JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Fungistatic and Bacteriostatic Activities of Alkamides from *Heliopsis longipes* Roots: Affinin and Reduced Amides

JORGE MOLINA-TORRES,*,† CARLOS JUNIOR SALAZAR-CABRERA,† CONCEPCIÓN ARMENTA-SALINAS,‡ AND ENRIQUE RAMÍREZ-CHÁVEZ†

Laboratorio de Fitobioquímica, Departamento de Biotecnología y Bioquímica de Plantas, CINVESTAV-IPN Unidad Irapuato, Km. 9.5 Libramiento Norte, Apdo. Postal 629, Irapuato, Gto. 36500 Mexico, and Instituto de Investigaciones Químico-Biológicas, Ciudad Universitaria, Universidad de San Nicolás de Hidalgo Edificio B-2, Morelia, Mich. 58030 Mexico

This work demonstrates the fungistatic and bacteriostatic activities of affinin, the main alkamide of *Heliopsis longipes* (Gray) Blake (Asteraceae) roots and two alkamides obtained by catalytic reduction of affinin: *N*-isobutyl-2*E*-decenamide and *N*-isobutyl-decanamide. The bioactivity was tested against *Rhizoctonia solani* groups AG3 and AG5, *Sclerotium rolfsii*, *Sclerotium cepivorum*, *Fusarium* sp., *Vertcillium* sp., phytopathogenic fungi; *Phytophthora infestans*, a phytopathogenic Chromista; *Saccharomyces cerevisiae*, a nonphytopathogenic ascomycete; and *Escherichia coli*, *Erwinia carotovora*, and *Bacillus subtilis*, bacteria. Affinin, being the primary component of the lipidic fraction, is expected to be responsible for the fungitoxic activity observed in roots of this plant species. Four of the assayed fungi showed an important sensitivity to the presence of affinin: *S. rolfsii*, *S. cepivorum*, *P. infestans*, and *R. solani* AG-3 and AG-5, displaying a growth inhibition of 100%. *S. cerevisiae* showed a similar growth inhibition with affinin. None of the alkamides obtained by catalytic reduction of affinin showed a fungitoxic activity. Affinin had a definite negative effect on the growth of *E. coli* and *B. subtilis*, but *E. carotovora carotovora* was not sensitive to the highest dose of affinin assayed. *N*-Isobutyl-2*E*-decenamide displayed a higher bacteriostatic activity against *E. coli* and *E. carotovora carotovora*. In both cases, this alkamide was more potent than affinin. On the other hand, only *N*-isobutyl-decanamide displayed a significant activity on the growth of *B. subtilis*.

KEYWORDS: ***Heliopsis longipes*; affinin; alkamides; fungistatic; bacteriostatic**

INTRODUCTION

Plant diseases cause important losses in agriculture and in the decay of postharvest products. Synthetic fungicides stand out as the principal measure of control, often with residual effects that can be dangerous to the environment. Because of the development of new physiological strains of pathogens, fungicidal efficacy diminishes gradually with time (*1*). Over the past years, there has been a growing interest in plant extracts containing fungitoxic properties. Recent reports from Asia and the Pacific show that numerous plants possess biological control over a wide range of pests and diseases (*2*).

Secondary metabolites, once considered as unimportant products, are now known to mediate, among other factors, communication mechanisms including plant chemical defense against microbial and herbivorous predators. These natural products are beginning to show a clear connection between pharmaceuticals and agrochemicals. The future successful development and approval of these new chemicals will require the study of their mechanisms in toxicology and pharmacology regardless if they are applied to plants or animals (*3*).

In Mesoamerica, there exists a great biodiversity of plants used for medicinal purposes over a period of centuries. The Asteraceae family is one of the most numerous in number of species and diversity in secondary metabolism. *Heliopsis longipes*, belonging to this family, is endemic to the Sierra Gorda in the central highlands of Mexico. This plant is used traditionally as a condiment, a buccal anesthetic, and an antiparasitic. On the basis of the observed insecticidal activity in the roots of this species, affinin was isolated as a bioactive compound for the first time in 1945 (*4*). Affinin (*N*-isobutyl-2*E*,6*Z*,8*E*-decatrienamide) is the most abundant amide in the roots, representing 0.78% of its dry weight. Various unsaturated aliphatic amides named alkamides have been identified and characterized in *H. longipes* roots (*5*).

The fungicidal activity of sulfurated amides (*6*) and the bactericidal activity of alkamides in plant species used for human consumption have been demonstrated (*7*). Alkamides, even though restricted in distribution, are present as bioactive compounds in 10 plant families, namely, Aristolochiaceae, Asteraceae, Brassicaceae, Convolvulaceae, Euphorbiaceae,

* To whom correspondence should be addressed. Tel: (52)462 6239645. Fax: (52)462 6239611. E-mail: jmolina@ira.cinvestav.mx.
† CINVESTAV-IPN Unidad Irapuato.
‡ Ciudad Universitaria.

10.1021/jf034374y CCC: $27.50
Published on Web 06/25/2004

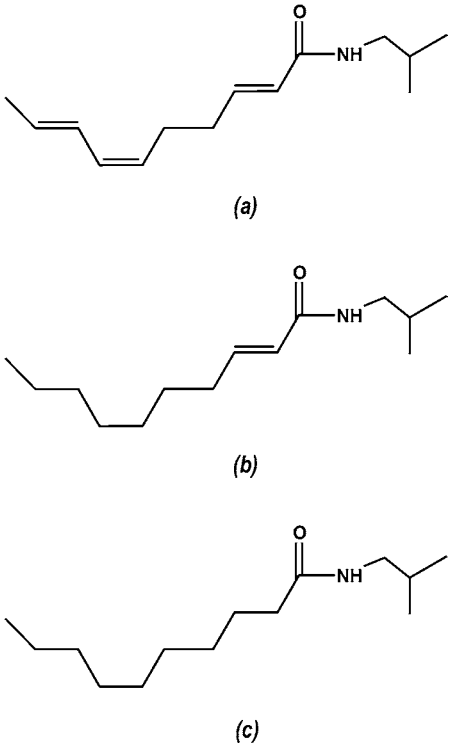

Figure 1. Structural formulas for (**a**) affinin, (**b**) *N*-isobutyl-2*E*-decenamide, and (**c**) *N*-isobutyl-decanamide.

Table 1. ^{1}H NMR (200 MHz) and ^{13}C NMR (50 MHz) Spectral Data of Reduced Amides[a]

	N-isobutyl-2*E*-decenamide		*N*-isobutyl-decanamide	
position	δ_C (ppm)	δ_H (ppm)	δ_C (ppm)	δ_H (ppm)
1	166.0		166.0	
2	123.5	5.72 d (15.0)	36.9	2.15 t (7.5)
3	144.9	6.85 m	29.6	1.3 m
4	31.9	2.16 dd (15.0; 7.5)	29.3	1.3 m
5	29.07	1.8 m	29.2	1.3 m
6	29.01	1.8 m	25.8	1.3 m
7	28.8	1.8 m	29.1	1.3 m
8	31.6	1.8 m	31.7	1.3 m
9	22.5	1.8 m	22.5	1.3 m
10	13.9	0.91 t (7.5)	14.0	0.91 t (7.5)
1′	46.7	3.17 t (6.6; 6.5)	46.7	3.17 t (6.5; 6.5)
2′	28.5	1.80 m	28.4	1.80 m
3′	20.0	1.80 m	20.0	0.93 d (7)
4′	20.0	1.80 m	20.0	0.93 d (7)

[a] Recorded in $CDCl_3$; TMS was used as the internal standard. Coupling constants (*J*) are in parentheses and are stated in Hz.

Menispermaceae, Piperaceae, Poaceae, Rutaceae, and Solanaceae. In the above-listed families, these metabolites may play a protective role. Many species containing alkamides have been used in traditional medicine in ancient cultures (*8*). Among alkamides, affinin is the most widely distributed in plants, being *H. longipes* roots, where the higher concentration is reported, as cited above (*5*). The objective of this study was to evaluate the importance of unsaturated bonds in the in vitro fungistatic and bacteriostatic activities of affinin and two alkamides obtained from partial and total catalytic reduction of the polyunsaturated amide.

MATERIALS AND METHODS

Materials. All solvents were reagent grade from Kem de León (Guanajuato, Mexico). *H. longipes* (Gray) Blake (Asteraceae) specimens, as authenticated by Dr. J. Rzedowski, Instituto de Ecología, Pátzcuaro, Michoacán, were collected at an altitude of 2500 m above sea level in Puerto de Tablas, in the municipality of Xichú, located in the Sierra Gorda of central Mexico, where annual precipitation varies between 500 and 1000 mm per year. The rainy season occurs from May to September. Voucher specimens were deposited at the Instituto de Ecología, Pátzcuaro (*H. longipes* JMT, IED).

Table 2. Phytopathogenic Fungi Mycelial Growth Inhibition as a Consequence of Different Concentrations of Affinin[a]

	concentration (μg/mL)			
species	0	50	75	150
Fusarium sp.	0[a]	22[b]	37[c]	38[c]
P. infestans	0[a]	52[b]	100[c]	100[c]
R. solani AG-3	0[a]	84[b]	99[c]	100[c]
R. solani AG-5	0[a]	87[b]	91[b]	91[b]
S. cepivorum	0[a]	94[b]	100[b]	100[b]
S. rolfsii	0[a]	100[b]	100[b]	100[b]
Verticillium sp.	0[a]	31[b]	34[b]	45[c]

[a] Growth inhibition expressed as percentage of the control. Above values are averages of three replicates. For each row, different letters imply significant differences (Tukey's test, $p = 0.05$).

Microorganisms Used. *Phytophthora infestans*, *Sclerotium rolfsii*, *Sclerotium cepivorum*, *Rhizoctonia solani* AG3 and AG5, *Fusarium* sp., *Verticillium* sp., *Saccharomyces cerevisiae* (wild stain 288C), *Escherichia coli* JM103, *Bacillus subtilis* (ssp. Kodiak), and *Erwinia carotovora* (ATCC 71) strains were provided through the courtesy of the Laboratorio de Bioquímica Ecológica del CINVESTAV-IPN Unidad Irapuato.

Extraction Process. Dry roots (1 kg) were mill ground and extracted in 10 L of 96% ethanol at room temperature for 1 week. The extract was then filtered, and the volume was reduced to 1 L using a rotatory evaporator. The concentration of affinin in the extract was quantified using gas chromatography coupled to a mass selective detector (GC/MSD).

Isolation of Affinin. Affinin was purified from the crude extract by thin-layer chromatography (TLC). Glass plates 20 cm × 20 cm with a thin layer of 0.5 mm of silica gel (Silica Gel 60 G, Merck) were prepared and developed with a solvent system of hexane:ethyl acetate (2:1 v/v). After development, the plates were air-dried, sprayed with a 0.02% ethanolic fluorescein solution, and studied under UV light. A dark band with $R_f = 0.5$ was collected, reextracted with ethyl acetate, and freed from the solvent under a stream of nitrogen. The TLC purification step was repeated three times. The content of affinin was monitored by GC/MSD.

Catalytic Reduction of Affinin and Isolation of Reduced Alkamides. To 10 mg of purified affinin dissolved in 2 mL of ethanol, 10 mg of platinum oxide was added. Then, a stream of hydrogen was bubbled into the reaction mixture in a water bath with the temperature maintained at 80 °C. The samples were collected every minute for 7 min and injected into GC/MS to monitor the reduction kinetics of affinin double bonds. After 3 min of reduction, a major intermediate compound was observed with a retention time in GC of 11.46 min. This component was isolated with the use of TLC plates impregnated with 10% silver nitrate and developed with the same solvent system as mentioned above. The separated compounds were located, on the TLC plate, under UV illumination after spraying with 0.02% fluorescein ethanolic solution. The main intermediate compound showed as a fluorescent band against a dark background with $R_f = 0.70$. The only final product was isolated after 7 min of reduction. The final reduced compound gave an $R_f = 0.61$ on TLC and a retention time of 10.01 min on GC. The TLC purification process was repeated three times. The MS and NMR spectra of these two amides were recorded.

Chromatographic and Spectroscopic Analysis. The samples were analyzed in a gas chromatograph (Hewlett-Packard GC model 5890), equipped with a capillary column HP-1MS (30 m × 0.25 mm i.d.; 0.25 μm film thickness) coupled to a MSD (Hewlett-Packard, model 5972 MSD). The operating conditions were as follows: the injector temperature was maintained at 200 °C. The oven temperature program

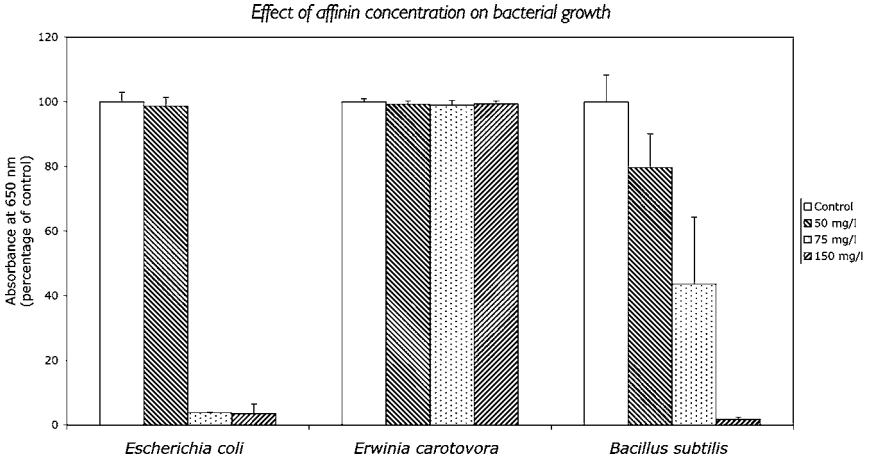

Figure 2. Effect of affinin on the growth of *E. coli* (Gram –), *E. carotovora* (Gram –), and *B. subtilis* (Gram +) in the range from 0 to 150 mg/L. Growth was estimated as the turbidity at 650 nm in a PDB liquid medium. All inocula were transferred from cultures growing at an exponential rate in the same media composition.

required maintaining an initial temperature 150 °C for 3 min and increasing at the rate of 4 °C/min to a final temperature of 300 °C maintained for 20 min. Helium was used as the carrier gas with a constant flow of 1 mL/min. One microliter of the sample was injected with a split ratio of 50:1. UV/vis spectra and turbidity at 650 nm were obtained with a Cary Varian model 3E spectrophotometer. NMR spectra were obtained in a Varian Gemini 200. Spectra were collected at frequencies of 200 MHz for ^{1}H and of 50 MHz for ^{13}C in deuterated chloroform and tetramethylsilane as the internal standards (99.8% deuterated chloroform containing 0.03% TMS, Aldrich).

Microbiological Assays. The poisoned food technique, as described by Hadacek and Greger (*9*), was adopted for toxicity studies. The minimum inhibitory concentration was estimated by testing different quantities of each compound incorporated into the PDA culture medium (potato dextrose agar, Difco Laboratories). The compound was incorporated into the medium at about 50°C, before it solidified in the Petri dishes. The solidified plates at room temperature were inoculated with a 5 mm mycelium disk, inverted, and incubated in darkness at 18−24 °C depending on the fungus strain. The mycelium dry weight was determined after 10 days of incubation. The statistical analysis of the results was accomplished by a completely randomized design where all treatments had three replicates. The mean separation was aided with Tukey's test ($p = 0.05$).

S. cerevisiae and bacterial growth were estimated as turbidity at 650 nm in a PDB liquid medium (potato dextrose broth, Difco Laboratories) in a double beam Cary Varian model 3E UV/visible spectrophotometer. All inocula were transferred from cultures growing at an exponential rate in the same media composition. To obtain the final specific concentration of the assayed compound, an ethanolic solution of the alkamide was added to the flasks. The ethanol was evaporated, and the residue was redissolved in the medium. Flasks containing 10 mL of PDB medium were inoculated with 0.1 mL of bacterial culture to be assayed. To estimate the bacteriostatic activity, liquid cultures were grown at 28 or 37 °C, depending on the microorganism, in the PDB medium with vigorous agitation. After 24 h of incubation, the turbidity was measured at 650 nm using the previously mentioned Cary Varian model 3E spectrophotometer. An optic density of 0.1, in a 1 cm long path cell, corresponds to 1.8×10^8 bacteria per milliliter.

RESULTS

Spectroscopic Analysis. Figure 1 illustrates the structures of the alkamides. The spectroscopic data were collected as follows. Affinin (**a**): retention time (R_t) = 12.03 min; retention index (R_i) = 1.000 in CG. The partially reduced amide *N*-isobutyl-2*E*-decenamide (**b**): R_t = 11.46 min; R_i = 0.953 in CG. UV (MeOH): λ_{max}= 211 nm. MS: *m*/*z* (%) = 225 (15.6), 210 (12.5), 170 (27.7), 153 (100), 126 (21.3), 55 (26.4). Totally reduced amide *N*-isobutyl-decanamide (**c**): R_t = 10.01 min; R_i = 0.832 in CG. MS: *m*/*z* (%) = 227 (6), 172 (30.1), 155 (31.1), 128 (25), 115 (100), 57 (24.6). The spectroscopic analytical results for the reduced alkamides for ^{1}H NMR (200 MHz) and ^{13}C NMR are presented in **Table 1**. The affinin resonance spectrum was as reported previously (*10*).

Fungistatic Activity. Mycelial growth inhibition at 10 days under the influence of affinin is illustrated in **Table 2**. *S. rolfsii* and *S. cepivorum* were more sensitive to this amide, with a mycelial growth inhibition of 100 and 94%, respectively, at 50 mg/L, the minimum concentration assayed. Following in sensitivity, *P. infestans*, *R. solani* AG-3 and AG-5 required 75 mg/L to show a similar inhibition of 100, 99, and 91%, respectively. A lower sensitivity was detected with *Verticillium* sp. and *Fusarium* sp. even at the higher concentration of affinin assayed. At 150 mg/L, the inhibition was less than 50%. *S. cerevisiae*, an ascomycete, growth was inhibited 100% at a concentration of 75 mg/L. It was grown in a liquid medium and was evaluated as the turbidity at 650 nm (data not shown). The partially and totally reduced amides did not have any significant influence on the mycelial growth of any of the fungi assayed (data not shown).

Bacteriostatic Activity. The bacteriostatic activity of affinin is presented in **Figure 2**. The growth of *E. coli* (Gram −) and *B. subtilis* (Gram +) was 100% inhibited at affinin concentrations of 75 and 150 mg/L, respectively. *E. carotovora* (Gram −) was not sensitive to the higher concentration of this amide. The partially reduced amide *N*-isobutyl-2*E*-decenamide was active against *E. coli*, *E. carotovora*, and *B. subtilis*, which showed a 100% growth inhibition at concentrations of 5, 5, and 50 mg/L, respectively (**Figure 3**). However, the totally reduced amide, *N*-isobutyl-decanamide, displayed a bacteriostatic activity against *B. subtilis* (100% growth inhibition at 150 mg/L) but was not inhibitory against *E. coli* or *E. carotovora* (**Figure 4**).

DISCUSSION

Even though the importance of the carbonyl conjugated double bond in amides to the insect toxicity has been observed (*11*, *12*), nothing has been suggested that relates to bacterial or fungal toxicity. To explain the relationship of the affinin structure activity to fungistatic and bacteriostatic activity, the molecule was modified by catalytic hydrogenation. The two main products obtained were *N*-isobutyl-2*E*-decenamide and *N*-isobutyl-decanamide. The structures were characterized by GC/MSD and NMR. Just as it is in the nervous system of insects

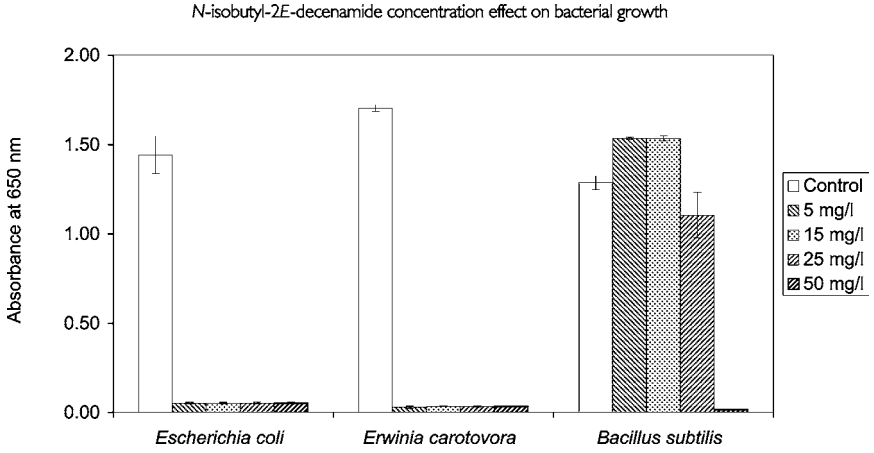

Figure 3. Effect of *N*-isobutyl-2*E*-decenamide on the growth of *E. coli* (Gram –), *E. carotovora* (Gram –), and *B. subtilis* (Gram +) in the range from 0 to 50 mg/L. Growth was estimated as the turbidity at 650 nm in a PDB liquid medium. All inocula were transferred from cultures growing at an exponential rate in the same media composition.

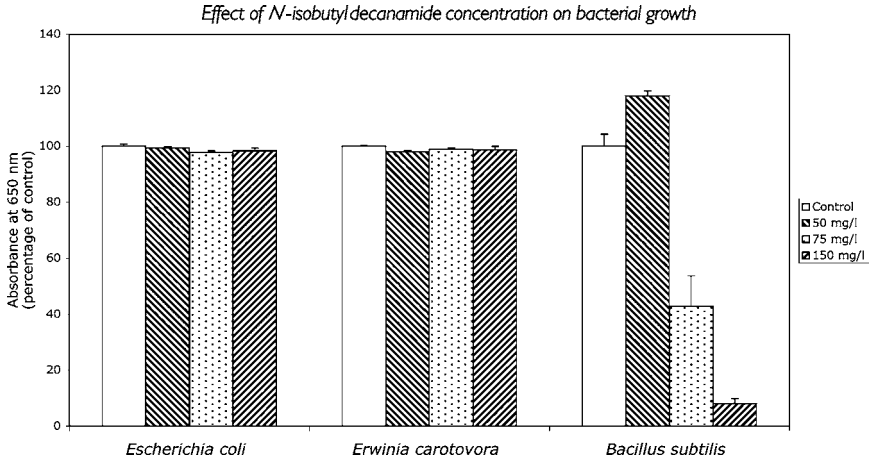

Figure 4. Effect of *N*-isobutyldecanamide on the growth of *E. coli* (Gram –), *E. carotovora* (Gram –), and *B. subtilis* (Gram +) in the range from 0 to 150 mg/L. Growth was estimated as the turbidity at 650 nm in a PDB liquid medium. All inocula were transferred from cultures growing at an exponential rate in the same media composition.

(*13*), these chemicals may have general or specific activities on key target sites in bacteria or fungi receptors.

The data indicate that the mechanism of action of the alkamides on the fungi and the bacteria may be different. Affinin was more active in fungi, which suggests that the conjugation of the carbonyl to the instauration 2*E* is insufficient for the fungitoxic action. It requires conjugation with the instauration in either positions 6*Z*, 8*E*, or both.

The results obtained show differences in the growth inhibition of the affinin depending on the phytopathogen. Fungi, which have hyaline mycelium such as *S. rolfsii*, *S. cepivorum*, and *P. infestans*, for some reason are more susceptible to growth inhibition than those that have colored mycelium. Singh and collaborators (*14*) observed that those fungi, which have colored conidia, require higher concentrations of the *Allium sativum* metabolite ajoene, to inhibit germination of the conidia.

There are few antecedents to the action of the fungicidal activity of the aliphatic amides. Vasques-da-Silva and collaborators (*15*) reported the inhibition in germination of the fungus *Cladosporium cladosporoides* spores with pellitorine (*N*-isobutyl-2*E*,4*E*-decadienamide) obtained from *Piper tuberculatum* seeds. A previous report on the fungicidal activity of sulfurated amides, isolated from plants of the genus *Glycosmis* (Rutaceae), showed germtube inhibition of *Cladosporium herbarum* spores at concentrations in the range of 8−200 mg/L. In that study, the most active structure was an amide unsaturated in position 2*E* (*6*). These values are comparable to our growth inhibition results, although here we study mycelial growth and not spore germination.

The growth inhibition effect of the affinin varies depending on the bacteria being tested; however, it has an inhibitory effect on the growth of Gram + bacteria as well as on Gram − bacteria. The amide *N*-isobutyl-decanamide with a totally saturated aliphatic chain only had an effect on *B. subtilis*, probably because this is a Gram + bacteria, and it had no effect on the Gram − bacteria assayed. The bacteriostatic activity of the partially saturated amide *N*-isobutyl-2*E*-decenamide maintained the conjugated trans double bound and was higher than that of affinin in both Gram + and Gram − bacteria. As most differences in antibacterial activity between Gram + and Gram − bacteria are due to membrane permeability, it would be interesting to know if alkamides interact with ion channels as in insects (*13*) and mammals (*16*).

The synthetic inhibitor of the β-hydroxydecanoyl dehydratase NAC (3-decynoyl-*N*-acetylcysteamine), blocking the synthesis of unsaturated fatty acids, displayed a strong activity against *E. coli* but only a slight and transient effect on the growth of *S. cerevisiae* (*17*). Affinin, having a structure resembling NAC, with an unsaturated 2*E* bound, displayed a similar but lower activity unlike to capsaicin, an amide not containing an unsaturated bound (*7*), which suggests that the affinin in bacteria may act by blocking the biosynthesis of unsaturated fatty acids

and then probably affecting the permeability of the membrane. However, a more detailed study of the action mechanism is required.

Finally, it is interesting to notice that this family of amides has an effect not only in animals and microorganisms but in plants where these reduced alkamides were recently shown to have a higher capacity to promote growth and alter root development in *Arabidopsis* seedlings than the polyunsaturated affinin (*18*).

LITERATURE CITED

(1) Wellman, R. Problems in development registration, and use of fungicides. *Annu. Rev. Phytopathol.* **1977**, *15*, 153−163.

(2) FAO. *Report of the FAO Expert Consultation on Regional Perspectives for Use of Botanical Pesticides in Asia and the Pacific*; FAO: 1995; p 57.

(3) Wedge, D.; Camper, D. Connections between Agrochemicals and Pharmaceuticals. In *Biologically Active Natural Products: Pharmaceuticals*; Cutler, H., Cutler, S., Eds.; CRC Press: Boca Raton, 1999; pp 1−15.

(4) Acree, F.; Jacobson, M.; Haller, H. An amide possessing insecticidal properties from the roots of *Erigeron affinis* DC. *J. Org. Chem.* **1945**, 236−242.

(5) Molina-Torres, J.; Salgado-Garciglia, R.; Ramírez-Chávez, E.; del-Rio, R. Purely olefinic alkamides in *Heliopsis longipes* and *Acmella (Spilanthes) oppositifolia*. *Biochem. Syst. Ecol.* **1996**, *24*, 43−47.

(6) Greger, H.; Zechner, G.; Hoffer, O.; Vajrodaya, S. Bioactive amides from *Glycosmis* species. *J. Nat. Prod.* **1996**, *59*, 1163−1168.

(7) Molina-Torres, J.; García-Chávez, A.; Ramírez-Chávez, E. Antimicrobial properties of natural alkamides traditionally used in Mesoamerica: Affinin and Capsaicin. *J. Ethnopharmacol.* **1999**, *64*, 241−248.

(8) Hashimoto, K.; Satoh, K.; Kase, Y.; Ishige, A.; Kubo, M.; Sasaki, H.; Nishikawa, S.; Kurosawa, S.; Yakabi, K.; Nakamura, T. Modulatory effect of aliphatic acid amides from *Zanthoxylum piperitum* on isolated gastrointestinal tract. *Planta Med.* **2001**, *67* (2), 179−181.

(9) Hadacek, F.; Greger, H. Testing of antifungal natural products: Methodologies, Comparability of results and assay choice. *Phytochem. Anal.* **2000**, *11*, 137−147.

(10) Correa, J.; Roquet, S.; Díaz, E. Multiple NMR analysis of the affinin. *Org. Magn. Reson.* **1971**, *3*, 1−5.

(11) Crombie, L.; Krasinski, A. Synthesis of N-isoButyldeca-*trans*-2,*cis*-6,*trans*-8- and *trans*-2,*cis*-6,*cis*-8-trienamide. *Chem. Ind.* **1962**, *June 2*, 983−984.

(12) Johns, T.; Graham, K.; Towers, G. Mulluscicidal activity of affinin and other isobutylamides from the Asteraceae. *Phytochemistry* **1982**, *21* (11), 2737−2738.

(13) Bloomquist, J. Ion channels as targets for insecticides. *Annu. Rev. Entomol.* **1996**, *41*, 163−190.

(14) Singh, U.; Pandey, V.; Wagner, K.; Singh, K. Antifungal activity of ajoene, a constituent of garlic (*Allium satuvum*). *Can. J. Bot.* **1990**, *68*, 1354−1356.

(15) Vasques-da-Silva, R.; Navickienea, H. D.; Kato, M.; Bolzani, V. d. S.; Méda, C.; Young, M.; Furlan, M. Antifungal amides from *Piper arboreum* and *Piper tuberculatum*. *Phytochemistry* **2002**, *59*, 521−527.

(16) Caterina, M.; Rosen, T.; Tominaga, M.; Brake, A.; Julius, D. A capsaicin-receptor homologue with a high threshold for noxious heat. *Nature* **1999**, *398*, 436−441.

(17) Kass, L. The antibacterial activity of 3-decynoyl-*N*-acetylcysteamine. Inhibition *in vivo* of b-hydroxydecanoyl thioester dehydrase. *J. Biol. Chem.* **1968**, *243*, 3223−3228.

(18) Ramírez-Chávez, E.; López-Bucio, J.; Herrera-Estrella, L.; Molina-Torres, J. Alkamides isolated from plants promote growth and alter root development in Arabidopsis. *Plant Physiol.* **2004**, *134*, 1−11.

Received for review April 11, 2003. Revised manuscript received May 18, 2004. Accepted May 19, 2004. We acknowledge the Consejo Nacional de Ciencia y Tecnología, Mexico, for the financial support of this work (Grants SHIGO 97-810 and CONACYT 26398N).

JF034374Y