Nematocidal Activity of Long Alkyl Chain Amides, Amines, and Their Derivatives on Dog Roundworm Larvae[1)]

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The nematocidal activity of amides and amines having a long alkyl chain against the second-stage larva of dog roundworm, *Toxocara canis*, was examined. Long chain acyl amides with smaller substituents on the nitrogen showed stronger activity and the activity of cyclic amine amides was stronger than that of acyclic ones. In a series of homologous amides, the activity was dependent on the alkyl chain length: it reached a maximum at an optimal chain length and decreased in both shorter and longer homologues. The relationship between the activity and hydrophobicity of the homologues was analysed by the use of the bilinear model. The hydrophobicity of a compound, which gives a maximal activity, was similar for all neutral amides, but amides which have an additional amine group in the molecule had different values. Tertiary amines and their salts having a long alkyl chain also showed nematocidal activities comparable to those of the corresponding amides. The salts killed the larva at concentrations lower than their critical micell concentration, suggesting that they behave as a single molecule for the nematocidal action.

Keywords long chain amide; long chain amine; bilinear model; critical micell concentration; hydrophobic/hydrophilic balance; nematocidal activity; structure–activity relationship; quantitative structure–activity relationship; tetradecanamide; *Toxocara canis*

In the course of screening and elucidation works of biologically active principles contained in traditional medicines and spices, we found that long chain fatty acids and fatty alcohols have a killing activity against the larve of dog roundworm, *Toxocara canis*, which is the major pathogenic parasite in visceral larva migrans.[2)] We also isolated long alkyl chain aralkyl amides, piperamides, as the nematocidal principles of pepper.[3)] A number of amides having a long acyl chain have been isolated from natural sources[4)] and biological activities such as insecticidal[5)] and molluscicidal[6)] activities have been reported for some of them. A wide variety of biological activities have also been reported for synthetic *N*-acyl and *N*-alkyl cyclic amines such as pyrrolidine, morpholine, and *N*-methylpiperazine.[7)] In this paper we describe nematocidal activity of simple amides and amines with a long alkyl chain and their derivatives on the second-stage larva of dog roundworm, *Toxocara canis*.

Results

I. Nematocidal Activity of Amide Derivatives. Nematocidal Activity of *N*-Acyl Cyclic Amines The nematocidal activity against the second-stage larva of *T. canis* was evaluated in terms of the relative mobility (RM) value and the minimal lethal concentration (MLC) (see Experimental). We first tested nematocidal activity of *N*-acyl cyclic amines (Table I) because piperamides that have pyrrolidine and piperidine as the amine moiety showed strong nematocidal activity, whereas those with isobutylamine were inactive.[3)] Since it is widely observed that the degree of a biological activity is dependent on the alkyl chain length in a series of homologous compounds,[8)] a series of homologues with various acyl chain lengths were prepared for testing the nematocidal activity. As expected, all series of amide homologues tested showed a similar tendency: in a series of homologues, the activity increased as the acyl chain became longer and reached a maximum around the chain length of C_{10} to C_{14}, then the activity decreased. The strongest activity was observed at C_{14} in azetidine amides (**1-*n***), at $C_{12}$to C_{13} in pyrrolidine homologues (**2-*n***), at C_{12} in piperidine (**3-*n***), and at C_{10} in hexamethyleneimine (**4-*n***) derivatives. Nematocidal activities of morpholine (**5-*n***), piperazine (**6-*n***), *N*-methylpiperazine (**7-*n***), *N*-methylhomopiperazine (**8-*n***), and *N*-fomylpiperazine (**9-*n***) amide homologues were also tested (Table

TABLE I. Nematocidal Activity of *N*-Acyl Cyclic Amines (1)

$X\text{–}CO(CH_2)_{n-2}CH_3$

Compd.	X[a)]	*n*	RM (100 μM)				MLC (μM)
			1 h	3 h	6 h	24 h	
1-10	AZ	10	100	100	100	96	200
1-12	AZ	12	33	0	0	0	40
1-14	AZ	14	40	0	0	0	10
1-16	AZ	16	100	53	37	0	100
1-18	AZ	18	100	100	92	63	400
2-8	PR	8	96	95	89	85	—
2-10	PR	10	84	24	0	0	100
2-11	PR	11	69	33	0	0	40
2-12	PR	12	32	0	0	0	20
2-13	PR	13	33	0	0	0	20
2-14	PR	14	33	0	0	0	40
2-15	PR	15	89	33	0	0	40
2-16	PR	16	100	99	36	0	80
2-18	PR	18	100	99	97	50	—
3-8	PP	8	100	100	100	100	—
3-10	PP	10	100	93	58	0	80
3-11	PP	11	100	39	38	0	80
3-12	PP	12	100	43	0	0	40
3-13	PP	13	100	85	0	0	80
3-14	PP	14	100	100	83	0	100
3-15	PP	15	100	95	95	66	—
3-16	PP	16	100	100	100	100	—
3-18	PP	18	100	98	98	97	—
4-7	HI	7	100	100	100	98	—
4-8	HI	8	100	100	100	96	—
4-9	HI	9	100	100	100	86	—
4-10	HI	10	100	100	44	0	80
4-12	HI	12	100	100	100	0	100
4-14	HI	14	100	100	100	100	>1000
4-16	HI	16	100	100	100	100	>1000
4-18	HI	18	100	100	100	100	>1000

a) AZ: azetidino; PR: pyrrolidino; PP: piperidino; HI: hexamethyleneimino.

TABLE II. Nematocidal Activity of *N*-Acyl Cyclic Amines (2)

$X\text{–}CO(CH_2)_{n-2}CH_3$

Compd.	X[a]	*n*	RM (100 μM) 1 h	3 h	6 h	24 h	MLC (μM)
5-8	MO	8	100	100	100	100	—
5-10	MO	10	96	90	68	95	—
5-12	MO	12	86	33	0	0	40
5-13	MO	13	89	33	0	0	20
5-14	MO	14	98	33	0	0	10
5-15	MO	15	33	33	0	0	20
5-16	MO	16	97	95	53	0	20
5-18	MO	18	100	100	100	100	—
6-12	HP	12	100	100	100	82	200
6-14	HP	14	33	0	0	0	40
6-16	HP	16	60	33	0	0	80
6-18	HP	18	100	33	0	0	100
7-8	MP	8	100	100	100	100	—
7-10	MP	10	100	91	86	71	250
7-12	MP	12	66	0	0	0	60
7-14	MP	14	31	0	0	0	15
7-16	MP	16	59	0	0	0	10
7-18	MP	18	69	67	19	0	20
8-12	HM	12	100	100	100	0	100
8-13	HM	13	100	100	75	0	80
8-14	HM	14	67	35	33	0	40
8-15	HM	15	67	33	33	0	20
8-16	HM	16	67	39	33	0	20
8-18	HM	18	71	35	0	0	20
8-20	HM	20	100	100	100	77	—
9-8	FP	8	100	100	100	36	—
9-9	FP	9	100	100	100	100	—
9-10	FP	10	100	100	100	44	—
9-11	FP	11	100	98	81	33	—
9-12	FP	12	100	75	38	0	80
9-13	FP	13	66	33	0	0	40
9-14	FP	14	62	33	0	0	40
9-15	FP	15	92	33	0	0	20
9-16	FP	16	98	58	33	0	20
9-17	FP	17	100	97	42	0	100
9-18	FP	18	100	96	40	0	100
9-20	FP	20	100	100	100	100	—

a) MO: morpholino; HP: piperazino; MP: *N*-methylpiperazino; HM: *N*-methylhomopiperazino; FP: *N*-formylpiperazino.

II). Maximal activities were again observed at the alkyl chain length between C_{12} to C_{16} in these derivatives. Thus all these amide homologues had optimal chain length(s) for the maximal activity, which became shorter when the ring size of amine became larger. It appears that the maximal activity is obtained where the hydrophobic/hydrophilic balance of the compound is optimized: when the hydrophobicity of the amine moiety increases (larger ring size), that of the acyl chain decreases (shorter chain length) to optimize the hydrophobic/hydrophilic balance (this will be discussed later). The maximal activity in a series of homologues became weaker as the ring size of the amine became larger (maximal activity: 10 μM for **1-*n***, 20 μM for **2-*n***, 40 μM for **3-*n***, and 80 μM for **4-*n***), showing that an amine with a smaller ring is preferable for the nematocidal activity.

Nematocidal Activity of Tetradecanamide Derivatives

TABLE III. Nematocidal Activity of *N*-Monosubstituted Tetradecanamides

$RNHCOC_{13}H_{27}$

Compd.	R	MLC (μM)	log V_R
10	H	>1000	3.74
11	CH_3	>1000	4.16
12	C_2H_5	>1000	4.37
13	C_3H_7	>1000	4.57
14	iso-C_3H_7	>1000	4.53
15	$CH_2=CH\text{–}CH_2$	>1000	4.43
16	C_4H_9	>1000	4.90
17	iso-C_4H_9	>1000	4.97
18	*sec*-C_4H_9	>1000	4.80
19	*tert*-C_4H_9	>1000	5.02
20	$C_{14}H_{29}$	>1000	9.67
21	Cyclohexyl	>1000	5.22
22	Ph	>1000	5.03
23	$PhCH_2$	>1000	4.09
24	Adamantyl	>1000	6.40
25		—[a]	—

a) RM = 100 at 100 μM after 24 h incubation.

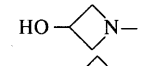
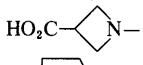
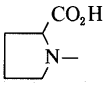
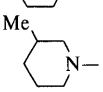
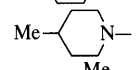
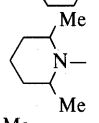
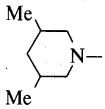
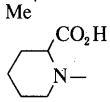
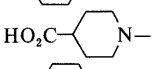
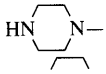
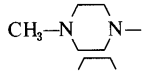
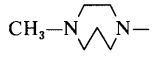
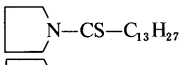
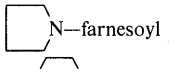
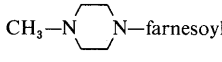
TABLE IV. Nematocidal Activity of *N*,*N*-Disubstituted Tetradecanamides

$R^1_{R^2}{>}NCOC_{13}H_{27}$

Compd.	R^1	R^2	RM (100 μM) 3 h	6 h	24 h	MLC (μM)	log V_R
26	CH_3	CH_3	0	0	0	40	4.67
27	CH_3	C_2H_5	49	35	0	40	5.13
28	CH_3	C_3H_7	100	93	56	300	5.28
29	CH_3	iso-C_3H_7	100	100	65	400	5.19
30	CH_3	$CH_2=CH\text{–}CH_2$	100	100	48	400	5.11
31	CH_3	C_4H_9	100	100	100	>1000	5.71
32	CH_3	iso-C_4H_9	100	100	100	>1000	5.64
33	CH_3	*sec*-C_4H_9	100	100	100	>1000	5.53
34	CH_3	*tert*-C_4H_9	100	100	100	>1000	5.81
35	CH_3	Cyclohexyl	100	100	100	>1000	6.10
36	CH_3	Ph	100	100	100	>1000	5.55
37	CH_3	$PhCH_2$	100	100	100	>1000	5.61
38	C_2H_5	C_2H_5	100	100	100	500	5.33
39	C_3H_7	C_3H_7	100	100	100	>1000	6.13
40	$HOCH_2CH_2$	$HOCH_2CH_2$	100	100	100	>1000	3.01

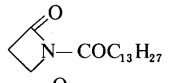
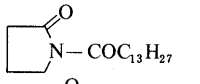
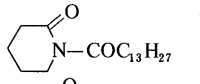
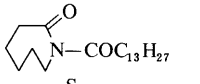
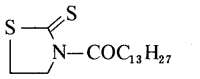
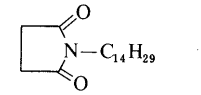
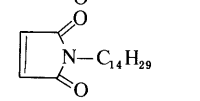
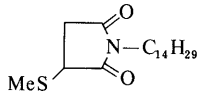
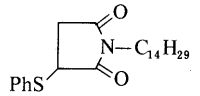
TABLE V. Comparison of Nematocidal Activity of *N*-Tetradecanoyl Cyclic Amines

$X\text{-}COC_{13}H_{27}$

Compd.	X	RM (100 μM) 3 h	6 h	24 h	MLC (μM)	log V_R
1-14		0	0	0	10	4.63
41		95	94	67	—	—
42		96	94	86	—	—
2-14		0	0	0	40	4.99
43		92	95	77	150	—
3-14		100	83	0	100	5.36
44		100	100	100	>1000	—
45		100	100	100	>1000	—
46		100	94	89	>1000	—
47		100	100	100	>1000	—
48		100	93	65	>1000	—
49		98	97	40	—	—
50		80	48	0	100	—
4-14		100	100	100	>1000	5.68
5-14		33	0	0	10	4.47
6-14		0	0	0	40	6.01
7-14		0	0	0	15	4.67
8-14		35	33	0	40	5.05
51		100	100	100	>1000	5.36
52		87	44	0	80	—
53		100	100	100	>1000	—

Since the maximal activities were observed for the acyl chain length between 12 to 16 in the above amides, we next compared the nematocidal activities of tetradecanamides with various *N*-substituents. Simple tetradecanamide (**10**) and all *N*-monosubstituted tetradecanamides tested were inactive up to 1 mM (Table III). Among the *N*,*N*-disubstituted tetradecanamides (Table IV), only those with small alkyl substituents showed the activity: *N*,*N*-dimethyltetradecanamide (**26**) showed the strongest activity, which decreased as the alkyl group became larger. *N*-Methyl-*N*-butyl derivatives (**31**—**34**) and those with larger alkyl groups (**35**—**37**) were inactive. *N*,*N*-Diethyl derivative (**38**) showed weak activity, and *N*,*N*-dipropyl (**39**) and *N*,*N*-bishydroxyethyl (**40**) derivatives were ineffective.

TABLE VI. Nematocidal Activity of Secondary Amides

Compd.		MLC (μM)	log V_R
54		>1000	4.98
55		>1000	5.27
56		>1000	5.63
57		>1000	6.13
58		>1000	—
59		>1000	5.22
60		>1000	5.92
61		>1000	—
62		>1000	—

Among the tetradecanamide of cyclic amines (Table V), those which have a smaller ring showed stronger activity as well. The azetidine amide (**1-14**) had a MLC of 10 μM and the activity decreased with the increase of the ring size to pyrrolidine (**2-14**, MLC=40 μM), piperidine (**3-14**, MLC=100 μM) and hexamethyleneimine (**4-14**, MLC> 1 mM). This relation was also valid for *N*-methylpiperazine (**7-14**, MLC=15 μM) and its higher homologue, *N*-methylhomopiperazine (**8-14**, MLC=40 μM). When a methyl group(s) was introduced to the piperidine ring (**44**—**48**), the activity largely decreased, suggesting that bulkiness of the amine moiety strongly affects the nematocidal activity and smaller alkyl substituents are preferable for the activity. In support of this, the activity of amides of acyclic amine was weaker than that of amides of cyclic amine, when the number of carbons on the nitrogen was similar (**1-14** *vs.* **26** and **27**; **2-14** *vs.* **28** and **38**; **3-14** *vs.* **31**, **38**, and **39**). Introduction of a carboxy group to azetidine (**42**), pyrrolidine (**43**), and piperidine (**49**) ring reduced the activity, whereas that to the piperidine ring at a position far from the amide group (**50**) scarcely affected the activity. Conversion of the carbonyl group of *N*-tetradecanoylpyrrolidine (**2-14**) to the thiocarbonyl group (**51**) led to a loss of the activity. *N*-Farnesoylpyrrolidine (**52**) showed a comparable activity to that of **2-16**, but 1-farnesoyl-4-methylpiperazine (**53**) was ineffective (Table V).

TABLE VII. Nematocidal Activity of *N*-Acyl Tetradecylamines

$R^1CONC_{14}H_{29}$
R^2

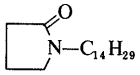
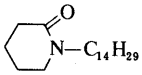
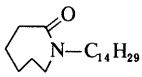
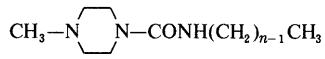
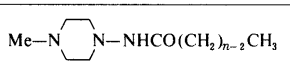
Compd.	R^1	R^2	RM (100 μM) 3 h	6 h	24 h	MLC (μM)	log V_R
63	H	H	44	0	0	20	4.81
64	H	CH_3	34	0	0	40	4.46
65	H	C_2H_5	100	46	0	80	5.07
66	CH_3	H	62	33	0	40	4.79
67	CH_3	CH_3	33	0	0	20	5.19
68	CH_3	C_2H_5	100	88	0	40	5.70
69	CH_3	C_3H_7	100	100	100	>1000	6.06
70	CH_3	$C_{14}H_{29}$	100	100	100	>1000	11.60
71	C_2H_5	H	100	100	95	>1000	4.97
72	C_2H_5	CH_3	100	100	100	700	5.56
73	$C_{13}H_{27}$	H	100	100	100	>1000	10.68
74	$HO_2CCH=CH$	H	99	98	97	>1000	—
75	$HO(CH_2)_3$	H	100	100	100	>1000	4.63
76	Ph	H	100	100	100	>1000	5.41
77			36	0	0	10	5.18
78			41	42	0	40	5.58
79			100	100	43	700	5.80

TABLE VIII. Nematocidal Activity of Urea and Hydrazino Type Compounds



Compd.	*n*	RM (100 μM) 3 h	6 h	24 h	MLC (μM)
80-8	8	100	100	97	>1000
80-10	10	100	89	75	400
80-12	12	73	34	0	40
80-14	14	64	35	0	20
80-16	16	81	34	0	25
80-18	18	100	71	50	300



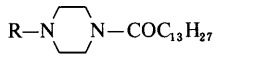
81-*n* (*n*=8—20)

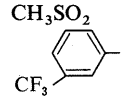
81-8—**81-20**: RM = 100 at 100 μM after 24 h incubation.

N-Tetradecanoyllactams (**54**—**58**) and *N*-tetradecyl imides (**59**—**62**) were all inactive (Table VI).

Nematocidal Activity of *N*-Acyl Tetradecylamines Next, we tested the nematocidal activity of amides having a tetradecylamine moiety (Table VII). In contrast to the tetradecanoylamines, *N*-tetradecylamides such as *N*-tetradecyl formamide (**63**) and acetamide (**66**) showed appreciable nematocidal activity. However, when the acyl group became larger than acetyl (**71**—**76**), the activity was greatly diminished. Introduction of a methyl group on the nitrogen increased the activity (**66** *vs.* **67**; **71** *vs.* **72**) as was seen in *N*,*N*-disubstituted tetradecanoylamines except for the *N*-formyl derivatives (**63** *vs.* **64**). However, a substituent larger than the methyl group on the nitrogen decreased the activity (**63** *vs.* **65**; **66** *vs.* **68**, **69**, and **70**). *N*-Tetradecyllactams (**77**—**79**) were also active and, as in the case of *N*-tetradecanoyl cyclic amines, the activity decreased as the ring size became larger. Urea type homologues (**80-*n***) also showed nematocidal activity (Table VIII), to give the highest activity for a tetradecylamine derivative (**80-14**) with an MLC of 20 μM. The compounds, in which the positions of the carbonyl and the nitrogen were swapped (**81-*n***), did not show activity up to 1 mM.

TABLE IX. Nematocidal Activity of *N*-Tetradecanoylpiperazine Derivatives



Compd.	R	RM (100 μM) 3 h	6 h	24 h	MLC (μM)	log V_R
6-14	H	0	0	0	40	6.01
7-14	CH_3	0	0	0	15	4.67
82	C_2H_5	0	0	0	10	4.82
83	C_3H_7	38	0	0	10	5.22
9-14	CHO	33	0	0	40	3.86
84	CH_3CO	33	0	0	20	3.96
85	C_2H_5CO	33	0	0	20	4.14
86	$C_5H_{11}CO$	33	0	0	20	5.07
87	$C_{13}H_{27}CO$	100	100	100	>1000	9.01
88	C_2H_5OCO	69	33	0	40	5.10
89	$HOCH_2CH_2$	33	0	0	40	3.95
90	CH_3SO_2	100	100	100	>1000	3.67
91		100	100	100	>1000	6.43

Nematocidal Activity of *N*-Tetradecanoylpiperazine Derivatives Since 1-tetradecanoylpiperazines (**6-14**, **7-14**, **9-14**) showed very strong nematocidal activity, further derivatives with various substituents at position 4 were examined (Table IX). The alkyl derivatives, R = methyl (**7-14**), ethyl (**82**), and propyl (**83**), showed strong activity (MLC = 15, 10, and 10 μM, respectively). The activity of these compounds was stronger than that of the unsubstituted compound (**6-14**). Among the acyl derivatives tested, formyl (**9-14**), acetyl (**84**), propanoyl (**85**), and hexanoyl (**86**) derivatives showed similar activity, whereas the tetradecanoyl derivative (**87**) did not show any activity, indicating that the nematocidal activity is not sensitive to the length of the substituent at position 4 as long as it is not too bulky. Ethoxycarbonyl (**88**) and hydroxyethyl (**89**) derivatives also showed appreciable activity (MLC = 40 μM), but methanesulfonyl (**90**) and 3-trifluoromethylphenyl (**91**) derivatives were inactive, suggesting that a bulky substituent at this position decreases the activity.

Relationship between Nematocidal Activity and Hydrophobicity of the Molecule As described above, nematocidal activity of *N*-acyl derivatives of cyclic amines largely depends on the acyl chain length: the activity reaches a maximum in a compound with an optimal chain length and decreases in both shorter and longer homologues. In order to explain such a non-linear relationship between biological activity and alkyl chain length, several models have been proposed which correlate biological activity with the hydrophobic character of a molecule, *i.e.*, partition coefficient.[9] Among these models, the bilinear model (Eq. 1) proposed by Kubinyi has been successfully applied to describe the bilinear nature of the observed relationship

between a biological activity and partition coefficient.[9c)]

$$\log(1/C) = a\log P - b\log(\beta P + 1) + c \quad (1)$$

In Eq. 1, P is the partition coefficient of a molecule and β is the volume ratio of the aqueous phase and lipid phase of the barriers. In this model, it is postulated that 1) the biological activity of a molecule is a function of its intrinsic activity, which is assumed to be identical for all members in a series of homologues, and of its probability to reach the receptor site; 2) the molecule has to pass through barriers consisting of the alternating of aqueous and lipid phases to reach the receptor site and the probability of a molecule to pass these barriers is determined by the partition coefficient between these phases.[9c,d)]

For application of this model to analyze our results, a hydrophobic parameter of the amides was determined by the high performance liquid chromatography (HPLC) method.[10)] First, the retention volumes of each member of 1-acyl-4-methylpiperazine homologues (**7-*n***) were determined on an octadesyl silica gel (ODS) column with methanol containing various concentrations of water as a solvent. The logarithm of the retention volume of a molecule showed a linear relationship with the water concentration in the solvent and the extrapolated retention volume at 100% water ($\log V_R$) was calculated. The $\log V_R$ of each member in the series of homologues showed a linear relationship with the acyl chain length and the $\log V_R$ increment for one methylene group was determined to be 0.477 for this HPLC system. Next, $\log V_R$ values of *N*-tetradecanoyl derivatives in Table I and II were determined and the values of the members of each homologous series were calculated from the CH_2 group increment. Using this $\log V_R$ value as a substitute of $\log P$ in Eq. 1, we analysed the relationship between the nematocidal activity evaluated in terms of MLC and the hydrophobic parameter by the bilinear model (Table X, Fig. 1).

TABLE X. Bilinear Model Analysis of *N*-Acyl Cyclic Amines[a)]

Homologue	1	2	3	5	7	8	9
a	1.041	1.303	0.431	1.508	0.690	0.520	0.610
b	2.014	1.801	1.102	1.958	1.211	0.832	1.469
c	−2.152	−2.900	−0.390	−3.664	−1.310	−1.180	−0.664
$\log\beta$	−4.39	−3.87	−4.57	−3.98	−5.34	−6.13	−4.46
$n^{b)}$	5	7	5	5	5	6	7
$r^{c)}$	0.9647	0.9692	0.7544	0.9038	0.9997	0.9798	0.8666
$s^{d)}$	0.3290	0.0932	0.1987	0.1822	0.0259	0.1010	0.2143
$\log V_{opt}{}^{e)}$	4.42	4.29	4.38	4.51	5.46	6.35	4.31
MLC_{opt} (μM)	15	20	56	13	9.7	17	24

a) Model: $\log(1/MLC) = a\log V_R - b\log(\beta V_R + 1) + c$. *b*) Number of observations. *c*) Correlation coefficient. *d*) Residual standard deviation. *e*) Optimal $\log V_R$ value: $\log V_{opt} = \log a - \log\beta - \log(b-a)$.

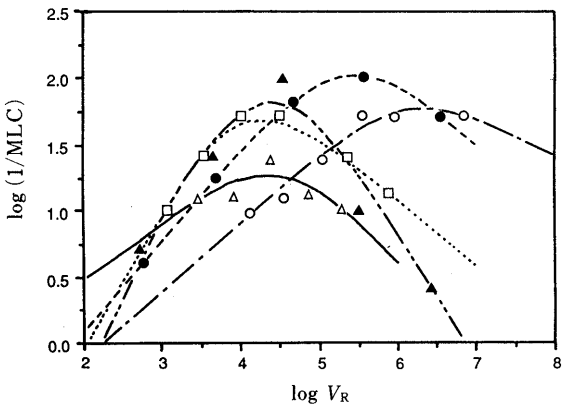


Fig. 1. Bilinear Model Analyses of the Relationship between Nematocidal Activity and Hydrophobic Parameter

——·—· and ▲, azetidine homologues (**1-*n***); ------ and □, pyrrolidine homologues (**2-*n***); —— and △, piperidine homologues (**3-*n***); – – – and ●, *N*-methylpiperazine homologues (**7-*n***); —·— and ○, *N*-methylhomopiperazine homologues (**8-*n***).

Although the acyl chain length of the homologue that shows the maximal activity was different for various amides, the optimal $\log V_R$ values ($\log V_{opt}$) of azetidine (**1-*n***), pyrrolidine (**2-*n***), and piperidine (**3-*n***) amide homologues for nematocidal activity were very similar (*ca.* 4.3—4.4). This indicates that these three homologues are a subject of the same transportation process and only the partition coefficient of the compound determines the probability of the molecule to reach the action site. The highest activity of each series predicted by the model (MLC_{opt}) indicates the relative magnitude of the intrinsic activity of these compounds, clearly showing that a smaller amine moiety is preferable for the activity. The $\log V_{opt}$ values of morpholine (**5-*n***) and *N*-formylpiperazine (**9-*n***) homologues also fall in the same range as those of the above series, suggesting that basically the same process is operating in the transportation of these neutral compounds. On the contrary, $\log V_{opt}$ values of compounds having an additional amino group in the molecule (**7-*n*** and **8-*n***) were very different from those of the above group, suggesting that the basicity of these compounds has a great effect on the transportation process.

$\log V_R$ values of other amides described above (Tables III—VII and IX) were also determined. However, no apparent relationship between the $\log V_R$ value and MLC was found among these compounds. Thus, although the nematocidal activity of a compound in a series of homologues is determined by its hydrophobic/hydrophilic balance, the intrinsic activity of the series is determined by the substituents on the nitrogen. It seems that a small, moderately hydrohpilic group at the end of an appropriately long alkyl chain is necessary for the activity.

II. Nematocidal Activity of Long Chain Alkylamines and Their Salts. Nematocidal Activity of Long Chain Alkylamines Next, we examined the nematocidal activity of *N*-alkyl cyclic amines (Table XI, XII). As in the case of *N*-acyl derivatives, *N*-alkyl cyclic amines showed nematocidal activity and the degree of the activity was dependent on the alkyl chain length. In these compounds, the optimal chain lengths which give maximal activity in terms of RM value and that in terms of MLC were not always identical. For example, RM values of **95-12**, **95-14**, and **95-16** at 100 μM after 3 h of incubation were 11, 37, and 95, respectively, and those after 6 h were 0, 4, and 53, showing that the activity of **95-12** is the strongest. However, when the incubation was prolonged for 24 h, all of these RM values became zero and the smallest MLC value (after 24 h incubation) among these homologues was observed for **95-14** and **95-16** (20 μM), whereas the MLC of **95-12** was higher (80 μM). It seems that a compound with a shorter alkyl chain exerts the effect faster than the higher homologues, but the higher homologues show the nematocidal activity at a lower concentration in prolonged incubation. The optimal chain length in terms of MLC was C_{18} or

TABLE XI. Nematocidal Activity of Tertiary Amines (1)

$X-(CH_2)_{n-1}CH_3$

Compd.	X[a]	n	RM (100 μM) 1 h	3 h	6 h	24 h	MLC (μM)
92-8	PR	8	100	100	93	87	—
92-10	PR	10	100	99	95	99	—
92-12	PR	12	100	89	20	0	100
92-14	PR	14	33	0	0	0	40
92-16	PR	16	0	0	0	0	20
92-18	PR	18	3	0	0	0	15
93-8	PP	8	100	100	94	90	—
93-10	PP	10	98	98	97	74	—
93-12	PP	12	70	0	0	0	80
93-14	PP	14	33	0	0	0	20
93-16	PP	16	31	0	0	0	8
93-18	PP	18	100	84	50	0	8
94-8	HI	8	100	100	67	67	—
94-10	HI	10	100	100	62	53	—
94-12	HI	12	100	47	0	0	100
94-14	HI	14	100	33	0	0	20
94-16	HI	16	100	67	33	0	40

a) PR: pyrrolidino; PP: piperidino; HI: hexamethyleneimino.

TABLE XII. Nematocidal Activity of Tertiary Amines (2)

$X-(CH_2)_{n-1}CH_3$

Compd.	X[a]	n	RM (100 μM) 1 h	3 h	6 h	24 h	MLC (μM)
95-8	MO	8	100	100	100	100	—
95-10	MO	10	100	88	60	99	—
95-12	MO	12	84	11	0	0	80
95-14	MO	14	100	37	4	0	20
95-16	MO	16	100	95	53	3	20
95-18	MO	18	100	100	100	100	—
96-12	HP	12	100	47	33	0	80
96-14	HP	14	51	33	0	0	40
96-16	HP	16	67	39	0	0	40
96-18	HP	18	100	84	48	0	80
97-7	MP	7	100	100	100	87	—
97-8	MP	8	100	100	100	70	1000
97-10	MP	10	100	82	54	4	200
97-12	MP	12	53	0	0	0	30
97-14	MP	14	0	0	0	0	10
97-16	MP	16	26	0	0	0	10
97-18	MP	18	57	21	4	0	15
98-12	HM	12	100	60	33	0	80
98-14	HM	14	66	33	0	0	20
98-16	HM	16	68	33	0	0	20
98-18	HM	18	100	46	20	0	20
99-12	FP	12	100	33	0	0	40
99-14	FP	14	33	33	0	0	10
99-16	FP	16	100	33	0	0	10
99-18	FP	18	100	100	100	0	20

a) MO: morpholino; HP: piperazino; MP; *N*-methylpiperazino; HM: *N*-methylhomopiperazino; FP: *N*-formylpiperazino.

longer for pyrrolidine homologues (**92-*n***), C_{16} to C_{18} for piperidine series (**93-*n***), and C_{14} for hexamethyleneimine derivatives (**94-*n***). These lengths were 4 to 6 carbons longer and the maximal activities were slightly stronger than those of corresponding *N*-acyl cyclic amines. It seems that an increase of hydrophilicity due to the conversion of the amide to an amine group is compensated by the longer alkyl chain. However, the chain length was not so different from that of the corresponding acyl derivatives in morpholine, piperazine, and homopiperazine series (**95-*n*—99-*n***).

TABLE XIII. Nematocidal Activity of Tertiary Amine Hydrochloride

$X-(CH_2)_{n-1}CH_3 \cdot HCl$

Compd.	X[a]	n	RM (100 μM) 1 h	3 h	6 h	24 h	MLC (μM)
92-8 ·HCl	PR	8	100	100	100	100	—
92-10·HCl	PR	10	100	100	100	96	—
92-12·HCl	PR	12	100	49	0	0	80
92-14·HCl	PR	14	33	0	0	0	8
92-16·HCl	PR	16	33	0	0	0	8
92-18·HCl	PR	18	17	0	0	0	8
93-8 ·HCl	PP	8	100	100	100	100	—
93-10·HCl	PP	10	100	101	91	83	—
93-12·HCl	PP	12	67	13	0	0	80
93-14·HCl	PP	14	33	0	0	0	10
93-16·HCl	PP	16	33	0	0	0	10
93-18·HCl	PP	18	58	14	0	0	10
95-8 ·HCl	MO	8	86	71	68	100	—
95-10·HCl	MO	10	67	70	60	50	—
95-12·HCl	MO	12	36	45	9	0	80
95-14·HCl	MO	14	33	0	0	0	20
95-16·HCl	MO	16	48	40	5	0	40
95-18·HCl	MO	18	67	60	9	13	—
97-8 ·2HCl	MP	8	100	97	97	97	—
97-10·2HCl	MP	10	100	85	80	90	—
97-12·2HCl	MP	12	98	24	0	0	—
97-14·2HCl	MP	14	40	2	0	0	—
97-16·2HCl	MP	16	24	0	0	0	—
97-18·2HCl	MP	18	67	0	0	0	—

a) PR: pyrrolidino; PP: piperidino; MO: morpholino; MP: *N*-methylpiperazino.

TABLE XIV. Nematocidal Activity of Tertiary Amine Hydrochloride

Compd.	RM (100μM) 1 h	3 h	6 h	24 h	MLC (μM)
6-14·HCl	100	100	98	0	80[a]
7-10·HCl	100	100	100	100	—
7-11·HCl	100	100	100	91	—
7-12·HCl	100	97	61	0	—
7-13·HCl	92	34	0	0	—
7-14·HCl	38	0	0	0	20[b]
7-15·HCl	37	0	0	0	—
7-16·HCl	51	0	0	0	—
7-17·HCl	68	23	0	0	—
7-18·HCl	99	26	0	0	—
8-14·HCl	100	90	89	58	30[c]
80-8 ·HCl	100	100	100	100	>1000
80-10·HCl	100	100	94	98	1000
80-12·HCl	89	17	0	0	500
80-14·HCl	72	31	0	0	15
80-16·HCl	92	65	56	0	500
80-18·HCl	100	100	100	96	1000

a) cmc in 0.75% saline was 2 mM at 19 °C. *b*) cmc in 0.75% saline was 1 mM at 21 °C. *c*) cmc in 0.75% saline was 1 mM at 35 °C.

Nematocidal Activity of Long Chain Alkylamine Salts Hydrochlorides of the above amines were also tested for nematocidal activity (Table XIII). These showed very similar activity as that of corresponding free amines. Hydrochlorides of the compound having both an amine and amide (or urea) group in the same molecule (**7-*n*·HCl** and **80-*n*·HCl**) also showed nematocidal activity similar to that of free amines (Table XIV). Unexpectedly, 1-

tetradecanoyl-4-methylhomopiperazine hydrochloride (**8-14**·HCl) showed very weak activity at 100 μM (RM = 58 after 24 h incubation). However, the activity of this compound at lower concentrations was stronger than that at 100 μM, showing a peculiar concentration dependence (Fig. 2). The MLC of this compound was determined to be 30 μM, which is about the same level as that of a corresponding free base. A similar phenomenon was observed in 1-dodecanoyl-4-methylpiperazine hydrochroride (**7-12**·HCl), for which the RM value was 73 at 1 mM and 0 at 0.1 mM after 24 h incubation. Since such phenomenon was not observed in corresponding free bases, we supposed that it was due to a property of the compound as a surfactant, *i.e.*, the ability to form micells.

In order to confirm this hypothesis, we determined the critical micell concentration (cmc) of **8-14**·HCl by measuring surface tension of the solution in 0.75% saline that was used in the assay. The surface tension of the solution decreased as the concentration of the compound increased and then became constant at about 100 μM and higher concentrations (Fig. 2), indicating that micell formation began at around 100 μM. Thus, the decrease of the nematocidal activity of **8-14**·HCl around 100 μM is ascribable to the decrease of concentration of the free molecule in the solution due to micell formation.

Quaternary ammonium salts of 1-tetradecanoylpiperazine derivatives were also examined (Table XV). All these ammonium salts showed moderate nematocidal activity (MLC = 80 to 100 μM). It is known tha the antibacterial activity of cationic surfactants such as benzalkonium varies depending on the counter anion. However, such phenomenon was not observed in the nematocidal activity of these compounds. For example, the activity of methosalts of **7-14** was almost identical for Cl^-, Br^-, and I^-. All MLC values of these compounds were smaller than their cmc values, again suggesting that they behave as a single molecule for exhibiting nematocidal activity.

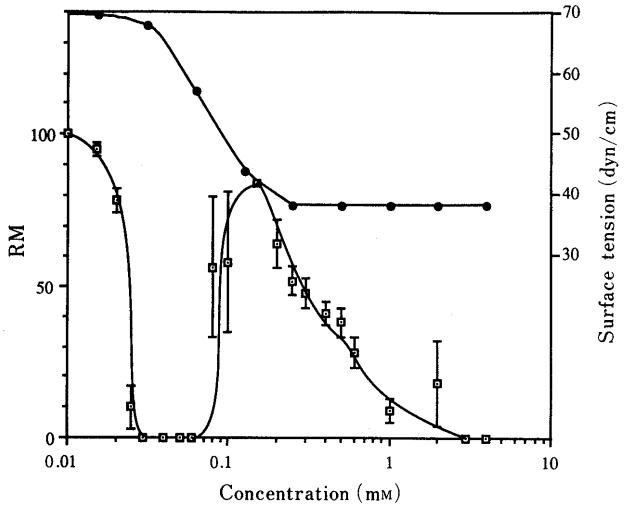
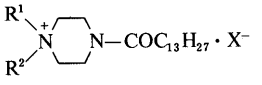


Fig. 2. Concentration Dependence of the RM Value of **80-14**·HCl and the Change of Surface Tension of the Solution

—⊡—, RM value after 24 h incubation; —●—, surface tension.

TABLE XV. Nematocidal Activity of Quaternary Ammonium Salts



Compd.	R^1	R^2	X	RM (100 μM) 1 h	3 h	6 h	24 h	MLC (μM)	cmc[a] (μM)
100	CH_3	CH_3	Cl	100	100	71	0	100	700[b]
101	CH_3	CH_3	Br	100	100	69	0	100	300[c]
102	CH_3	CH_3	I	100	100	70	0	100	600[c]
103	CH_3	$PhCH_2$	Cl	98	71	54	0	100	200[d]
104	CH_3	$PhCH_2$	Br	95	69	47	0	80	300[c]

a) 0.75% saline was used as a solvent. *b*) 23 °C. *c*) 16 °C. *d*) 19 °C.

Discussion

A wide variety of biological activities have been reported for amides, amines, and amine oxides having a long alkyl chain. Among these activities, the most remarkable are toxic action to various organisms and insect repellent activity.[7] Ishizuka *et al.*[7a] examined nematocidal activity of various amines with a long alkyl chain against the larvae of dog hookworm, *Anchylostoma canninum*. They found that tertiary amines having C14 to C18 alkyl chain had strong nematocidal activity. De Villiers and Rossouw[7c] reported molluscicidal activity of pyrrolidine, piperidine, morpholine, and dimethylamine amide homologues against a snail, *Bulinus tropicus*, showing that the activity depended on the acyl chain length and maximal activities were observed for C12 to C14 homologues. Similar results were also reported for antimicrobial[7d] and hemolytic[7e] activity of 4-alkylmorpholine-*N*-oxides. These reports are in good agreement with our observations suggesting the presence of a common mechanism or at least a common process which determines the degree of the activity of these compounds. On the contrary, shorter homologues (C8 to C9) of cyclic amine amides were reported to show maximal activities as an insect repellent.[7g] It is probably because moderate volatility is necessary for an efficient insect repellent.

As for the mechanism of the toxic action of these compounds, Šubík *et al.*[7d] found that 4-dodecylmorpholine-*N*-oxide induced significant changes in the permeability of the plasma membrane in yeast cells, resulting in a rapid loss of intracellular K^+, and proposed that the antimicrobial activity of such amine oxides is due to the disturbance of biological membrane systems. On the other hand, Miller *et al.*[11] observed that, when being applied to baby hamster kidney cells, *N*-dodecylimidazole and *N*-dodecylmorpholine (**5-12**) accumulated in lysosomes inducing a release of cell contents. They concluded that these amines were trapped by a pH gradient in lysosomes, in which the pH is low, causing the release of the contents, and that it is this release of lysosomal enzymes into the cytoplasma that kills the cells. In our experiments, the amide homologues showed very similar activity to that of the amines. Since it is not likely that these amides are trapped specifically in lysosomes like the amines by a pH gradient, it should not be a specific action of these compounds to lysosomes but a general effect to membrane systems that kills a wide variety of organisms. This consideration is supported by the fact that some amides such as **2-*n***, **3-*n***, and **4-*n*** have been reported to enhance the dermal penetration of various drugs,[12] which indicates the ability of these compounds to alter the permeability of membrane systems.

Conclusion

Amides, tertiary amines and their hydrochlorides having a long alkyl chain showed nematocidal activity against the larva of dog roundworm, *Toxocara canis*. Long chain acyl amides with smaller substituents on the nitrogen show stronger activity and the activity of cyclic amine amides is stronger than that of acyclic ones. In a series of alkyl homologues, the activity is dependent on the alkyl chain length: it reaches a maximum at an optimal chain length and decreases in both shorter and longer homologues. Quantitative structure–activity relationship analysis suggests that, in a series of homologues, the hydrophobic/hydrophilic balance of the compound controls the degree of the activity, whereas the intrinsic activity of the series is determined by the bulkiness of the substituents on the nitrogen atom.

Tertiary amines and their salts show comparable activities to those of the corresponding amides, suggesting that they share a common mechanism in killing the nematode. The salts kill the larva at a concentration lower than their cmc, suggesting that they behave as a single molecule for the nematocidal activity.

Experimental

General Melting points were taken on a Yanagimoto micro hot-stage melting point apparatus and were not corrected. Infrared (IR) spectra were taken in chloroform solution on a JASCO A-202 spectrometer and data (ν_{max}) are given in cm^{-1}. Proton nuclear magnetic resonance (^1H-NMR) spectra were measured in $CDCl_3$ solution with tetramethylsilane as an internal standard on a JEOL PMX-100 spectrometer and the chemical shifts are given in δ values. Mass spectra (MS) were taken on a Hitachi M-80 spectrometer and data are given by m/z (%). All organic extracts were washed with brine and dried over anhydrous sodium sulfate before concentration. Column chromatography was performed on Fuji-Davison BW-820MH (silica gel). HPLC was performed using a Toso CCPM pump and the column temperature was controled by a Toso CO-8000 column oven. Compounds were detected by a refractive index detector (Toso RI-8000) and chromatograms were processed by a Shimadzu Chromatopack C-R4A. Regression analyses were performed by a Statistical analysis system (SAS) program package[13] on a Fujitsu M760/20 computer. Surface tension was determined by the drop weight method[14] by the use of Traube's stalagmometer. All compounds were fully characterized by physical and spectral methods (data for new compounds are summarized in Tables XVI—XIX). All new compounds gave satisfactory elementary analyses or high resolution MS data.

Primary Amides I. Acylation of Amines Method I-A: When an acid chloride was commercially available, it was reacted with an appropriate amine in a mixture of ether and 5% K_2CO_3 solution according to the Schotten–Baumann procedure.

Method I-B: If an acid chloride was not available, a corresponding carboxylic acid was converted to a mixed acid anhydride by ethyl chloroformate and Et_3N in acetone and reacted with an appropriate amine.

Method I-C: Compounds **40**—**42** were prepared by acylation of an appropriate amine[15] with tetradecanoyl chloride in MeOH in the presence of Et_3N.

Method I-D: 1-Acylpiperazines (**6-*n***) were prepared as follows. An acid chloride (1 eq) in dry ether was added dropwise to a MeOH solution of piperazine (3 eq) at room temperature with stirring. The mixture was stirred for 2 h at 0 °C and concentrated to dryness. The residue was parti-

TABLE XVI. Physical and Spectral Data of the Samples (1)

Compd.	Method (Yield %)	mp or bp[a] (°C)	Formula	M^+ m/z (%)	IR (CO) cm^{-1}
1-10	I-A (56)	O	$C_{13}H_{25}NO$	211 (7)	1625
1-12	I-A (68)	C, 34—36	$C_{15}H_{29}NO$	239 (14)	1625
1-14	I-A (15)	C, 45.5—46	$C_{17}H_{33}NO$	267 (2)	1625
1-16	I-A (54)	C, 52—53	$C_{19}H_{37}NO$	295 (11)	1625
1-18	I-A (68)	C, 44—46	$C_{21}H_{41}NO$	323 (6)	1625
6-12	I-D (41)	O	$C_{16}H_{32}N_2O$	268 (6)	1627
6-14	I-D (67)	C, 38—40	$C_{18}H_{36}N_2O$	296 (6)	1625
6-16	I-D (25)	C, 69—71	$C_{20}H_{40}N_2O$	324 (12)	1625
6-18	I-D (37)	C, 122—124	$C_{22}H_{44}N_2O$	352 (4)	1625
7-8	I-A (72)	O	$C_{13}H_{26}N_2O$	226 (18)	1625
7-10	I-A (61)	O	$C_{15}H_{30}N_2O$	254 (10)	1640[b]
7-12	I-A (93)	O	$C_{17}H_{34}N_2O$	282 (14)	1640[b]
7-14	I-A (83)	C, 36—37	$C_{19}H_{38}N_2O$	310 (13)	1630
7-16	I-A (58)	C, 46—47	$C_{21}H_{42}N_2O$	338 (10)	1630
7-18	I-A (60)	C, 53—54	$C_{23}H_{46}N_2O$	366 (10)	1630
8-12	I-A (63)	O	$C_{18}H_{36}N_2O$	296 (7)	1623
8-13	I-B (76)	O	$C_{19}H_{38}N_2O$	310 (3)	1623
8-14	I-A (50)	O	$C_{20}H_{40}N_2O$	324 (5)	1623
8-15	I-B (94)	O	$C_{21}H_{42}N_2O$	338 (7)	1623
8-16	I-A (92)	O	$C_{22}H_{44}N_2O$	352 (7)	1623
8-18	I-A (51)	C, 32—33	$C_{24}H_{48}N_2O$	380 (10)	1623
8-20	I-B (72)	C, 43—43.5	$C_{26}H_{52}N_2O$	408 (6)	1625
9-8	I-A (76)	O, 248 (2 mmHg)	$C_{13}H_{24}N_2O_2$	240 (17)	1668, 1642
9-9	I-B (62)	O	$C_{14}H_{26}N_2O_2$	254 (12)	1670, 1642
9-10	I-A (91)	C, 43.5—44	$C_{15}H_{28}N_2O_2$	268 (19)	1668, 1640
9-11	I-B (87)	N, 39—41	$C_{16}H_{30}N_2O_2$	282 (9)	1668, 1640
9-12	I-A (71)	C, 33.5—35	$C_{17}H_{32}N_2O_2$	296 (8)	1670, 1640
9-13	I-B (82)	C, 53—54	$C_{18}H_{34}N_2O_2$	310 (7)	1670, 1640
9-14	I-A (80)	N, 65—66	$C_{19}H_{36}N_2O_2$	324 (7)	1668, 1640
9-15	I-B (89)	N, 63.5—64	$C_{20}H_{38}N_2O_2$	338 (6)	1672, 1640
9-16	I-A (79)	C, 71—72	$C_{21}H_{40}N_2O_2$	352 (5)	1670, 1640
9-17	I-B (68)	C, 69—71	$C_{22}H_{42}N_2O_2$	366 (8)	1670, 1640
9-18	I-A (82)	C, 60—62	$C_{23}H_{44}N_2O_2$	380 (5)	1670, 1640
9-20	I-B (99)	C, 70—72	$C_{25}H_{48}N_2O_2$	408 (5)	1670, 1640

a) C: colorless crystalline solid; N: colorless needles; O: colorless oil. *b*) Film.

TABLE XVII. Physical and Spectral Data of the Samples (2)

Compd.	Method (Yield %)	mp or bp[a] (°C)	Formula	M^+ m/z (%)	IR (CO) cm^{-1}
13	I-A (86)	N, 69—70	$C_{17}H_{35}NO$	269 (5)	1660
14	I-A (90)	N, 73—74	$C_{17}H_{35}NO$	269 (7)	1660
16	I-A (90)	N, 60—61	$C_{18}H_{37}NO$	283 (3)	1660
18	I-A (63)	N, 67—69	$C_{18}H_{37}NO$	283 (4)	1660
19	I-A (70)	P, 58—60	$C_{18}H_{37}NO$	283 (4)	1665
23	I-A (65)	S, 91—93	$C_{21}H_{35}NO$	317 (24)	1665
24	I-A (71)	N, 84—85	$C_{24}H_{43}NO$	361 (4)	1662
25	I-A (90)	O	$C_{20}H_{34}N_2O$	318 (55)	1665
27	II-A (63)	O	$C_{17}H_{35}NO$	269 (1)	1625
28	II-A (64)	O	$C_{18}H_{37}NO$	283 (6)	1625
29	II-A (66)	O	$C_{18}H_{37}NO$	283 (7)	1620
30	II-A (67)	C, <30	$C_{18}H_{35}NO$	281 (4)	1635
31	II-A (55)	O	$C_{19}H_{39}NO$	297 (3)	1630
32	II-A (61)	P, 33—36	$C_{19}H_{39}NO$	297 (2)	1630
33	II-A (79)	O	$C_{19}H_{39}NO$	297 (4)	1623
34	II-A (78)	C, <30	$C_{19}H_{39}NO$	297 (5)	1635
35	II-A (34)	O	$C_{21}H_{41}NO$	323 (12)	1625
36	II-A (60)	O	$C_{21}H_{35}NO$	317 (3)	1640
37	II-A (69)	C, 39—41	$C_{22}H_{37}NO$	331 (20)	1635
40	I-C (62)	N, 52—55	$C_{18}H_{37}NO_3$	315 (1)	1610
41	I-C (97)	N, 80—81	$C_{17}H_{33}NO_2$	283 (3)	1615
42	I-C (9)	N, 84—85	$C_{18}H_{33}NO_3$	311 (1)	1720, 1633
47	I-A (72)	C, <30	$C_{21}H_{41}NO$	323 (10)	1605
48	I-A (78)	O, 228—230 (3 mmHg)	$C_{21}H_{41}NO$	323 (11)	1620
51	[b] (83)	N, <30	$C_{18}H_{35}NS$	297 (4)	—
52	I-B (86)	O	$C_{19}H_{31}NO$	289 (13)	1597
53	I-B (39)	O	$C_{20}H_{34}N_2O$	318 (34)	1609
54	V (65)	N, 52—53	$C_{17}H_{31}NO_2$	281 (2)	1780, 1690
55	V (52)	N, 43—44	$C_{18}H_{33}NO_2$	295 (3)	1730, 1690
56	V (88)	C, 38—40	$C_{19}H_{35}NO_2$	309 (5)	1685
57	V (64)	C, <30	$C_{20}H_{37}NO_2$	323 (1)	1685
58	[b] (68)	Y, 51—53	$C_{17}H_{31}NOS_2$	329 (15)	1695
59	[b] (99)	S, 61—62	$C_{18}H_{33}NO_2$	295 (18)	1700
60	[b] (41)	N, 59—61	$C_{18}H_{31}NO_2$	293 (100)	1708
61	[b] (17)	N, <30	$C_{19}H_{35}NO_2S$	341 (19)	1700
62	[b] (4)	N, 48—50	$C_{24}H_{37}NO_2S$	403 (30)	1710

a) C: colorless crystalline solid; N: colorless needles; O: colorless oil; P: colorless plates; S: colorless scales; Y: yellow needles. *b*) See Experimental.

tioned between ether and water, the ether layer was concentrated to dryness, and the residue was purified by column chromatography.

II. Alkylation of Amides Method II-A: A mixture of a monosubstituted amide and NaOH (excess) in dry dimethylformamide (DMF) was stirred for 1—2 h. CH_3I or C_2H_5Br (excess) was added and the stirring was continued for an additional 3—4 h. DMF was removed under reduced pressure and the residue was extracted with hexane. The extract was purified by column chromatography.

Method II-B: A mixture of a lactim ether (1 eq) and tetradecyl bromide (1 eq) was heated at 150 °C for 10 h and the product (**77—79**) was purified by column chromatography.

III. 4-Alkyl-1-tetradecanoylpiperazines (82, 83, and 89) Method III: A mixture of tetradecanoylpiperazine (**6-14**, 1 eq) and an alkyl bromide (C_2H_5Br, C_3H_7Br, or $HOCH_2CH_2Br$) (1 eq) in EtOH was stirred in the dark at room temperature for 1—7 d and concentrated to dryness. The residue was purified by column chromatography. The methanesulfonyl derivative (**90**) was prepared by mesylation of **6-14** with $MeSO_2Cl$ in pyridine.

IV. 1-Alkyl-4-formylpiperazines (9-*n*) Method IV: Acetic anhydride (0.7 ml) was added to a solution of 1-alkylpiperazine (1 mmol) in formic acid (2.1 ml) and the mixture was stirred at room temperature for 1 h. The reaction mixture was neutralized with saturated $NaHCO_3$ solution and extracted with ether (3 times). The combined extracts were concentrated and the residue was purified by column chromatography and crystallization from hexane.

Secondary Amides Method V[16]: $RuO_2 \cdot xH_2O$ (5 mg) in 10% aqueous $NaIO_4$ was added to a solution of *N*-acyl cyclic amine (100 mg) in AcOEt (10 ml) and the mixture was stirred vigorously for 2 h at room temperature. The AcOEt layer was separated and the water layer was extracted with AcOEt. A few drops of isopropanol were added to the combined organic layers and the mixture was stirred at room temperature for 2—3 h. The black precipitates were filtered off and the filtrate was washed with brine, dried over Na_2SO_4, and concentrated to dryness. The residue was purified by column chromatography to give **54—57**.

Urea Type Compounds (80-*n*) Method VI: A mixture of *N*-methylpiperazine (1 g) and Et_3N (1.01 g) in dry CH_2Cl_2 (10 ml) was added dropwise to a stirred solution of trichloromethyl chloroformate (TCF) (1.2 ml) in dry CH_2Cl_2 (20 ml) at 0 °C and the stirring was continued for an additional 1 h at room temperature. The mixture was concentrated to dryness and the residue was suspended in dry toluene (20—30 ml). To this suspension was added a mixture of an amine (2 eq), Et_3N (1.2 eq), and *N*,*N*-dimethylaminopyridine (DMAP) (100 mg) and the mixture was stirred at 80 °C for 1—6 h. The precipitaes were filtered off and the filtrate was diluted with $CHCl_3$ washed with 1 N NaOH and brine, dried over Na_2SO_4, and concentrated to dryness. The residue was purified by column chromatography.

***N*-Alkyl Cyclic Amines** Method VII: To a stirred suspension of $LiAlH_4$ (16 mmol) in dry tetrahydrofuran (THF) (30 ml) was added dropwise a solution of H_2SO_4 (8 mmol) in dry THF (5 ml) at 0 °C and the stirring was continued for 30—60 min. An amide (8 mmol) in THF (20 ml) was added dropwise to the above suspension with stirring at 0 °C. After additional stirring for 1—2 h at 0 °C, the excess AlH_3 was decomposed by adding saturated KF solution. The precipitates were filtered and washed thoroughly with ether. The combined filtrate and washings were washed with 1 N NaOH and extracted with 1 N HCl (3 times).

For **92-*n*—94-*n***, the acidic aqueous layer was extracted with $CHCl_3$ (3 times) and the combined extracts were concentrated to dryness. The residue was crystallized from AcOEt or AcOEt–EtOH to give an amine hydrochloride. A solution of the hydrochloride in $CHCl_3$ was washed with

TABLE XVIII. Physical and Spectral Data of the Samples (3)

Compd.	Method (Yield %)	mp or bp[a] (°C)	Formula	M^+ m/z (%)	IR (CO) cm^{-1}
65	II-A (83)	O	$C_{17}H_{35}NO$	269 (18)	1662
67	II-A (81)	O	$C_{17}H_{35}NO$	269 (1)	1630
68	II-A (83)	O	$C_{18}H_{37}NO$	283 (6)	1622
69	II-A (39)	O	$C_{19}H_{39}NO$	297 (8)	1630
70	II-A (25)	O	$C_{30}H_{61}NO$	451 (4)	1630
72	II-A (48)	O	$C_{18}H_{37}NO$	283 (4)	1635
73	I-A (82)	C, 82—84	$C_{28}H_{57}NO$	423 (31)	1660
74	[c] (10)	C, 83—95	$C_{18}H_{33}NO_3$	293 (34)[b]	1718, 1580
75	[c] (3)	P, 85—86	$C_{18}H_{37}NO_2$	299 (31)	1650
76	I-A (94)	S, 73—75	$C_{21}H_{35}NO$	317 (22)	1650
77	II-B (28)	C, <30	$C_{18}H_{35}NO$	281 (12)	1660
78	II-B (21)	O	$C_{19}H_{37}NO$	295 (12)	1620
79	II-B (18)	O	$C_{20}H_{39}NO$	309 (26)	1625
80-8	VI (61)	O	$C_{14}H_{29}N_3O$	255 (20)	1635
80-10	VI (79)	P, 43—44	$C_{16}H_{33}N_3O$	283 (16)	1635
80-12	VI (84)	P, 48—50	$C_{18}H_{37}N_3O$	311 (14)	1635
80-14	VI (88)	P, 48—51	$C_{20}H_{41}N_3O$	339 (12)	1635
80-16	VI (80)	P, 60.5—61	$C_{22}H_{45}N_3O$	367 (8)	1638
80-18	VI (70)	P, 65.5—67.5	$C_{24}H_{49}N_3O$	395 (3)	1638
81-8	I-A (29)	S, 116—117	$C_{13}H_{27}N_3O$	241 (9)	1667
81-9	I-B (72)	S, 114—116	$C_{14}H_{29}N_3O$	255 (9)	1667
81-10	I-A (13)	I, 118—119	$C_{15}H_{31}N_3O$	269 (10)	1667
81-11	I-B (60)	N, 117—118	$C_{16}H_{33}N_3O$	283 (10)	1667
81-12	I-A (33)	N, 116—117	$C_{17}H_{35}N_3O$	297 (11)	1667
81-13	I-B (42)	N, 117—118	$C_{18}H_{37}N_3O$	311 (11)	1667
81-14	I-A (43)	I, 116—118	$C_{19}H_{39}N_3O$	325 (14)	1667
81-15	I-B (12)	I, 119—120	$C_{20}H_{41}N_3O$	339 (15)	1667
81-16	I-B (91)	N, 119—120	$C_{21}H_{43}N_3O$	353 (10)	1665
81-17	I-B (77)	S, 120—121	$C_{22}H_{45}N_3O$	367 (12)	1665
81-18	I-A (45)	N, 119—120	$C_{23}H_{47}N_3O$	381 (6)	1665
81-20	I-B (75)	N, 122—123	$C_{25}H_{51}N_3O$	409 (11)	1665
82	III (37)	O	$C_{20}H_{40}N_2O$	324 (4)	1630
83	III (42)	O	$C_{21}H_{42}N_2O$	338 (19)	1625
84	I-A (61)	N, 61—65	$C_{20}H_{38}N_2O_2$	338 (16)	1635
85	I-A (73)	S, 58—59	$C_{21}H_{40}N_2O_2$	352 (20)	1635
86	I-A (64)	S, 49—50	$C_{24}H_{46}N_2O_2$	394 (19)	1635
87	I-A (80)	N, 73—75	$C_{32}H_{62}N_2O_2$	506 (16)	1630
88	I-A (68)	C, 36—38	$C_{21}H_{40}N_2O_3$	368 (10)	1685, 1630
89	III (30)	C, 47—50	$C_{20}H_{40}N_2O_2$	340 (4)	1630
90	[c] (83)	S, 122—123	$C_{19}H_{38}N_2O_3S$	374 (3)	1640
91	I-A (30)	O	$C_{25}H_{39}N_2OF_3$	440 (21)	1635
98-12	VII (88)	O	$C_{18}H_{38}N_2$	282 (19)	
98-14	VII (76)	O	$C_{20}H_{42}N_2$	310 (9)	
98-16	VII (76)	O	$C_{22}H_{46}N_2$	338 (12)	
98-18	VII (84)	O	$C_{24}H_{50}N_2$	366 (4)	
99-12	IV (72)	O	$C_{17}H_{34}N_2O$	282 (10)	1621
99-14	IV (80)	N, 46—47.5	$C_{19}H_{38}N_2O$	310 (5)	1623
99-16	IV (86)	N, 50—52.5	$C_{21}H_{42}N_2O$	338 (3)	1621
99-18	IV (50)	I, 53—56	$C_{23}H_{46}N_2O$	366 (16)	1623

a) C: colorless crystalline solid; I: colorless prisms; N: colorless needles; O: colorless oil; P: colorless plates; S: colorless scales. *b*) $M^+ - 18$. *c*) See Experimental.

1 N NaOH and water, dried over Na_2SO_4, and concentrated to yield a free amine, which was purified by distillation or crystallization.

For **95-*n*—98-*n***, the HCl layer was basified with NaOH and extracted with ether (3 times). The combined extracts were concentrated to dryness and the residue was purified by distillation or crystallization.

Amine Hydrochlorides Dry HCl gas was introduced to an EtOH solution of an amine at 0 °C and the precipitates were collected and crystallized from AcOEt or AcOEt–EtOH.

Quaternary Ammonium Salts (100—104) A mixture of 1-tetradecanoyl-4-methylpiperazine (**7-14**) and CH_3I or $C_6H_5CH_2Br$ (excess) in EtOH was stirred for 1—3 d at room temperature. The precipitates were collected and crystallized from acetone and then from benzene. The counter anion of the above compounds was exchanged by passing a solution of the compound in EtOH–water through a column of Amberlite IRA-400 of an appropriate anion form.

1-(1-Thioxotetradecyl)pyrrolidine (51) Compound **2-14** (213 mg) was heated under reflux with Lauesson's reagent (181 mg) in dry toluene (18 ml) for 1.5 h.[17] The mixture was washed with saturated $NaHCO_3$ solution and water, dried over Na_2SO_4 and concentrated to dryness. The residue was purified by column chromatography to give **51** as colorless needles.

3-Tetradecanoyl-2-thioxothiazolidine (58) A mixture of 2-mercaptothiazoline (3 g), tetradecanoyl chloride (7.4 g), Et_3N (3.5 g), and DMAP (0.1 g) in dry THF was heated under reflux for 6 h.[18] The mixture was concentrated to dryness and the residue was purified by column chromatography and crystallized from hexane to give **58** (5.56 g) as yellow needles.

***N*-Tetradecyl Imides (59—62)** A mixture of an acid anhydride (maleic anhydride or succinic anhydride) and tetradecylamine was heated at 100—120 °C for 1—3 h. Acetic anhydride was added to the mixture and the mixture was heated at 120—130 °C for 1.5 h. After decomposition of the excess acetic anhydride with water, the mixture was extracted with ether and the extract was concentrated to dryness. The residue was purified by column chromatography to give an imide (**59** and **60**). Compound

TABLE XIX. Physical Properties of Amine Salts

Compd.	mp[a] (°C)	Formula
97-8·2HCl	S, 217 (dec.)	$C_{13}H_{30}Cl_2N_2$
97-10·2HCl	S, 241 (dec.)	$C_{15}H_{34}Cl_2N_2$
97-12·2HCl	S, 192 (dec.)	$C_{17}H_{38}Cl_2N_2$
97-14·2HCl	C, 241—243 (dec.)	$C_{19}H_{42}Cl_2N_2$
97-16·2HCl	N, 232—234 (dec.)	$C_{21}H_{46}Cl_2N_2$
97-18·2HCl	N, 225—228 (dec.)	$C_{23}H_{50}Cl_2N_2$
7-10·HCl	N, 160—163	$C_{15}H_{31}ClN_2O$
7-11·HCl	N, 172—173	$C_{16}H_{33}ClN_2O$
7-12·HCl	N, 184—186	$C_{17}H_{35}ClN_2O$
7-13·HCl	N, 183.5—185.5	$C_{18}H_{37}ClN_2O$
7-14·HCl	N, 187—190	$C_{19}H_{39}ClN_2O$
7-15·HCl	N, 177—179	$C_{20}H_{41}ClN_2O$
7-16·HCl	N, 184—186	$C_{21}H_{43}ClN_2O$
7-17·HCl	N, 180—184	$C_{22}H_{45}ClN_2O$
7-18·HCl	N, 179—184	$C_{23}H_{47}ClN_2O$
80-8·HCl	C, 225—229	$C_{14}H_{30}ClN_3O$
80-10·HCl	S, 234—236	$C_{16}H_{34}ClN_3O$
80-12·HCl	S, 231—240	$C_{18}H_{38}ClN_3O$
80-14·HCl	S, 230—238	$C_{20}H_{42}ClN_3O$
80-16·HCl	S, 228—235	$C_{22}H_{46}ClN_3O$
80-18·HCl	C, 215—220	$C_{24}H_{50}ClN_3O$
100	N, 175	$C_{20}H_{41}ClN_2O$
101	S, 175	$C_{20}H_{41}BrN_2O$
102	N, 175	$C_{20}H_{41}IN_2O$
103	P, 195	$C_{24}H_{45}ClN_2O$
104	N, 195	$C_{24}H_{45}BrN_2O$

a) C: colorless crystalline solid; N: colorless needles; P: colorless plates; S: colorless scales.

61 and **62** were prepared by a reaction of *N*-tetradecylmaleinimide (**60**) and methanethiol or benzenethiol in ether at room temperature.

Compound 74 A mixture of maleic anhydride (3 g) and tetradecylamine (6.4 g) was heated under argon at 100—110 °C for 1 h. The mixture was separated by column chromatography to give **74** (0.31 g) as a colorless crystalline solid.

***N*-(4-Hydroxybutanoyl)tetradecylamine (75)** A mixture of tetradecylamine (1 g) and γ-butyrolactone (0.4 g) was heated under argon at 120 °C for 2 h, 150 °C for 2 h, and then 170 °C for 2 h. The reaction mixture was separated by column chromatography to give **75** (39 mg) as colorless plates.

Assay Method Nematocidal activity was determined according to the method previously described.[2] For one assay, 20 second-stage larvae of *Toxocara canis* were incubated with a 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. All assays were done in duplicate. The test sample was dissolved in 0.75% saline and applied to the assay. When the sample is not soluble in water, it was dissolved in a small volume of dimethyl sulfoxide (DMSO), which was diluted with 0.75% saline at an appropriate concentration to keep the DMSO concentration in the solution at 2%. The nematocidal activity was evaluated in terms of the RM value: a smaller RM value indicates a stronger nematocidal activity, and when all the larvae die, this value is zero. MLC was defined as the lowest concentration giving an RM of 0 after 24 h incubation.

Determination of a Hydrophobic Parameter HPLC retention times were determined at 37 °C on an ODS column (Toso ODS-120T, 4.6 × 150 mm) using a H_2O–MeOH system containing 1% Et_3N as a solvent. The retention volume was caluculated from the retention time and the flow rate (1 ml/min) and the retention volume of formamide was subtracted as a void volume. The retention volumes of compounds **7-10**—**7-18** were determined at H_2O concentrations of 0, 2, 5, 8, 10, 15, 20, and 25% whenever possible. The relationship between the logarithm of retention volume and water concentration was determined by regression analysis for each compound and retention volumes at 100% water ($\log V_R$) were calculated by extrapolation. The relationship between the number of carbons in the acyl chain and the $\log V_R$ value was determined by regression analysis: $\log V_R = 0.477N - 1.268$ (N = number of carbons in the acyl chain, $n=5$, $r=0.999$, $s=0.046$). The $\log V_R$ values of other compounds were determined by measuring the retention volumes at H_2O concentrations of 0, 2, 5, and 10%, followed by regression analysis.

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