JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

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Synthesis of a Combinatorial Library of Amides and Its Evaluation against the Fall Armyworm, *Spodoptera frugiperda*

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ABSTRACT: The fall armyworm *Spodoptera frugiperda* is a polyphagous pest that causes important damage in different regions of America and mainly affects corn crops in both tropical and subtropical areas. Currently, control relies on both transgenic plants and/or chemical pesticides. In this work we describe the preparation of an indexed combinatorial library of amides and its toxic effect by contact against *S. frugiperda*. (*E*)-1-(1-Piperidinyl)-3-[4-(trifluoromethoxy)phenyl]-2-propen-1-one was the most active compound with an LD_{50} = 0.793 μg mg^{-1} of larva. This amide was also evaluated by ingestion and at the lowest concentration (1 mg kg^{-1}) achieved 83.3% mortality.

KEYWORDS: *Spodoptera frugiperda*, Lepidoptera, insecticidal activity, combinatorial library, piperamides

INTRODUCTION

The fall armyworm *Spodoptera frugiperda* Smith and Abbott (Lepidoptera: Noctuidae) is a polyphagous pest that causes important damage in different regions of America and mainly affects corn crops in both tropical and subtropical areas.[1−3] Currently, control relies on both transgenic plants and/or chemical pesticides.[4] In the two last decades, emphasis has been placed on the discovery of new, ecologically safe pesticides, in the hope that the use of more damaging synthetic pesticides can be reduced. Thus, several alternative methods have been investigated, as biological control,[5] pheromones,[6] and natural products from plants.[2,7]

The genus *Piper* (Piperaceae) has been widely studied, due to the biological properties of secondary metabolites from these plants.[8] Dyer et al. tested the effect of three amides isolated from *Piper cenocladum*: piplartine (**1**), 4′-desmethylpiplartine (**2**), and cenocladamide (**3**) (Figure 1).[9,10] On *S. frugiperda* they observed that amide mixtures caused decreased pupal weights and survivorship and increased development times. Castro et al. evaluated the insecticidal potential of the aqueous extract of fresh fruit dehydrated *P. tuberculatum* on *S. frugiperda*.[11] We have synthesized 10 amides, including two natural isolated from *P. piresii*, and evaluated their toxic effect against *S. frugiperda*. The most active amide was the natural piperidine derivative **4** with an LD_{50} of 1.07 μg mg^{-1} of larva.[12]

On the basis of these results, we describe herein the preparation of an indexed combinatorial library of amides and its insecticidal activity against the fall armyworm, *S. frugiperda*.

RESULTS AND DISCUSSION

In order to screen a large number of candidates and evaluate the potential of this class of compounds as insecticides, we prepared an indexed solution phase combinatorial library[13] of amides (Table 1). The possibility to prepare the library through a simple, highly effective coupling protocol, an amidation reaction between an acyl chloride and an amine, which does not generate any significant byproduct that could interfere with the biological assay, was of major relevance. Therefore, this methodology is a very simple approach to readily identify lead compounds from mixture screenings. Thus, a 200-member library of amides **7***[1−10,1−10]* was prepared employing 10 amines **5***[1−10]* and 10 acyl chlorides **6***[1−10]*, as shown in Scheme 1 and Table 1.

The library was prepared in a 0.2 mmol scale of each component in the mixture and was fully analyzed by GC-MS. The analysis of the GC-MS chromatograms confirms that most of the designed library members were formed, although this validation strategy does not allow the quantitative determination of each of their components. Figure 2 shows a chromatogram of one of the prepared mixtures, **7***[1−10,3]*, and the MS spectrum of one of the compounds of this mixture, **7***[2,3]*. In Figure 3, GC-MS analysis of the mixture **7***[1,1−10]* and the assignments of the peaks to the correspondent piperidine amine are shown in Table 2. Mixture **7***[1−10,2]* presented the worst results among all, as shown in Figure 4. Only one of the desired amides could be observed (16.01 min), probably due to the low acyl chloride reactivity.

The toxic effects of the amide library on second instar larvae *S. frugiperda* were determined. In the topical application bioassays were verified that mixtures **7***[1,1−10]* and **7***[1−10,8]* caused statistically significant mean mortality, 68 and 20%, respectively (Tables 3 and 4). Thus, of the library deconvolution through the cross-analysis of the results we propose that amide **7***[1,8]*, containing the piperidine and 4-(trifluoromethoxy)benzoyl groups, would

Received: December 22, 2010
Accepted: March 28, 2011
Revised: March 28, 2011
Published: March 28, 2011

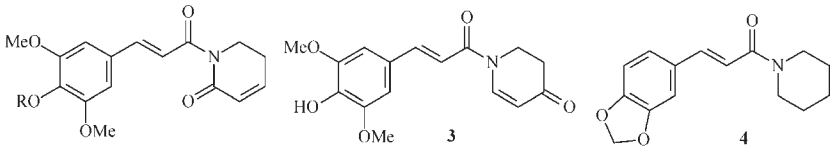


Figure 1. Natural piperamides with activity against *S. frugiperda*.

Table 1. Building Blocks Used in the Library Preparation

[N]	amine **5**	acyl chloride **6**
1	piperidine	3-(3,4-methylenedioxyphenyl)-2-propenoyl
2	pyrrolidine	3-(2,4-dimethoxyphenyl)-2-propenoyl
3	cyclohexylamine	benzoyl
4	di-*n*-butylamine	cinnamoyl
5	diethylamine	4-methoxybenzoyl
6	benzylamine	2,4-dimethoxybenzoyl
7	isobutylamine	piperonyloyl
8	2-methylbutylamine	4-(trifluoromethoxy)benzoyl
9	morpholine	4-(trifluoromethoxy)cinnamoyl
10	aniline	4-methoxycinnamoyl

be responsible for the activity in these mixtures. In order to determine its toxic effect, amide **7***[1,8]* should be prepared. On the basis of our previous work, which showed the importance of the double bond in the piperamides,[12] we have also found interesting to synthesize compound **7***[1,9]*. Thus, amides **7***[1,8]* and **7***[1,9]* (Figure 5) were prepared in 88 and 73% yields over two steps from the corresponding acids, fully characterized, and evaluated against *S. frugiperda*.

Probit analysis showed that *S. frugiperda* was more susceptible to **7***[1,9]* than **7***[1,8]*; the corresponding LD_{50} values were respectively 0.793 and 8.15 μg mg^{-1} weight of larva (Table 5). The activity of amide **7***[1,9]* was shown to be comparable with piperine against *Ascia monuste orseis*[9] and the toxicity of piperine analogues and piperonyl butoxyde to third instar larvae of *A. monuste orseis* and *S. frugiperda* by topical application.[14] The **7***[1,9]* amide also demonstrated a higher LD_{50} in comparison to amide **4** (1.07 μg mg^{-1} larva).[12] Therefore, from these results, **7***[1,9]* amide was tested to verify its insecticidal activity and/or activity related to the feeding of the insect.

The results of the present study showed that amide **7***[1,9]* was active by ingestion to first instar larvae (1 day old) of *S. frugiperda*. The toxicity for this insect, when administered in the diet, did not vary with concentration. The lowest concentration (1 mg kg^{1-}) achieved 83.3% mortality, and at the highest concentration (100 mg kg^{1-}), 86.6% mortality was recorded. It was verified that the toxicity of the amide began acting at the larval stage, because at this stage there was a mortality rate above 55%, whereas that in the control was 6.6% (Table 6). Similar results of larval mortality were also obtained by Ewete et al.[15] for the piperine extract, in *Ostrinia nubilalis* Hubner (Lepidoptera: Pyralidae), and amide **7***[1,9]* has been demonstrated to be more active than amide **4** (Figure 1).[12]

EXPERIMENTAL SECTION

Unless otherwise noted, all commercially available reagents were purchased from Aldrich Chemical Co. (Milwaukee, WI). Reagents and solvents were purified when necessary according to the usual procedures described in the literature. ^{1}H and ^{13}C NMR spectra were recorded on Bruker ARX-200 and ARX-400 instruments (200 and 100 MHz, respectively). The IR spectra refer to films and were measured on a Bomem M102 spectrometer. Mass spectra were recorded on a Shimadzu GCMS-QP5000 instrument. Analytical thin-layer chromatography was performed on a 0.25 μm film of silica gel containing fluorescent indicator UV_{254} supported on an aluminum sheet (Sigma-Aldrich). Flash column chromatography was performed using silica gel (Kieselgel 60, 230−400 mesh, E. Merck). Gas chromatography was performed in a Shimadzu GC-17A instrument with H_2 as carrier and using a DB-5 column (30 m × 0.25 mm i.d., 0.25 μm film thickness, J and W Scientific). Gas chromatography−mass spectrometry was performed in an Agilent 5890/6973, with He as carrier and using a MDN-5S column (30 m × 0.25 mm ID, 0.25 μm film thickness, Agilent Technologies). The initial oven temperature was 150 °C for 1 min and was then increased to 250 °C at a rate of 7 °C min^{-1}. The final temperature was retained for 5 min. Electron impact mass spectra were monitored at 70 eV in the *m*/*z* range of 20−500. HRMS were obtained by direct injection in a ESI(+)-MS and ESI(+)-MS/MS, electrospray ionization positive mode ions and analysis by mass spectrometry and tandem mass spectrometry equipment in QTOF Micromass (Micromass, U.K.).

General Procedure. In 10 oven-dried test tubes were added separately each of the amine **5***[N]* (2.0 mmol). In parallel, in a 50 mL volumetric flask containing anhydrous methylene chloride (20 mL) was added an equimolar mixture of the same compounds **5***[1−10]* (2.0 mmol each) and the volume of the flask was completed with methylene chloride. In 10 test tubes were added separately each of the acyl chlorides **6***[N]* (2.2 mmol), and in a 50 mL volumetric flask containing anhydrous methylene chloride was added a mixture of these acyl chlorides **6***[1−10]* (2.2 mmol each) and the volume was completed with the solvent. In each of the 20 test tubes was added methylene chloride (2.5 mL). In each test tube containing the amine **5***[N]* was added the solution of the 10 acyl chlorides **6***[1−10]* in methylene chloride (2.5 mL), and in each test tube containing the acyl chlorides **6***[N]* was added the solution of the 10 amines **5***[1−10]* (2.5 mL). Then, in each of the 20 test tubes was added triethylamine (430 μL, 4.0 mmol). The resulting mixtures were stirred in a shaker at room temperature for 12 h. Then, water (5 mL) was added. The organic layers were washed with a saturated solution of $NaHCO_3$ (5 mL) and water (5 mL), dried over $MgSO_4$, and concentrated under vacuum. All of the resulting mixtures **7***[N,1−10]* and **7***[1−10,N]* were analyzed by GC-MS and then submitted to biochemical evaluation against *S. frugiperda*. Amides **7***[1,8]* and **7***[1,9]* were prepared by parallel synthesis from the corresponding commercially available carboxylic acids.

1-Piperidinyl[4-(trifluoromethoxy)phenyl]methanone (7*[1,8]*)[16]**.** Yield: 88% (0.49 g; 2.02 mmol). Clear uncolored viscous liquid. IR (ν_{max}, film): 2918, 2850, 1647, 1502, 1488, 1446, 1353, 1247, 1189, 1101, 1037, 975, 912, 808, 746 cm^{-1}. GC-MS (relative abundance, %): *m*/*z* 272 (M^+), 189 (100), 161, 95, 84, 69, 55. ^{1}H NMR (200 MHz,

dx.doi.org/10.1021/jf104903t |*J. Agric. Food Chem.* 2011, 59, 4822–4827

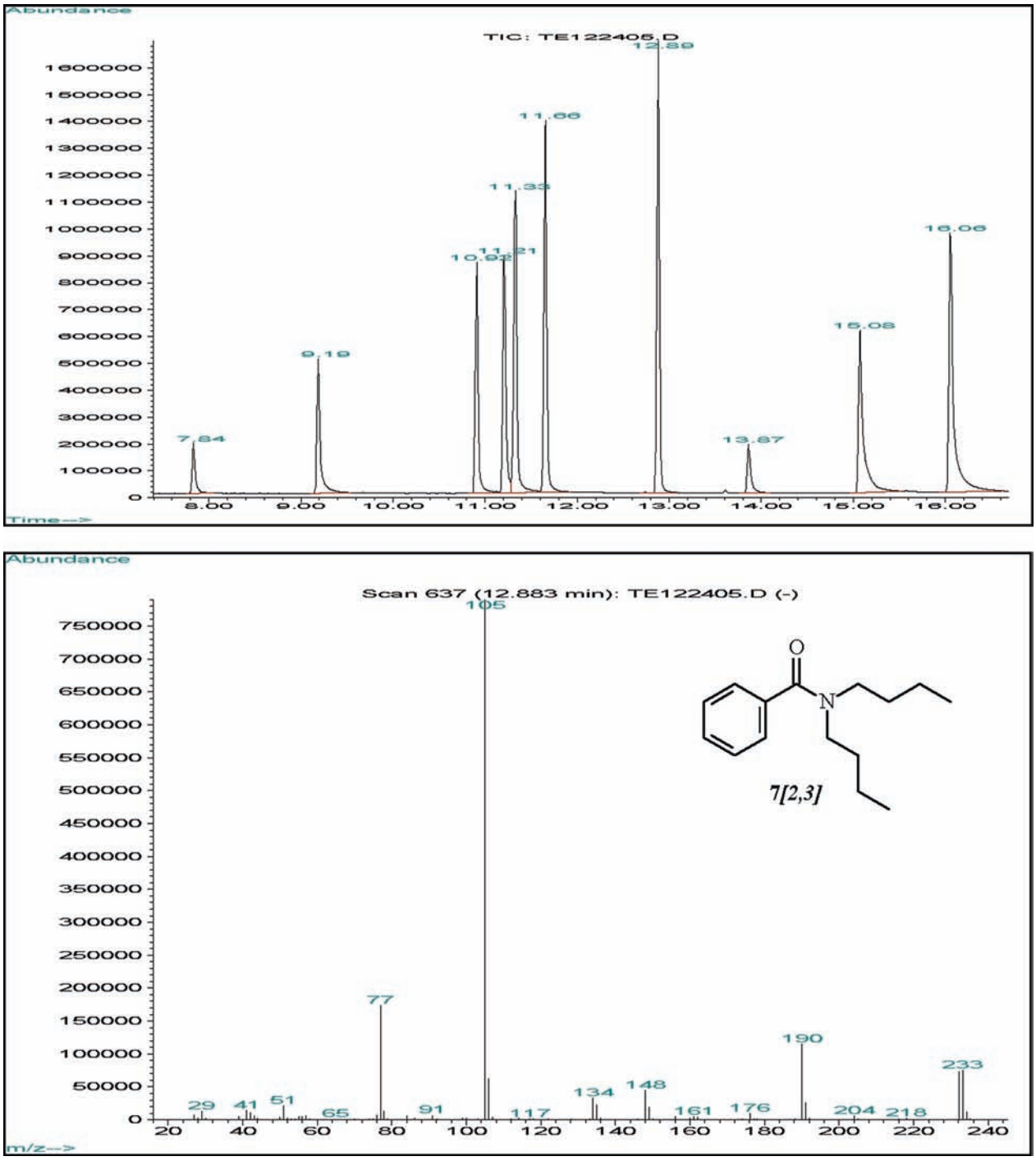


Figure 2. FID-GC chromatogram of the mixture 7*[1−10,3]* and MS spectrum of the component 7*[2,3]* (retention time 12.88 min).

$CDCl_3$): δ 7.44 (d, J = 8 Hz, 2H), 7.24 (d, J = 8.0 Hz, 2H), 3.70 (s, 2H), 3.35 (s, 2H), 1.68 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.50, 25.51, 26.52, 43.15, 48.54, 119.11, 120.85, 121.64, 128.61, 135.05, 149.76, 168.94.

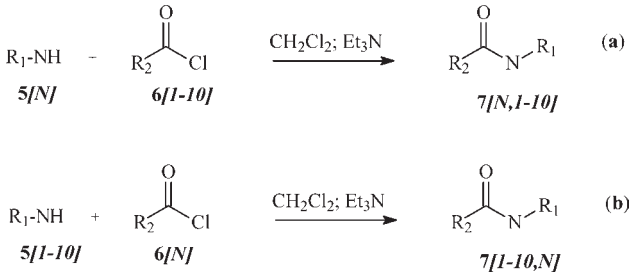
(*E*)-1-(1-Piperidinyl)-3-[4-(trifluoromethoxy)phenyl]-2-propen-1-one (7[1,9]). Yield: 73% (0.20 g; 0.67 mmol). Mp: 56.9−57.2 °C. IR (ν_{max}, film): 3070, 3002, 2939, 2858, 1647, 1602, 1508, 1442, 1278, 1213, 1161, 1018, 979, 921, 835, 808 cm^{-1}. GC-MS (relative abundance, %): m/z 299 (M^+), 215 (100), 187, 138, 101, 84. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.59−1.72 (m, 6H), 3.57−3.60 (m, 4H), 6.86 (d, J = 15.6 Hz, 1H), 7.60 (d, J = 15.6 Hz, 1H), 7.40−7.64 (m, 2H), 7.17−7.25 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.26, 25.23, 26.43, 43.04, 46.71, 118.44, 120.76, 128.68, 133.86, 140.10, 149.40, 164.60. HRMS (ESI): m/z calcd for $C_{15}H_{16}F_3NO_2$ $[M + 1]^+$ 300.1211, found 300.1211.

Biological Activity. Larvae of *S. frugiperda* were obtained from the Insect Bioassay Laboratory of Universidade Federal de São Carlos, São Carlos, Brazil, and reared on artificial diets.[17,18] They were maintained in an incubation chamber with a photophase of 12 h, 70 ± 5% relative humidity, and temperature 25 ± 1 °C.

In a first experimental stage, the contact toxicity of synthetic amides was evaluated.[19] Groups of 10 larvae in the second instar (5 day old) of *S. frugiperda* were transferred to glass Petri dishes. The average weight of insect was obtained by measuring on an analytical balance, the mass of five groups containing 10 insects each. To each individual insect was applied 1 μL of solution of the test compound in acetone topically, via a microsyringe, at concentrations of 10^{-2}, 10^{-1}, 1, and 10 mg mL^{-1} for all amides. In order to avoid the possible insect inanition, each group of larvae was supplied with a small amount (300 mg) of artificial diet. This procedure was performed 1 h after application of the test compound. The control was carried out under the same conditions; 1 μL of acetone was applied on each insect. The mortality counts were made after 48 h. All experiments and the respective controls were carried out in five replicates, and the LD_{50} was determined by analysis using Polo Software. This program uses Abbott's transformation for control mortality and calculates log dose probit lines according to the process described by Finney.[20]

In a second experimental stage, amide **7***[1,9]*, which caused the highest mortality in the first experiment, was tested to verify its insecticidal activity and/or activity related to the feeding of the insect. For each treatment and control, 30 first instar larvae (1 day old) of *S. frugiperda* were used. Amide **7***[1,9]*was administered by incorporation into an artificial diet in which bean and wheat germ are the basic ingredients.[17,18] In order to ensure uniformity, amide **7***[1,9]* (dissolved in acetone) was mixed with 1.8 g of ascorbic acid (a component of the diet) and dried, using vacuum at 40 °C, in a rotary evaporator prior to its incorporation into the diet. The mixture was incorporated to the artificial diet at the final contents of 1, 10, 50, and 100 mg kg^{-1}. The control was prepared similarly as above, but without

Scheme 1. Strategy Used for the Indexed Library Preparation[a]



[a] In the first set of mixtures (a) the amine portion of the amides was kept constant (mixtures **5***[N,1−10]*), whereas in the second set of mixtures (b) the acyl chlorides were kept constant (mixtures **6***[1−10,N]*).

Table 2. Assignments of the Peaks to the Correspondent Piperidine Amine (5*[1]*)

retention time (min)	acyl chloride **6** *[N]*
8.1	*8*
8.5	*3*
11.5	*5*
12.1	*9*
12.4	*7*
12.5	*4*
12.7	*6*
14.2	*1*
15.3	*10*
16.7	*1*

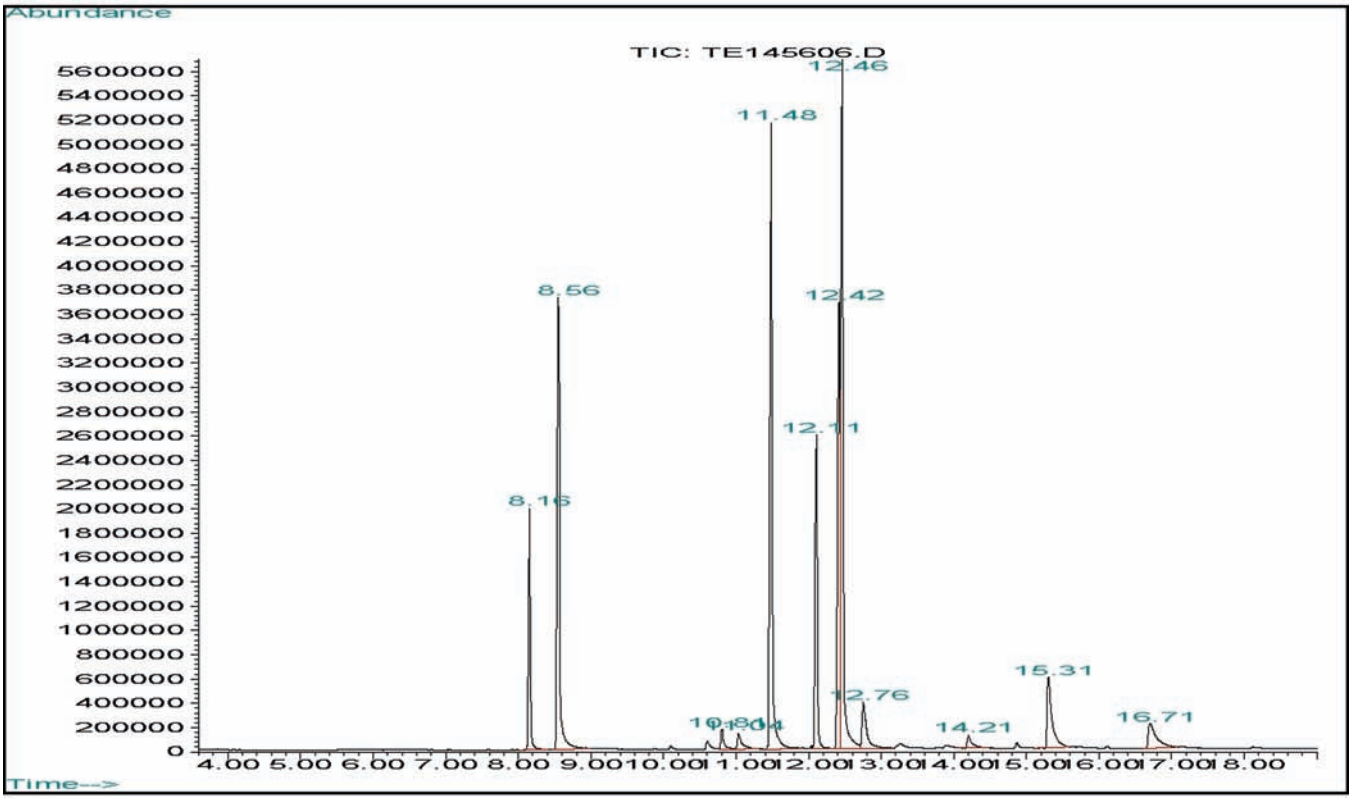


Figure 3. GC-MS analysis of mixture **7***[1,1−10]*.

dx.doi.org/10.1021/jf104903t |*J. Agric. Food Chem.* 2011, 59, 4822–4827

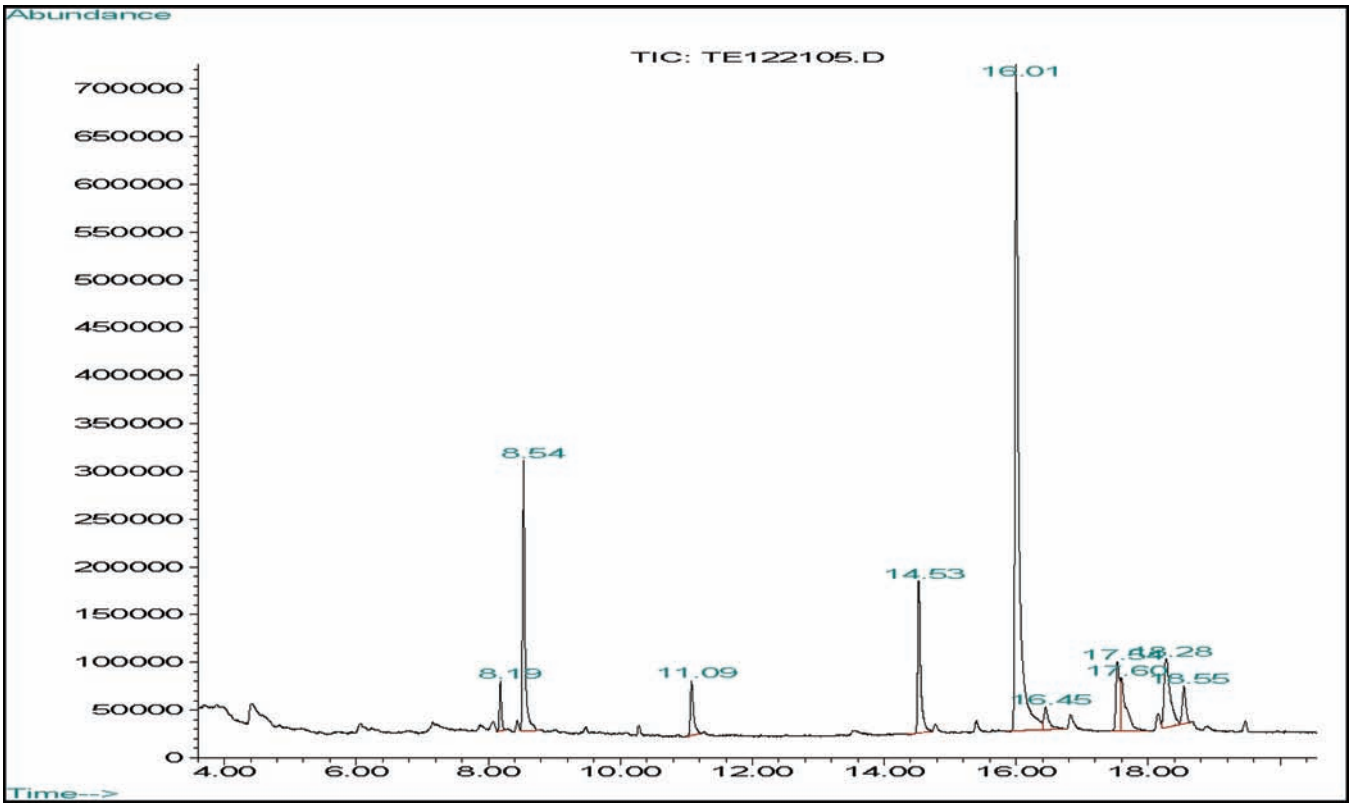


Figure 4. Chromatogram of the mixture 7*[1−10,2]*.

Table 3. Toxic Effects of Amide Library on Second Instar Larvae of *S. frugiperda*

library	mean mortality[a]
7*[1−10,3]*	8.0 ± 4.0^{bc}
7*[1−10,4]*	14.0 ± 17.0^{ab}
7*[1−10,5]*	8.0 ± 8.0^{bc}
7*[1−10,6]*	6.0 ± 9.0^{cd}
7*[1−10,7]*	12.0 ± 13.0^{b}
7*[1−10,8]*	20.0 ± 20.0^{a}
7*[1−10,9]*	12.0 ± 13.0^{b}
7*[1−10,10]*	12.0 ± 16.0^{b}
control	0.0^{d}

[a] Means followed by the same letters, in this column, indicate no significant difference ($P \leq 0.05$) in the Tukey test. Concentration of each sample containing a mixture of amides: 2000 mg L^{-1}.

amide 7*[1,9]*. The diets were placed in previously sterilized glass tubes (8.5 × 2.5 cm), into which larvae of *S. frugiperda* were introduced individually. Daily observations were made, and the following parameters were evaluated percentage of dead insects (mortality) at the end of each phase. Data were submitted to an analysis of variance (ANOVA), and the averages were compared by applying the Tukey test ($P \leq 0.05$). Each tube containing one insect, independent of the developing phase, was considered as one replicate; therefore, the number of the replicates was different for each treatment. For evaluation of the mortality of the larval and pupal phases and total cycle, the experimental unit was constituted by mean five tubes with one larva each, with ten replications by treatment.

Table 4. Toxic Effects of Amide Library on Second Instar Larvae of *S. frugiperda*

library	mean mortality[a]
7*[1,1−10]*	68.0 ± 15.0^{a}
7*[2,1−10]*	28.0 ± 18.0^{c}
7*[3,1−10]*	46.0 ± 29.0^{b}
7*[4,1−10]*	20.0 ± 12.0^{cd}
7*[5,1−10]*	2.0 ± 4.0^{fg}
7*[6,1−10]*	2.0 ± 4.0^{fg}
7*[7,1−10]*	10.0 ± 7.0^{ef}
7*[8,1−10]*	16.0 ± 13.0^{de}
7*[9,1−10]*	17.0 ± 5.0^{de}
7*[10,1−10]*	4.0 ± 9.0^{f}
control	0.0^{g}

[a] Means followed by the same letters, in this column, indicate no significant difference ($P \leq 0.05$) in the Tukey test. Concentration of each sample containing a mixture of amides: 2000 mg L^{-1}.

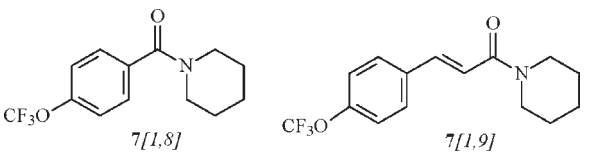


Figure 5. Synthetic amides.

Table 5. LD_{50} (μg mg^{-1} Weight of Larva) of Amides Inhibiting Growth (Second Instar) of *S. frugiperda* Administered Topically[a]

amide	slope ± SE	LD_{50} (CI 90%) (μg mg^{-1} larva)	χ^2	*G* (0.95)
7[1,8]	1.26 ± 0.254	8.15 (7.041−34.19)[b]	2.41	0.16
7[1,9]	0.593 ± 0.123	0.793 (0.054 − 1.182)[c]	0.38	0.19

[a] Definitions: SE = standard error; LD = lethal dose; CI = confidence interval; *G* = significance index. [b] *N* = 300 insects. [c] *N* = 245 insects.

Table 6. Mortality (%) of Larval and Pupal Phases and Total Cycle of *S. frugiperda* with amide *7[1,9]* Administered in the Diet

	mortality (%)[a]		
concn (mg kg^{-1})	larval phase	pupal phase	total cycle
100	55.0[a]	75.0[a]	86.6[a]
50	73.3[a]	69.4[ab]	93.3[a]
10	63.3[a]	48.6[ab]	86.6[a]
1	65.8[a]	61.4[ab]	83.3[a]
control	6.66[b]	13.3[b]	13.3[b]

[a] Means followed by the same letters, in each column, indicate no significant difference ($P \leq 0.05$) in the Tukey test.

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ACKNOWLEDGMENT

We are grateful to the FAPESP, CNPq, and CAPES for financial support and Prof. M. N. Eberlin, IQM-UNICAMP for the HRMS analysis.

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