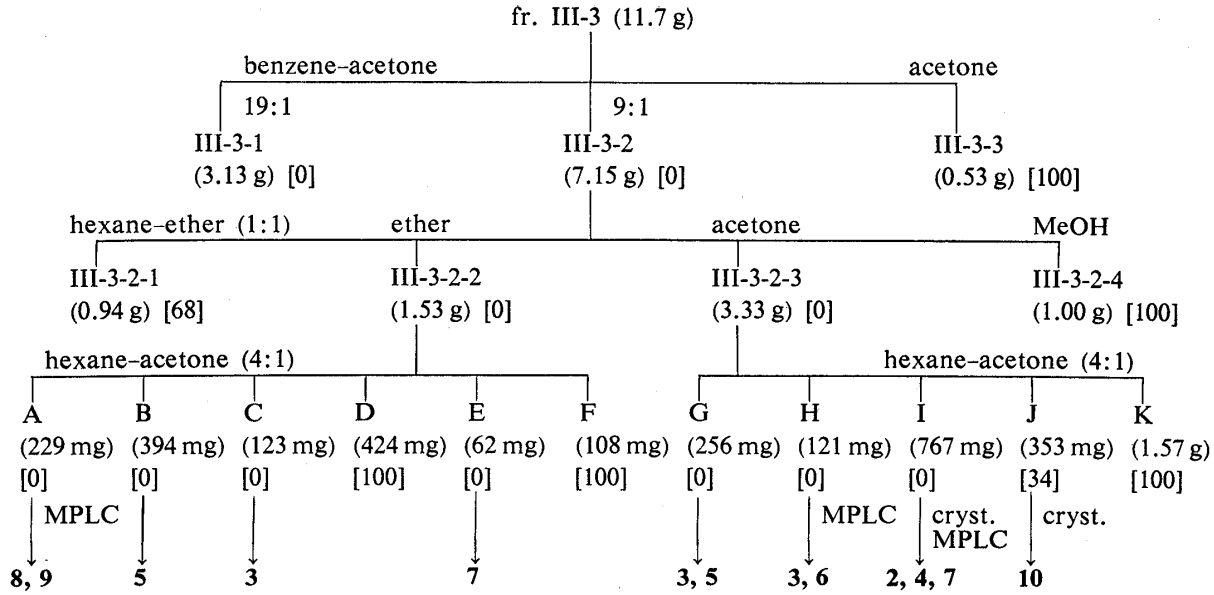


( ): yield.
[ ]: RM value at 0.1 mg/ml after 24 h incubation.

Chart 2. Fractionation of fr. III

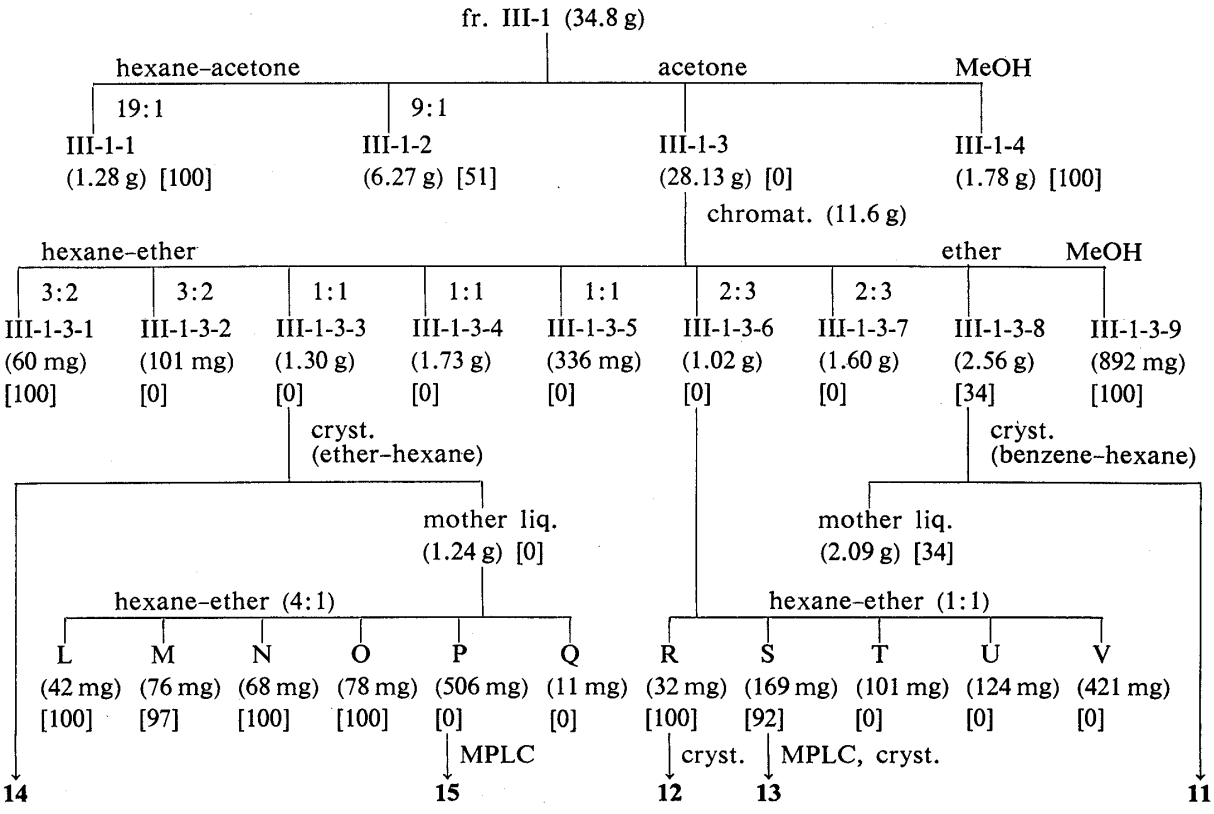
methanol-soluble (fr. IV) fractions, as shown in Chart 1. Among these fractions, fr. III showed strong larvicidal activity, and deposited crystals of piperine (**1**) when kept in ethyl acetate. However, piperine was inactive (1 mM, 24 h, relative mobility (RM) = 100), and the activity was concentrated in the mother liquor (0.1 mg/ml, 24 h, RM = 0). This active fraction was fractionated by silica gel column chromatography with monitoring by thin layer chromatography (TLC) and in terms of RM value[6] at a concentration of 0.1 mg/ml, thus yielding two active fractions (III-1 and III-3) (Chart 2).

The fr. III-3 (more polar fraction than piperine) was repeatedly chromatographed on



( ): yield.
[ ]: RM value at 0.1 mg/ml after 24 h incubation.

Chart 3. Chromatographic Separation of fr. III-3



( ): yield.
[ ]: RM value at 0.1 mg/ml after 24 h incubation.

Chart 4. Chromatographic Separation of fr. III-1

silica gel with three different combinations of solvent systems (Chart 3) to obtain seven strongly active (A, B, C, E, G, H, and I), one moderately active (J), and three inactive fractions (D, F, and K). Each active fraction was further subjected to separation and/or

purification by means of normal and reversed-phase preparative medium-pressure liquid chromatographies (MPLC) resulting in the isolation of eight new compounds (**2**—**9**). Six of them (**3**, **4**, **5**, **6**, **8**, and **9**) showed larvicidal activity, but two (**2** and **7**) were inactive (see below). The moderately active fraction (J) gave yellow needles, mp 148—149 °C; this product was identical with piperylin (**10**)[3a] and was found to be inactive.

The fr. III-1 (less polar fraction than piperine) was separated by two chromatographies (Chart 4) into six active (III-1-3-2—III-1-3-7), one moderately active (III-1-3-8), and two inactive (III-1-3-1 and III-1-3-9) fractions. The moderately active fraction (III-1-3-8) gave yellow needles, mp 147—153 °C; this product was identified as piperettine (**11**)[3b] and was found to be inactive. The active fraction III-1-3-6 gave two compounds, colorless prisms (mp 139—142 °C) and colorless needles (mp 122—128 °C), both of which were, however, inactive. The former compound is a new amide as a pepper constituent, but was found to be identical with retrofractamide-A (**12**)[7] previously isolated from *Piper retrofractum* on the basis of comparisons of the spectral data. The latter compound was identical with pipercide (**13**).[3c] Another active fraction III-1-3-3 was subjected to further separation resulting in two compounds; one (colorless needles, mp 118—120 °C) was inactive and the other (oil) was an active compound. These were identified as guineensine (**14**)[3d] and piperolein-B (**15**),[3a] respectively.

Thus, we have isolated seven active (**3**, **4**, **5**, **6**, **8**, **9**, and **15**) and eight inactive constituents (**1**, **2**, **7**, **10**, **11**, **12**, **13**, **14**) from pepper. Among these, compounds **1**, **10**, **11**, **12**, **13**, **14**, and **15** are known and the others are new compounds.

Although some other fractions in fr. III-1 showed strong activity, isolation of the active principle was not achieved in the present investigation. This will be a subject of future investigations.

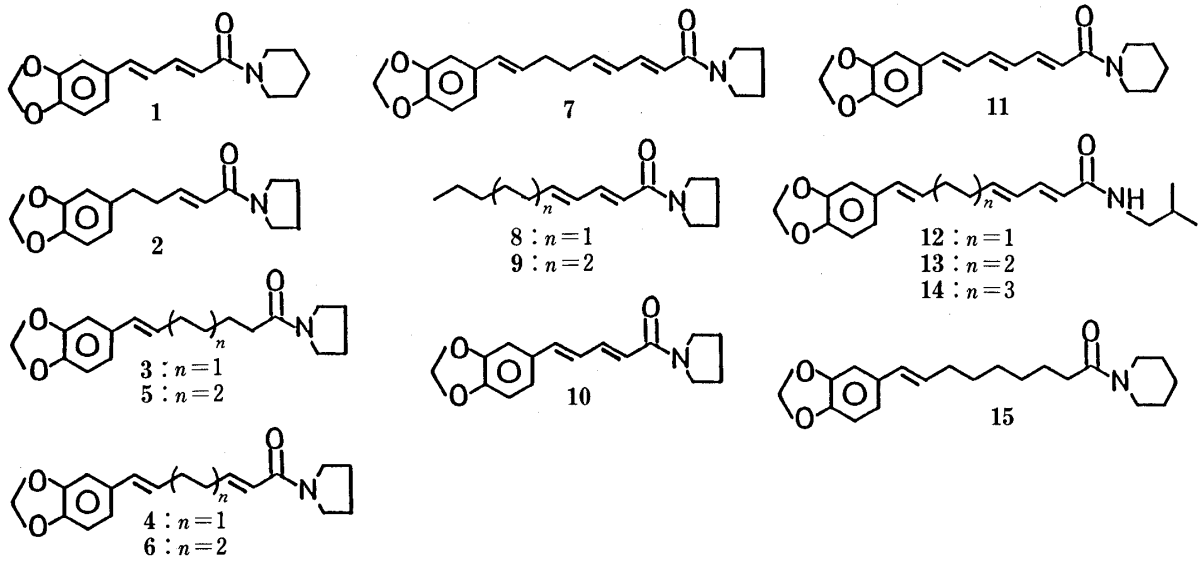


Chart 5

Proposal of a Systematic Nomenclature for Piperaceous Amides

As seen in the forthcoming section most of the amides we have isolated from pepper have very similar structures to each other. Further, because many amides with closely related structures have been isolated from Piperaceous plants and various names given to them from time to time without any system, it is necessary to avoid confusion in designating them.

Here, we wish to propose a systematic nomenclature adopting the general name "piperamide" for the compounds carrying an aromatic group and an amide group. The

compounds possessing a methylenedioxyphenyl group as an aromatic nucleus are designated as "piperamide-A, -B, or -C *m*:*n*," where A, B, and C indicate piperidin-, *N*-isobutyl-, and pyrrolidin-amide, respectively, and *m* indicates the number of carbon atoms between the nitrogen and the aromatic nucleus, while *n* is the number of double bond(s) therein. The position and configuration of the double bond(s) can be shown in parentheses. Thus, piperine (**1**), piperylin (**10**), piperettine (**11**), piperolein-B (**15**), retrofractamide-A (**12**), pipercide (**13**), and guineensine (**14**) are piperamide-A5:2(*E*,*E*), piperamide-C5:2(*E*,*E*), piperamide-A7:3-(*E*,*E*,*E*), piperamide-A9:1(8*E*), piperamide-B9:3(2*E*,4*E*,8*E*), piperamide-B11:3(2*E*,4*E*,10*E*), and piperamide-B13:3(2*E*,4*E*,12*E*), respectively. The amides carrying a new amine moiety, when isolated, may be designated as piperamide-D, -E, and so on.

Structure Determinations of Active Principles

The structures of the above isolated new amides (including inactive ones) were determined as follows.

Compound **2** was an oil and had the formula $C_{16}H_{19}NO_3$ as judged from the high-resolution mass spectrum (HRMS). The ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectrum indicated a 3,4-methylenedioxyphenyl group and a double bond, these being not conjugated as judged from the ultraviolet (UV) spectrum (λ_{max} 286 nm). The NMR signals characteristic of a pyrrolidine ring [δ 1.8—2.0 (4H), 3.4—3.5 (4H)] and infrared (IR) absorptions at 1600 and 1655 cm^{-1} indicated that the compound has a conjugated pyrrolidin-amide moiety. The configuration of the double bond was assigned as *E* from the coupling constant of 15.0 Hz. Hence, compound **2** was elucidated as 1-[(2*E*)-5-(3,4-methylenedioxyphenyl)-2-pentenoyl]-pyrrolidine, or piperamide-C5:1(2*E*) (**2**).

Compounds **3**—**7** possess a methylenedioxyphenyl group which is conjugated with a double bond as suggested by their UV spectra (λ_{max} 305—306 nm). They also have a pyrrolidin-amide group as indicated from the characteristic NMR signals [δ 1.8—2.0 (4H), 3.4—3.6 (4H)] and a mass spectral (MS) fragment ion at *m/z* 70 (see below).

Compound **3** was an oil and had the formula $C_{18}H_{23}NO_3$. The amide group in this compound is saturated (1620 cm^{-1}) and the double bond [δ 6.04 (1H), 6.29 (1H)] conjugated to the aromatic ring has *E*-configuration (J=15.5 Hz). The signals at δ 2.21 (2H) and 2.38 (2H) were assigned to the methylene groups connected to the double bond and the carbonyl group, respectively. The remaining signals at δ 1.51 (2H) and 1.71 (2H) are assignable to CH_2CH_2 between the above methylene groups. Thus it is 1-[(*E*)-7-(3,4-methylenedioxyphenyl)-6-heptenoyl]pyrrolidine, or piperamide-C7:1(6*E*) (**3**).

Compound **4** was an oil and had the formula $C_{18}H_{21}NO_3$. It is an unsaturated amide (1600, 1655 cm^{-1}). Two methylene groups (δ 2.31—2.39, 4H) are located between the two double bonds [δ 6.04 (1H), 6.14 (1H), 6.33 (1H), 6.94 (1H)]. The configuration of both double bonds was assigned as *E* from the coupling constants (J=15.0, 15.5 Hz). It was thus concluded to be 1-[(2*E*,6*E*)-7-(3,4-methylenedioxyphenyl)-2,6-heptadienoyl]pyrrolidine, or piperamide-C7:2(2*E*,6*E*) (**4**).

Compound **5** was an oil and had the formula $C_{20}H_{27}NO_3$. It has a double bond (δ 5.98, dt, J=15.5 and 6.5 Hz, and 6.28, d, J=15.5 Hz) and a saturated amide (1635 cm^{-1}). The spectral pattern of this compound was quite similar to that of piperolein-B (**15**) except that the NMR signals characteristic of a pyrrolidine group instead of a piperidine group are present. The *E*-configuration of the double bond assigned from ^{1}H-NMR (J=15.5 Hz) was confirmed by the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum, since the C-7 signal of this compound appeared at δ 32.8.[8] Hence it was identified as 1-[(*E*)-9-(3,4-methylenedioxyphenyl)-8-nonenoyl]pyrrolidine, or piperamide-C9:1(8*E*) (**5**).

In 1976, Singh *et al.*[9] isolated an amide constituent named tricholein from *Piper trichostachyon* and proposed the structure **5**. However, the reported IR absorption (1608,

1655 cm^{-1}) for tricholein is not consistent with the proposed structure, instead showing the presence of a conjugated amide (see accompanying paper). Moreover, the base peak at *m/z* 175 for tricholein reported by the previous authors was completely absent in our compound. Although the reported NMR spectrum (at 60 MHz) of tricholein is not in conflict with our 400 MHz spectrum of piperamide-C9:1(8*E*),[10] we suspect that "tricholein" reported by the previous authors may be a compound of a different structure or a mixture.

Compound **6**, an oil, $C_{20}H_{25}NO_3$, has an additional double bond (compared to **5**), which is conjugated with the amide group (1600, 1655 cm^{-1}). The *E*-configurations of the two double bonds assigned from ^{1}H-NMR (J = 14.5, 15.5 Hz) were confirmed by ^{13}C-NMR of this compound; δ 32.2 and 32.6 for C-4 and C-7, respectively. Hence the compound is 1-[(2*E*,8*E*)-9-(3,4-methylenedioxyphenyl)-2,8-nonadienoyl]pyrrolidine, or piperamide-C9:2(2*E*,8*E*) (**6**).

Compound **7**, mp 105—106 °C, $C_{20}H_{23}NO_3$, has three double bonds, two of which are conjugated with the amide group (1590, 1625, 1650 cm^{-1}). The strong UV absorption at 270 nm (log ε 4.60) supported this assignment. Since all the double bonds were assigned to be of *E*-configuration (J = 14.5, 14.5, 15.5 Hz), the compound was elucidated as 1-[(2*E*,4*E*,8*E*)-9-(3,4-methylenedioxyphenyl)-2,4,8-nonatrienoyl]pyrrolidine, or piperamide-C9:3(2*E*,4*E*,8*E*) (**7**).

Compound **8**, an oil, $C_{14}H_{23}NO$, is also a pyrrolidin-amide as indicated from the NMR signals at δ 1.8—2.0 (4H) and 3.5—3.6 (4H) and a diagnostic fragment ion at *m/z* 70 in the MS. Unlike the above compounds, it lacks a methylenedioxyphenyl group, and instead has a straight chain aliphatic acyl group (CH_3: δ 0.89, 3H, t) which contains two double bonds [δ 6.08 (2H), 6.18 (1H), 7.27 (1H)]. The amide group of compound **8** is conjugated with these double bonds [λ_{max} 265 nm (log ε 4.32), IR: 1600, 1620, 1650 cm^{-1}]. Since the configuration of the double bonds was assigned as *E* for both Δ^2 and Δ^4 (J = 14.5, 15.0 Hz), the structure was concluded to be 1-[(2*E*,4*E*)-2,4-decadienoyl]pyrrolidine (**8**). This seems to be the first report of the occurrence of **8** as a natural product, although it has been already obtained by synthesis.[11]

Compound **9** was not obtained in a completely pure form. However, comparisons of its ^{1}H-NMR spectrum and MS with those of **8** allowed us to elucidate its structure as 1-[(2*E*,4*E*)-2,4-dodecadienoyl]pyrrolidine (**9**). It was suggested to be active from the assay result of the crude fraction (RM = 0, 24 h, 0.1 mg/ml).

MS of Piperamides

The above elucidated structures of piperamides (abbreviated as PA hereafter) are supported by their MS, which can be classified into several types reflecting the structural features. Figure 1 schematically shows the bonds which are susceptible to fragmentation and the ions derived therefrom.

Non-conjugated amides (type I) always exhibit the base peak l through McLafferty rearrangement of the amide moiety. As in usual alkyl amides,[12] ion n due to the cleavage of the γ-bond at the carbonyl group is also appreciable. The other peaks indicative of an amine moiety are ions i, j, and k. Among these, ion i is particularly useful as a diagnostic peak for the kind of amine, *i.e.*, *m/z* 70 for pyrrolidin-, 72 for *N*-isobutyl-, and 84 for piperidin-amide.

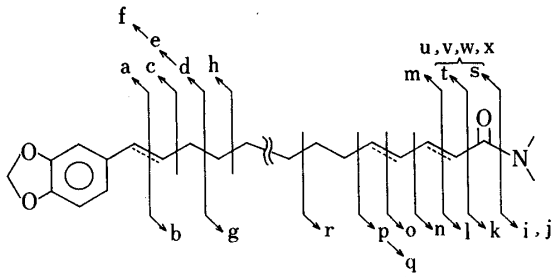


Fig. 1. General Scheme of Mass Fragmentation of Piperamides and Ions
Details see Figs. 2—4 and text.

TABLE II. Mass Spectral Data for Piperamides (EI, at 20 eV)

Type	Piperamide	Ion (intensity %)																								
		M^+	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	q	r	s	t	u	v	w	x
I-a	A5:0[a]	84	39	38	37	—	—	—	—	—	50	24	35	100	—	71	—	—	—	—	—	—	40	—	—	—
	C5:0[a]	56	34	19	27	—	—	—	—	—	19	14	19	100	—	62	—	—	—	—	—	—	17	—	—	—
	B5:0[a]	86	92	24	67	—	—	—	—	—	27	26	—	100	—	47	—	—	—	—	—	—	55	—	—	—
	C7:0[a]	26	27	29	—	—	—	—	—	—	11	9	9	100	—	56	—	—	—	—	—	—	—	—	—	—
	C9:0[a]	30	31	63	—	—	—	—	—	—	12	10	7	100	—	61	—	—	—	—	—	—	—	—	—	—
I-b	C7:1 (6*E*)	31	7	9	3	3	8	8	3	11	6	7	8	100	—	19	—	—	—	—	—	—	5	—	—	—
	A9:1 (8*E*)	40	24	25	11	9	23	10	15	4	20	22	14	100	—	58	—	—	—	—	—	—	5	—	—	—
	C9:1 (8*E*)	59	26	32	14	10	25	10	17	—	35	30	20	100	—	64	—	—	—	—	—	—	8	—	—	—
II	C7:2 (2*E*,6*E*)	17	—	—	—	63	100	35	—	—	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	C9:3 (2*E*,4*E*,8*E*)	9	—	—	—	81	100	37	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	B9:3 (2*E*,4*E*,8*E*)	6	—	—	—	100	99	28	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	C5:1 (2*E*)	11	100	—	—	—	—	—	—	—	3	—	—	—	—	—	—	—	—	—	—	—	—	5	—	—
III-a	C9:2 (2*E*,8*E*)	34	29	46	11	14	39	27	—	—	22	11	33	49	12	27	29	100	—	—	—	11	—	16	—	—
	A9:2 (2*E*,8*E*)[b]	29	40	49	16	8	45	29	—	—	61	16	36	37	10	58	27	100	—	—	—	14	—	26	—	—
	B9:2 (2*E*,8*E*)[b]	47	100	62	23	25	94	53	—	—	—	—	—	20	33	15	45	79	20	—	—	45	—	32	—	—
III-b	B11:3 (2*E*,4*E*,10*E*)	35	100	49	21	23	52	23	—	—	—	—	—	—	—	—	—	40	—	12	—	11	—	—	—	—
	B13:3 (2*E*,4*E*,12*E*)	74	71	60	34	55	100	29	—	—	—	—	—	—	—	—	—	90	12	43	—	—	—	—	—	—
IV	A5:2 (*E*,*E*)	66	—	—	—	—	—	—	—	—	29	—	8	—	—	—	—	—	—	—	100	32	—	20	26	71
	B5:2 (*E*,*E*)[a]	72	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	100	72	—	27	25	57
	C5:2 (*E*,*E*)	59	—	—	—	—	—	—	—	—	7	—	10	—	—	—	—	—	—	—	100	29	—	17	17	47
	A7:3 (*E*,*E*,*E*)	94	—	—	—	—	—	—	—	—	25	—	74	—	—	—	—	—	—	—	33	55	39	30	83	100

a) Synthetic compound (see N. Nakamura, F. Kiuchi, and Y. Tsuda, *Chem. Pharm. Bull.*, **36**, 2647 (1988)). *b*) Recently synthesized in our laboratory (to be published).

Type I amides can be further classified into two groups based on the absence or presence of a double bond conjugated to the aromatic group; type I-a and I-b. They are distinguishable by the absence or presence of the ions due to the fragmentation process B discussed below. The fully saturated compounds (type I-a) only show the tropylium ion a (*m/z* 135) and the ion b (corresponding to the opposite half to the ion a) with appreciable intensities, and these are attributable to the cleavage at the benzylic position. On the other hand, the compounds in which a double bond is conjugated to the aromatic group (type I-b) give, besides the ions a and b which, we consider, are due to the prior migration of the double bond (process A), several additional ions d, e, and f with appreciable intensities. They are the ions due to the fragmentation process B as suggested by Grewe *et al.*[3a] The other ions observed for type I

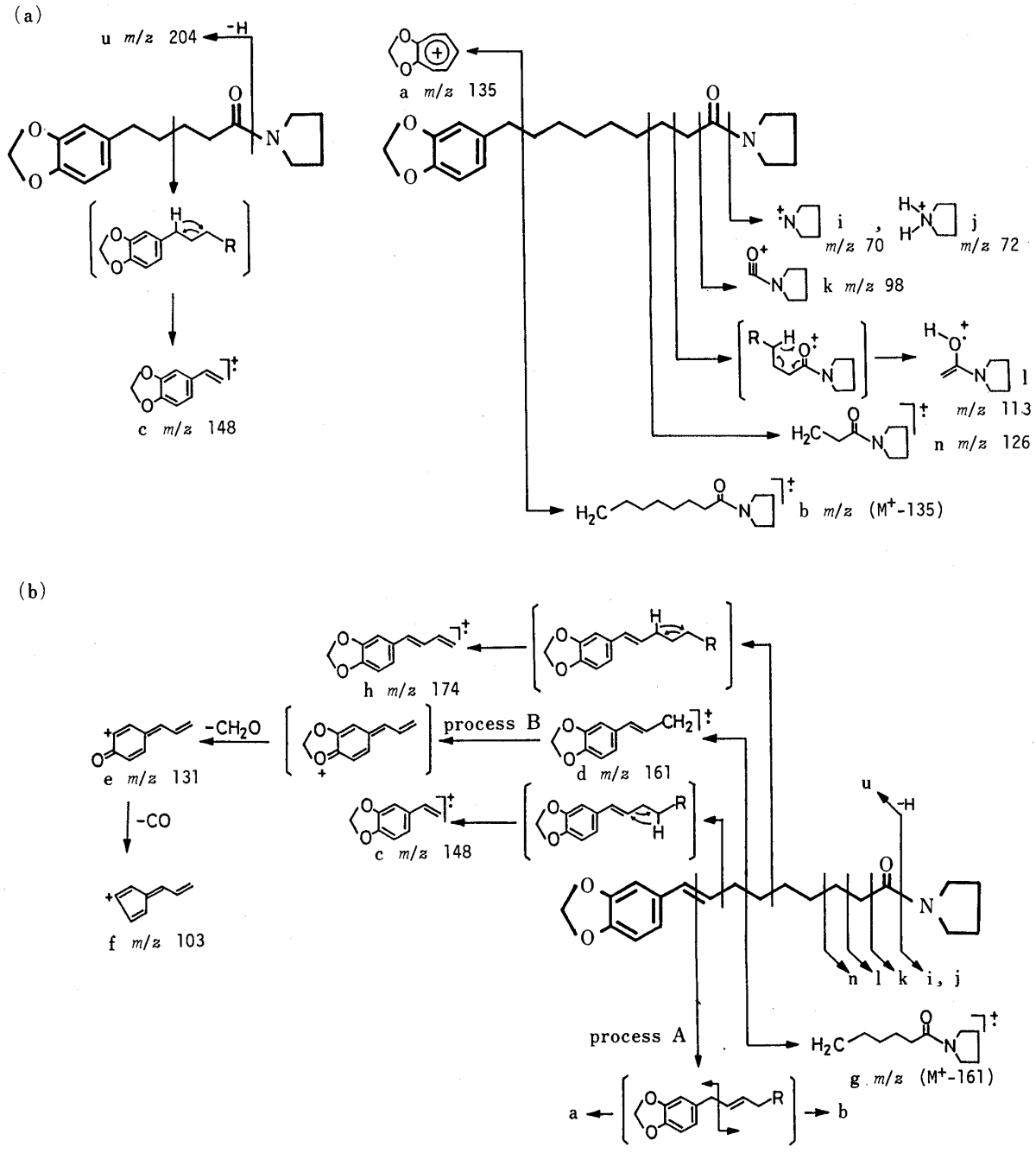


Fig. 2. Fragmentation of Type I Piperamides

2-a, piperamide-C5 : 0, piperamide-C9 : 0 (type I-a); 2-b, piperamide-C9 : 1 (8*E*) (type I-b).

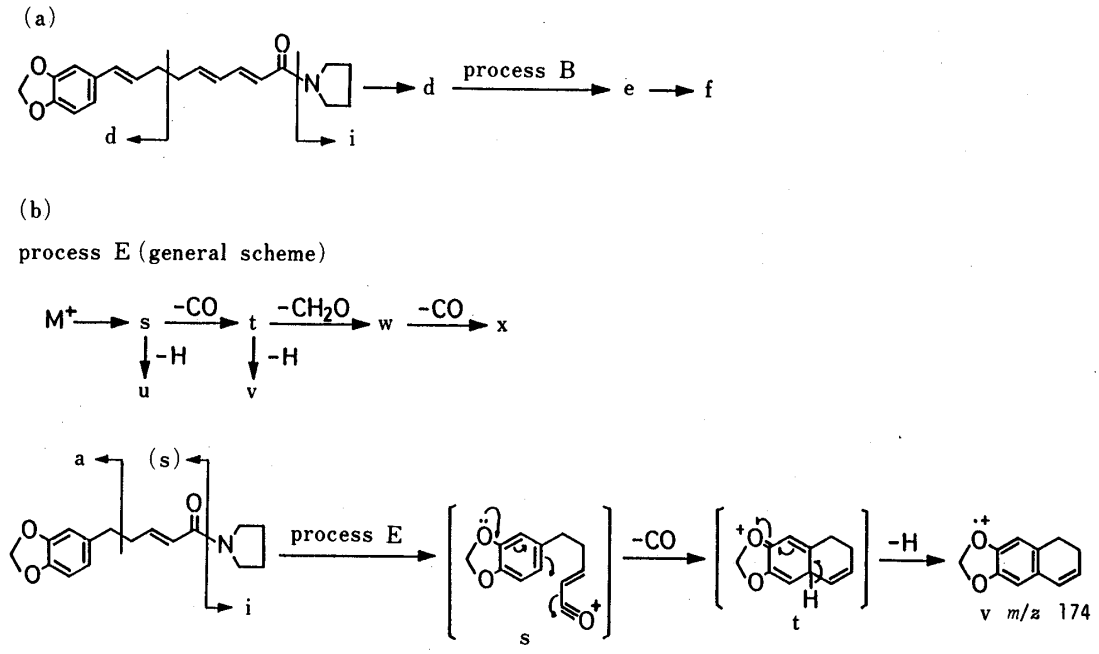


Fig. 3. Fragmentation of Type II Piperamides

3-a, piperamide-C9 : 3 (2*E*,4*E*,8*E*); 3-b, piperamide-C5 : 1 (2*E*).

amides are c and u, which are particularly significant for PA-X5:0 but are negligible for PA-X7:0 and PA-X9:0. Although the ion c from type I-a and I-b amides has the same mass number, it must be derived by a different pathway, as depicted in Fig. 2.

The MS of conjugated amides are variable. For the compounds possessing two methylene groups between the double bonds (type II), cleavage of the bond allylic to both double bonds (thus the fragmentation according to process B) predominates, giving ion d or e as the base peak and very simple spectra.

Piperamide-C5:1(2*E*) is a variant of this type, in which the cleavage at the benzylic position is predominant. The ion diagnostic of the amine moiety (ion i) and that probably derived from the acyl moiety (ion v) were also observed, though the intensities were very weak.

The compounds possessing four or more methylene groups between the double bonds (type III) show complex spectra. In these compounds, the ions due to processes A and B as well as ion c are always significant. The spectra of monoene-amides with four methylene groups between the double bonds (type III-a) show the greatest number of peaks, particularly those for the amine moiety. Among these, cleavage at the bond γ to both double bonds (ion p) sometimes gives the base peak (for piperidin- and pyrrolidin-amides). Ions l and m (opposite half of ion l) are also observed in these amides, and, we consider, are due to prior migration of the double bond followed by McLafferty rearrangement (process C). In *N*-isobutyl diene-amides (type III-b), the ions due to the amine moiety are relatively few and weak compared to those from aromatic portions. They give the base peak by process A or B (ion a or e). The piperidone peak (ion q) suggested by Loder *et al.*[13] (process D) was also observed.

The fully conjugated amides (type IV) show a completely different pattern, in which the ions due to cleavage of the α-bond to the carbonyl (ions s and t) and/or those of further fragmentation (process E) are predominant. Some of these peaks were discussed by Chatterjee and Dutta.[14]

Larvicidal Activity of Piperamides

The *in vitro* minimal lethal concentrations (MLC) of the above isolated piperamides and

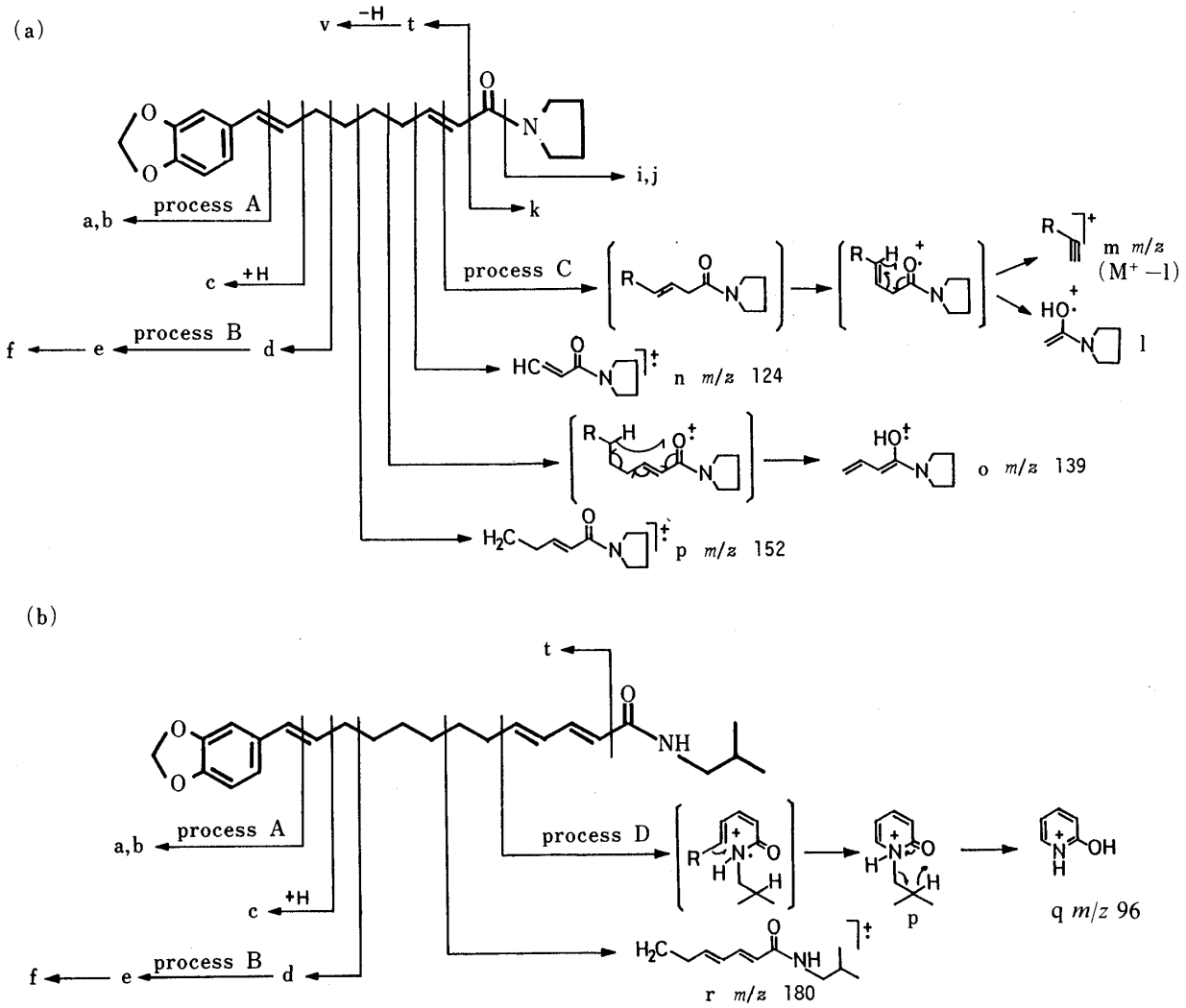


Fig. 4. Fragmentation of Type III Piperamides

4-a, piperamide-C9 : 2 (2*E*,8*E*) (type III-a); 4-b, piperamide-B13 : 3 (2*E*,4*E*,12*E*) (type III-b).

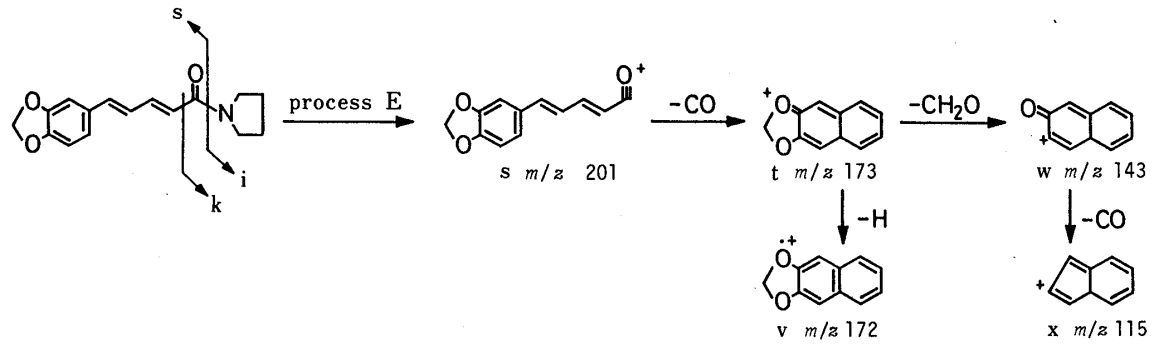


Fig. 5. Fragmentation of Type IV Piperamides

Piperamide-C5 : 2 (*E*,*E*).

some aliphatic amides against the second-stage larva of *Toxocara canis* are listed in Table III.

Table III suggests that the appropriate carbon lengths (7—9) between the methylenedioxyphenyl and the amine moiety, together with flexibility of the molecule, may be necessary for the larvicidal activity. It is also suggested that piperidine and pyrrolidine amides are active

TABLE III. Minimal Lethal Concentrations (MLC) of the Amide Constituents Isolated from Pepper against *Toxocara canis* (mM, after 24 h)

Compound	MLC	Compound	MLC
Piperamide-A5 : 2 (*E,E*) (**1**)	>1	Piperamide-C9 : 1 (8*E*) (**5**)	0.05
Piperamide-C5 : 1 (2*E*) (**2**)	>1	Piperamide-C9 : 2 (2*E*,8*E*) (**6**)	0.15
Piperamide-C5 : 2 (*E,E*) (**10**)	>1	Piperamide-C9 : 3 (2*E*,4*E*,8*E*) (**7**)	>1
Piperamide-A7 : 3 (*E,E,E*) (**11**)	>1	Piperamide-B11 : 3 (2*E*,4*E*,10*E*) (**13**)	>1
Piperamide-C7 : 1 (6*E*) (**3**)	0.25	Piperamide-B13 : 3 (2*E*,4*E*,12*E*) (**14**)	>1
Piperamide-C7 : 2 (2*E*,6*E*) (**4**)	0.3	1-[(2*E*,4*E*)-2,4-Decadienoyl]pyrrolidine (**8**)	1.5
Piperamide-A9 : 1 (8*E*) (**15**)	0.3	1-[(2*E*,4*E*)-2,4-Dodecadienoyl]pyrrolidine (**9**)	<0.5[a]
Piperamide-B9 : 3 (2*E*,4*E*,8*E*) (**12**)	>1		

a) Not exactly determined.

and *N*-isobutyl amides are inactive.

Miyakado *et al.*[15] reported that the insecticidal activity of the Piperaceous amides against *Callosobruchus chinensis*, adzuki bean weevil, is the strongest when the compounds are *N*-isobutyl dienamides with 11 to 13 carbon atoms between the aromatic and amino groups, *i.e.*, piperamide-B11:3 (**13**) and piperamide-B13:3 (**14**). This is in remarkable contrast to our result and suggests that our larvicidal activity on *Toxocara canis* works through a different mechanism.

To our great interest, the aliphatic amides **8** and **9** are also larvicidal. Since some aliphatic acids and alcohols with an appropriate carbon chain length are known to be larvicidal,[6] the above result suggests that suitably designed aliphatic amides may also be strong larvicides.

The structure–activity relationship of piperamides and aliphatic amides will be discussed in more detail in a future publication.

Experimental

General——Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. UV spectra were measured with a Hitachi 323 spectrophotometer in methanol and are given as λ_{max} (ε). IR spectra were taken on a JASCO A-202 spectrometer and are given in cm^{-1}. ^1H- and ^{13}C-NMR spectra were measured on a JEOL GX-400 and/or JEOL FX-100 spectrometer in $CDCl_3$ with tetramethylsilane as an internal standard, and chemical shifts are given in δ. MS and HRMS were measured with a Hitachi M-80 machine at an ionization voltage of 20 or 70 eV, respectively. Fuji Davison BW-820MH (silica gel) was used for column chromatography. For preparative MPLC, LiChroprep Si-60 size B (Si-60) and LiChroprep RP-8 size B (RP-8) were used for normal and reversed-phase chromatography, respectively. For TLC, Macherey–Nagel precoated TLC plates SIL G-25 UV_{254} and Merck HPTLC precoated plates RP-8 F254 were used for normal and reversed-phase, respectively.

Assay Method——Larvicidal activity of each fraction was tested by the method previously described.[6] For one assay, 20 second-stage larvae of *Toxocara canis* were incubated with the test solution in a Corning cell well at 37 °C and the behavior of the larvae was observed under a microscope at 1, 3, 6, and 24 h after the start of incubation. All assays were done in duplicate. The effect of each test material was assessed according to the state of the larvae and the larvicidal activity was evaluated in terms of the RM value described in the previous paper.[6] A smaller RM value indicates stronger larvicidal activity, and when all larvae die, this value becomes 0. Minimal lethal concentration (MLC) was determined as the lowest concentration with an RM value of 0 after 24 h of incubation.

Extraction of Pepper——Powdered Indonesian pepper (2 kg) was extracted four times with 2 l portions of EtOH under reflux and the combined extract was concentrated *in vacuo* to give a residue (129 g). This was dissolved in MeOH–H_2O (10 : 1, 660 ml) and extracted with hexane (300 ml). The hexane layer, on concentration, gave a residue (fr. II, 30 g). The MeOH layer was concentrated to 300 ml and shaken with $CHCl_3$–H_2O (3 : 2, 500 ml). The $CHCl_3$ layer was concentrated to give a residue (fr. III, 92 g) and the aqueous layer, on concentration, gave fr. IV (7 g) (see Chart 1).

Fr. III, when dissolved in warm AcOEt and kept standing overnight, deposited crystals, which were recrystallized from AcOEt to give piperine **1** (31.6 g). The mother liquor (59 g) from piperine was column-

chromatographed; elution with hexane (Hx)–benzene (1 : 2), benzene (Bz), benzene–acetone (9 : 1), acetone (Ac), and MeOH (Me) gave frs. III-1 (Hx–Bz, Bz), III-2 (Bz), III-3 (Bz, Bz–Ac), III-4 (Bz–Ac, Ac), III-5 (Me), of which frs. III-1 and III-3 were active and frs. III-2, III-4, and III-5 were inactive. Fr. III-2 gave a further crop of piperine (**1**) (see Chart 2).

Fr. III-3 (11.7 g) was also column-chromatographed; elution with benzene–acetone (19 : 1), benzene–acetone (9 : 1), and acetone gave frs. III-3-1, III-3-2, and III-3-3, respectively, of which frs. III-3-1 and III-3-2 were active. Rechromatography of fr. III-3-2 (7.15 g) with hexane–ether (1 : 1), ether, acetone, and MeOH gave fr. III-3-2-1 to III-3-2-4, in which the activity was concentrated in frs. III-3-2-2 and III-3-2-3. Frs. III-3-2-2 (1.53 g) and III-3-2-3 (3.33 g) were each further chromatographed with hexane–acetone (4 : 1), with monitoring of fractions by TLC, to obtain fr. A—K.

MPLC of fr. A (229 mg) on Si-60 with hexane–acetone (3 : 1) gave **9** (19 mg) then **8** (60 mg). Fr. B (394 mg) gave **5**. Frs. C (123 mg) and E (62 mg) were compounds **3** and **7**, respectively, and fr. G (256 mg) was a mixture of **5** and **3**, as revealed by gas chromatography (GC) and high performance liquid chromatography (HPLC). MPLC of fr. H (121 mg) on Si-60 with benzene–acetone (9 : 1), then on RP-8 with EtOH–H_2O (2 : 1) gave **3** (72 mg) and **6** (10 mg). Fr. I (720 mg) gave **7** (67 mg) on crystallization from AcOEt. The mother liquor (620 mg) from **7** was subjected to MPLC on Si-60 with benzene–acetone (9 : 1), then on RP-8 (EtOH–H_2O, 2 : 1) to give **2** (150 mg) and **4** (160 mg). Fr. J, on crystallization from AcOEt–ether, gave piperylin (**10**), which was found to be inactive (see Chart 3).

Fr. III-1 (34.8 g) was column-chromatographed, and elution with hexane–acetone, acetone, and MeOH gave frs. III-1-1 (Hx–Ac 19 : 1), III-1-2 (Hx–Ac 9 : 1), III-1-3 (Hx–Ac 4 : 1, Ac), and III-1-4 (Me). A portion of fr. III-1-3 (11.6 g) was rechromatographed with hexane–ether, ether (Et), and MeOH to give frs. III-1-3-1 (Hx–Et 3 : 1), III-1-3-2 (Hx–Et 3 : 2), III-1-3-3 to III-1-3-5 (Hx–Et 1 : 1), III-1-3-6 to III-1-3-7 (Hx–Et 2 : 3), III-1-3-8 (Et), and III-1-3-9 (Me). Fr. III-1-3-3 gave guineensine **14** (62 mg) on crystallization from ether–hexane. The mother liquor from guineensine and fr. III-1-3-6 were chromatographed on Si-60 with hexane–ether (1 : 1), with monitoring by TLC, to separate frs. L—Q and R—V, respectively.

MPLC of fr. P (506 mg) on RP-8 with EtOH–H_2O (2 : 1) gave piperolein-B (**15**, 320 mg). Fr. R (32 mg) was crystallized from AcOEt–hexane to give retrofractamide-A (**12**, 7 mg). MPLC of fr. S (169 mg) on Si-60 with hexane–acetone (3 : 2) and crystallization of the eluate from AcOEt–hexane gave pipercide (**13**, 25 mg). Fr. III-1-3-8 (2.56 g), on crystallization from benzene–hexane, gave piperettine (**11**, 468 mg) (see Chart 4).

Homogeneity of the above isolated compounds except **9** was confirmed by TLC and GC and/or HPLC, and identifications of the known compounds were done by spectral comparisons.

Piperine (1)——Yellow needles, mp 132—133 °C (lit. mp 127 °C).[3a] UV: 245 (11100), 255 (11200), 262 (11100), 298 sh (18300), 311 (21700), 343 (34700). IR (KBr): 1635, 1610, 1585, 1250, 1030, 995, 925. ^{1}H-NMR (100 MHz): 1.38—1.78 (6H), 3.36—3.67 (4H), 5.94 (2H, s), 6.39 (1H, d, J = 15.0 Hz), 6.66—6.95 (5H), 7.37 (1H, ddd, J = 15.0, 7.0, 4.0 Hz). ^{13}C-NMR (25 MHz): 24.7 (t), 26.4 (t), 26.5 (t), 43.4 (t), 46.7 (t), 101.2 (t), 105.6 (d), 108.4 (d), 120.1 (d), 122.4 (d), 125.3 (d), 131.0 (s), 138.1 (d), 142.3 (d), 148.1 (s), 148.1 (s), 165.3 (s).

Compound 2 [Piperamide-C5:1(2*E*)] (2)——Oil. UV: 235 sh (16400), 286 (7800). IR ($CHCl_3$): 1655, 1600, 1240, 1035, 970, 900. ^{1}H-NMR (400 MHz): 1.82—1.99 (4H), 2.48 (2H, q, J = 7.0 Hz), 2.70 (2H, t, J = 7.0 Hz), 3.44—3.54 (4H), 5.92 (2H, s), 6.09 (1H, d, J = 15.0 Hz), 6.61—6.75 (3H), 6.91 (1H, dt, J = 15.0, 7.0 Hz). HRMS: Found: 273.1365. Calcd for $C_{16}H_{19}NO_3(M^+)$: 273.1364.

Compound 3 [Piperamide-C7:1(6*E*)] (3)——Oil. UV: 261 (11200), 268 (11100), 305 (5200), 321 sh (2800). IR ($CHCl_3$): 1620, 1240, 1035, 960, 925. ^{1}H-NMR (400 MHz): 1.51 (2H, m), 1.71 (2H, m), 1.87 (2H, m), 1.96 (2H, m), 2.21 (2H, q, J = 7.0 Hz), 2.28 (2H, t, J = 7.5 Hz), 3.40 (2H, t, J = 7.5 Hz), 3.46 (2H, t, J = 7.5 Hz), 5.93 (2H, s), 6.04 (1H, dt, J = 15.5, 7.0 Hz), 6.29 (1H, d, J = 15.5 Hz), 6.73—6.88 (3H). ^{13}C-NMR (25 MHz): 24.4 (t), 24.5 (t), 26.1 (t), 29.3 (t), 32.7 (t), 34.6 (t), 45.6 (t), 46.6 (t), 100.8 (t), 105.4 (d), 108.1 (d), 120.2 (d), 128.9 (d), 129.5 (d), 132.4 (s), 146.5 (s), 147.9 (s), 171.5 (s). HRMS: Found: 301.1688. Calcd for $C_{18}H_{23}NO_3(M^+)$: 301.1676.

Compound 4 [Piperamide-C7:2(2*E*,6*E*)] (4)——Oil. UV: 261 (18700), 268 sh (17400), 306 (7100), 321 sh (3700). IR ($CHCl_3$): 1655, 1600, 1240, 1035, 965, 930. ^{1}H-NMR (400 MHz): 1.82—2.00 (4H), 2.31—2.39 (4H), 3.47—3.55 (4H), 5.94 (2H, s), 6.04 (1H, dt, J = 15.5, 6.5 Hz), 6.14 (1H, d, J = 15.0 Hz), 6.33 (1H, d, J = 15.5 Hz), 6.74—6.88 (3H), 6.94 (1H, dt, J = 15.0, 6.5 Hz). HRMS: Found: 299.1519. Calcd for $C_{18}H_{21}NO_3(M^+)$: 299.1519.

Compound 5 [Piperamide-C9:1(8*E*)] (5)——Oil. UV: 261 (11900), 268 (11700), 306 (5400), 321 sh (3000). IR (film): 1635, 1240, 1030, 960, 925. ^{1}H-NMR (100 MHz): 1.20—1.79 (8H), 1.79—2.02 (4H), 2.02—2.36 (4H), 3.24—3.62 (4H), 5.91 (2H, s), 5.98 (1H, dt, J = 15.5, 6.5 Hz), 6.28 (1H, d, J = 15.5 Hz), 6.70—6.93 (3H). ^{13}C-NMR (25 MHz): 24.4 (t), 24.8 (t), 26.1 (t), 29.0 (t), 29.3 (t), 29.4 (t), 32.8 (t), 34.8 (t), 45.6 (t), 46.6 (t), 100.8 (t), 105.4 (d), 108.1 (d), 120.1 (d), 129.2 (d), 129.3 (d), 132.4(s), 146.5 (s), 147.9 (s), 171.7 (s). HRMS: Found: 329.1970. Calcd for $C_{20}H_{27}NO_3(M^+)$: 329.1989.

Compound 6 [Piperamide-C9:2(2*E*,8*E*)] (6)——Oil. UV: 261 (17600), 268 sh (16300), 306 (6000), 321 sh (3400). IR ($CHCl_3$): 1655, 1600, 1240, 1035, 965, 935. ^{1}H-NMR (400 MHz): 1.46—1.57 (4H), 1.81—2.00 (4H), 2.12—2.27 (4H), 3.48—3.55 (4H), 5.93 (2H, s), 6.02 (1H, dt, J = 15.5, 7.0 Hz), 6.10 (1H, d, J = 14.5 Hz), 6.28 (1H, d, J = 15.5 Hz), 6.74—6.88 (3H), 6.91 (1H, dt, J = 14.5, 7.0 Hz). ^{13}C-NMR (25 MHz): 24.3 (t), 26.1 (t), 27.9 (t), 28.9 (t), 32.2 (t), 32.6 (t), 45.7 (t), 46.4 (t), 100.8 (t), 105.4 (d), 108.1 (d), 120.2 (d), 121.8 (d), 128.8 (d), 129.6 (d), 132.3 (s), 145.4 (d), 146.5

(s), 147.9 (s), 164.8 (s). HRMS: Found: 327.1821. Calcd for $C_{20}H_{25}NO_3(M^+)$: 327.1832.

Compound 7 [Piperamide-C9:3(2*E*,4*E*,8*E*)] (7)——Yellow needles, mp 105—106 °C. UV: 260 sh (36600), 270 (39700), 306 sh (10800), 321 sh (4400). IR (KBr): 1650, 1625, 1590, 1245, 1030, 1005, 970, 925. ^{1}H-NMR (400 MHz): 1.82—2.01 (4H), 2.29—2.34 (4H), 3.49—3.57 (4H), 5.94 (2H, s), 6.02 (1H, dt, J=15.5, 6.5 Hz), 6.11 (1H, d, J=14.5 Hz), 6.11 (1H, dt, J=14.5, 7.0 Hz), 6.22 (1H, dd, J=14.5, 10.5 Hz), 6.31 (1H, d, J=15.5 Hz), 6.73—6.89 (3H), 7.28 (1H, dd, J=14.5, 10.5 Hz). ^{13}C-NMR (25 MHz): 25.2 (t), 25.2 (t), 32.2 (t), 32.9 (t), 46.2 (t), 46.2 (t), 100.9 (t), 105.4 (d), 108.2 (d), 120.1 (d), 120.3 (d), 127.7 (d), 129.2 (d), 130.2 (d), 132.1 (s), 141.8 (d), 142.0 (d), 146.7 (s), 147.9 (s), 165.1 (s). *Anal.* Calcd for $C_{20}H_{23}NO_3$: C, 73.82; H, 7.12; N, 4.30. Found: C, 73.55; H, 7.11; N, 4.26.

Compound 8 [1-[(2*E*,4*E*)-2,4-Decadienoyl]pyrrolidine] (8)——Oil. UV: 265 (21000). IR ($CHCl_3$): 1650, 1620, 1600. ^{1}H-NMR (400 MHz): 0.89 (3H, t, J=6.6 Hz), 1.23—1.47 (6H), 1.82—2.01 (4H), 2.15 (2H, q, J=7.0 Hz), 3.48—3.57 (4H), 6.08 (1H, d, J=14.5 Hz), 6.08 (1H, dt, J=15.0, 7.0 Hz), 6.18 (1H, dd, J=15.0, 10.5 Hz), 7.27 (1H, dd, J=14.5, 10.5 Hz). MS m/z (%): 221 (M^+, 36), 178 (17), 164 (19), 151 (42), 150 (100), 98 (13), 95 (14), 81 (59), 70 (30), 69 (17), 67 (17), 55 (18). HRMS: Found: 221.1784. Calcd for $C_{14}H_{23}NO(M^+)$: 221.1786.

Compound 9 [1-[(2*E*,4*E*)-2,4-Dodecadienoyl]pyrrolidine] (9)——This was not obtained in completely pure form. ^{1}H-NMR (400 MHz): 0.88 (3H, t, J=7.0 Hz), 1.18—1.46 (10H), 1.84—2.00 (4H), 2.15 (2H, q, J=7.0 Hz), 3.50—3.56 (4H), 6.08 (1H, d, J=14.5 Hz), 6.08 (1H, dt, J=15.0, 7.0 Hz), 6.18 (1H, dd, J=15.0, 10.5 Hz), 7.27 (1H, dd, J=14.5, 10.5 Hz). MS m/z (%): 249 (M^+, 29), 179 (23), 178 (16), 164 (17), 150 (100), 113 (17), 98 (16), 95 (16), 81 (45), 70 (27), 67 (11), 55 (21).

Piperylin (10)——Yellow needles, mp 148—149 °C (lit. mp 142—143 °C).[16] UV: 246 (10400), 255 (10700), 263 (10900), 299 sh (16600), 311 (19900), 345 (33400). IR (KBr): 1640, 1615, 1590, 1250, 1035, 990, 925. ^{1}H-NMR (100 MHz): 1.68—2.16 (4H), 3.30—3.66 (4H), 5.95 (2H, s), 6.12 (1H, d, J=15.0 Hz), 6.68—7.00 (5H), 7.38 (1H, ddd, J=15.0, 7.3, 3.8 Hz).

Piperettine (11)——Yellow needles, mp 147—153 °C (lit. mp 146 °C).[3b] UV: 258 (9300), 310 sh (19500), 324 sh (34200), 358 (42800). IR (KBr): 1625, 1600 sh, 1585 sh, 1575, 1250, 1005, 920. ^{1}H-NMR (400 MHz): 1.55—1.71 (6H), 3.46—3.68 (4H), 5.97 (2H, s), 6.38 (1H, d, J=14.5 Hz), 6.41 (1H, dd, J=14.0, 11.5 Hz), 6.60 (1H, d, J=15.0 Hz), 6.64 (1H, dd, J=14.0, 10.0 Hz), 6.68 (1H, dd, J=15.0, 10.0 Hz), 6.77 (1H, d, J=8.0 Hz), 6.86 (1H, dd, J=8.0, 2.0 Hz), 6.96 (1H, d, J=2.0 Hz), 7.35 (1H, dd, J=14.5, 11.5 Hz).

Retrofractamide-A (12)——Colorless prisms, mp 139—142 °C (lit. mp 129 °C).[7] UV: 263 (39300), 270 sh (37800), 306 sh (7600), 320 sh (4000). IR (KBr): 3325, 1655, 1630, 1615, 1550, 1250, 1040, 995, 965, 920. ^{1}H-NMR (400 MHz): 0.93 (6H, d, J=6.5 Hz), 1.80 (1H, m), 2.29—2.34 (4H), 3.17 (2H, t, J=6.4 Hz), 5.52 (1H, br), 5.77 (1H, d, J=15.0 Hz), 5.93 (2H, s), 6.02 (1H, dt, J=15.5, 6.7 Hz), 6.09 (1H, dt, J=14.5, 6.0 Hz), 6.17 (1H, dd, J=14.5, 10.0 Hz), 6.31 (1H, d, J=15.5 Hz), 6.72—6.90 (3H), 7.19 (1H, dd, J=15.0, 10.0 Hz).

Pipercide (13)——Colorless needles, mp 122—128 °C (lit. mp 114—115 °C).[3c] UV: 260 (38800), 269 sh (35800), 306 sh (5900), 321 sh (3200). IR (KBr): 3325, 1660, 1630, 1615, 1550, 1250, 1035, 995, 960, 920. ^{1}H-NMR (400 MHz): 0.93 (6H, d, J=6.5 Hz), 1.38—1.50 (4H), 1.80 (1H, m), 2.11—2.22 (4H), 3.17 (2H, t, J=6.4 Hz), 5.45 (1H, br), 5.74 (1H, d, J=15.0 Hz), 5.93 (2H, s), 6.02 (1H, dt, J=15.5, 7.0 Hz), 6.06 (1H, dt, J=14.5, 6.5 Hz), 6.14 (1H, dd, J=14.5, 10.0 Hz), 6.28 (1H, d, J=15.5 Hz), 6.73—6.90 (3H), 7.19 (1H, dd, J=15.0, 10.0 Hz).

Guineensine (14)——Colorless needles, mp 118—120 °C (lit. mp 113—115 °C).[17] UV: 261 (40300), 268 sh (36800), 305 sh (5700), 320 sh (3200). IR (KBr): 3300, 1655, 1630, 1615, 1545, 1250, 1035, 990, 960, 920. ^{1}H-NMR (400 MHz): 0.93 (6H, d, J=6.5 Hz), 1.25—1.50 (8H), 1.80 (1H, m), 2.12—2.21 (4H), 3.16 (2H, t, J=6.4 Hz), 5.48 (1H, br), 5.74 (1H, d, J=15.0 Hz), 5.93 (2H, s), 6.04 (1H, dt, J=15.5, 7.0 Hz), 6.06 (1H, dt, J=14.5, 6.0 Hz), 6.13 (1H, dd, J=14.5, 10.0 Hz), 6.28 (1H, d, J=15.5 Hz), 6.72—6.90 (3H), 7.19 (1H, dd, J=15.0, 10.0 Hz).

Piperolein-B (15)——Oil (lit. oil).[3a] UV: 261 (12000), 268 (11800), 306 (5500), 321 sh (3100). IR (film): 1635, 1240, 1030, 960, 925. ^{1}H-NMR (400 MHz): 1.20—1.30 (4H), 1.30—1.37 (2H), 1.37—1.47 (4H), 1.47—1.56 (4H), 2.05 (2H, q, J=7.0 Hz), 2.19 (2H, t, J=7.5 Hz), 3.26 (2H, t, J=5.5 Hz), 3.42 (2H, t, J=5.5 Hz), 5.81 (2H, s), 5.92 (1H, dt, J=15.5, 7.0 Hz), 6.16 (1H, d, J=15.5 Hz), 6.61—6.78 (3H). ^{13}C-NMR (25 MHz): 24.6 (t), 25.4 (t), 25.6 (t), 26.6 (t), 29.0 (t), 29.3 (t), 29.4 (t), 32.8 (t), 33.4 (t), 42.6 (t), 46.7 (t), 100.8 (t), 105.4 (d), 108.1 (d), 120.1 (d), 129.2 (d), 129.3 (d), 132.4 (s), 146.5 (s), 147.9 (s), 171.3 (s).

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References and Notes

1) Part III: F. Kiuchi, Y. Tsuda, K. Kondo, H. Yoshimura, I. Nishioka, and G. Nonaka, *Chem. Pharm. Bull.*, **36**, 1796 (1988).
2) Jiang-su-xin-yi-xue-yuan (ed.), "Zhong-yao-da-ci-dian (中葯大辞典)," Shang-hai-ke-xue-ji-shu-chu-ban-she,

Shang-hai, 1978, p. 1539.
3) *a*) R. Grewe, W. Freist, H. Neumann, and S. Kersten, *Chem. Ber.*, **103**, 3752 (1970); *b*) F. S. Spring and J. Stark, *J. Chem. Soc.*, **1950**, 1177; *c*) M. Miyakado, I. Nakayama, H. Yoshioka, and N. Nakatani, *Agric. Biol. Chem.*, **43**, 1609 (1979); *d*) N. Nakatani and R. Inatani, *Agric. Biol. Chem.*, **45**, 1473 (1981); *e*) R. Inatani, N. Nakatani, and H. Fuwa, *ibid.*, **45**, 667 (1981); *f*) N. Nakatani, R. Inatani, and H. Fuwa, *ibid.*, **44**, 2831 (1980).
4) M. Miyakado, I. Nakayama, N. Ohno, and H. Yoshioka, "Structure, Chemistry and Actions of the Piperaceae Amides: New Insecticidal Constituents Isolated from the Pepper Plant," in "Natural Products for Innovative Pest Management," ed. by D. L. Whitehead, Pergamon Press, New York, 1983, pp. 369—382.
5) P. C. Beaver, R. C. Jung, and E. W. Cupp, "Clinical Parasitology," 9th ed., Lea and Febiger, Philadelphia, 1984, p. 325.
6) F. Kiuchi, N. Miyashita, Y. Tsuda, K. Kondo, and H. Yoshimura, *Chem. Pharm. Bull.*, **35**, 2880 (1987).
7) A. Banerji, D. Bandyopadhyay, M. Sarkar, A. K. Siddhanta, S. C. Pal, S. Ghosh, K. Abraham, and J. N. Shoolery, *Phytochemistry*, **24**, 279 (1985).
8) I. Yasuda, K. Takeya, and H. Itokawa, *Chem. Pharm. Bull.*, **29**, 564 (1981).
9) J. Singh, D. D. Santani, and K. L. Dhar, *Phytochemistry*, **15**, 2018 (1976).
10) On comparison of the spectrum of our compound with that kindly provided by Prof. Singh, we could not reach any definite conclusion as to the identity of the two compounds.
11) M. Miyakado, I. Nakayama, N. Ohno, and H. Yoshioka, *Nippon Kagaku Kaishi*, **1981**, 886.
12) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Interpretation of Mass Spectra of Organic Compounds," Holden-Day, San Francisco, 1964, p. 75.
13) J. W. Loder, A. Moorhouse, and G. B. Russell, *Aust. J. Chem.*, **22**, 1531 (1969).
14) A. Chatterjee and C. P. Dutta, *Tetrahedron*, **23**, 1769 (1967).
15) M. Miyakado, I. Nakayama, A. Inoue, M. Hatakoshi, and N. Ohno, *J. Pesticide Sci.*, **10**, 11 (1985).
16) J. Singh, K. L. Dhar, and C. K. Atal, *Tetrahedron Lett.*, **1969**, 4975.
17) J. I. Okogun and D. E. U. Eknog, *J. Chem. Soc., Perkin Trans. 1*, **1974**, 2195.