Isolation, Structural Assignment, and Synthesis of *N*-(2-Methyl-3-oxodecanoyl)-2-pyrroline, a New Natural Product from *Penicillium brevicompactum* with in Vivo Anti-Juvenile Hormone Activity

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A new natural product with in vivo anti-juvenile hormone (JH) activity, *N*-(2-methyl-3-oxodecanoyl)-2-pyrroline (**2**), has been isolated from *Penicillium brevicompactum* Dierckx. Its structure has been tentatively assigned based on spectral data and unambiguously confirmed by alternative syntheses. Compound **2** has been prepared by means of a sequence of reactions beginning with acylation of Meldrum's acid by octanoyl chloride. The subsequent steps have been aminolysis of the resultant intermediate with pyrrolidine, alkylation of the active position with iodomethane, introduction of a methoxy group in the pyrrolidine ring by anodic oxidation, and final elimination of methanol through adsorption on SiO_2 and heating. The natural product **2** and its bicyclic isomer 2-heptyl-3-methyl-4-oxo-6,7,8,8a-tetrahydro-4*H*-pyrrolo[2,1-*b*]-1,3-oxazine (**3**) are also obtained. This reveals that **2** can be biogenetically related to the recently discovered brevioxime (**1**). Compound **2** and the synthetic precursors of **2** have shown important biological activities as insecticides (against *Oncopeltus fasciatus* Dallas) and fungicides. The natural product induces precocious metamorphosis in the target insect (70% precocious adults at 10 μg/nymph). In view of the above results, these products could be useful as lead molecules for the synthesis of analogues with enhanced biological activities.

Introduction

One of the most important and challenging aspects of pesticide research is the urgent need to develop new and effective methods of controlling plagues; these methods should cause no harm to human health and the environment, and they must be accepted as safe by the general public.[1] Natural products, with their tremendous structural diversity, are an important source of new alternatives. Many natural products showing fungicidal, bactericidal, or insecticidal activities are isolated every year. If their properties allow and if sufficient quantities can be obtained from natural sources or by synthesis, such compounds may be used as agricultural chemicals. Alternatively, they may constitute useful starting points as lead molecules for the synthesis of analogues with improved biological and physical properties.[2]

In recent times, investigations by several research groups have shown that one of the most important sources of bioactive compounds is fungus. The secondary metabolites of fungal origin exhibit a wide range of potentially useful biological activities.[3,4]

Penicillium is one of the genera, together with *Aspergillium* and *Fusarium*, which produce metabolites known to be toxic to insects.[5] Particularly, the fungus *Penicillium brevicompactum* Dierckx has been described as one of the most prolific producers of secondary metabolites. These include mycophenolic acid and related compounds,[6] the Raistrick phenols,[7−9] the pebrolides[10] or the *N*-benzoyl derivatives of phenylalanine, phenylalaninol, and its ester, asperphenamate.[11] In addition, the fungus also produces brevigellina,[12] several piperazine-2,5-dione derivatives, a drimane diterpenoid,[13] the brevianamides,[14,15] and compactin.[16] The latter is a reported hypocholesterolaemic agent that was shown to be a reversible, competitive inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase). Previous work on the effects of this product on

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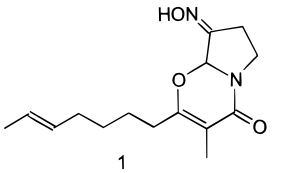
insects has shown that it is able to produce a potent in vitro juvenile hormone (JH) biosynthesis inhibition, with IC_{50} of the order of $10^{-7}-10^{-9}$ M in lepidoptera[17,18] and dictioptera.[19,20] However, as a general rule, the morphological effects of compactin are scarce.

Recently we have reported the isolation and identification of brevioxime (**1**), a new metabolite from *P. brevicompactum*, which exhibits a very high activity as a JH biosynthesis inhibitor.[21] Its chemical structure contains an unusual heterobicyclic skeleton and an oxime functionality.

In this paper we report the isolation, identification, and alternative synthesis of a new natural product (**2**) from *P. brevicompactum*, with a high in vivo anti-JH activity.



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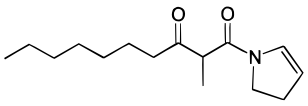

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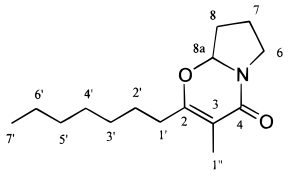


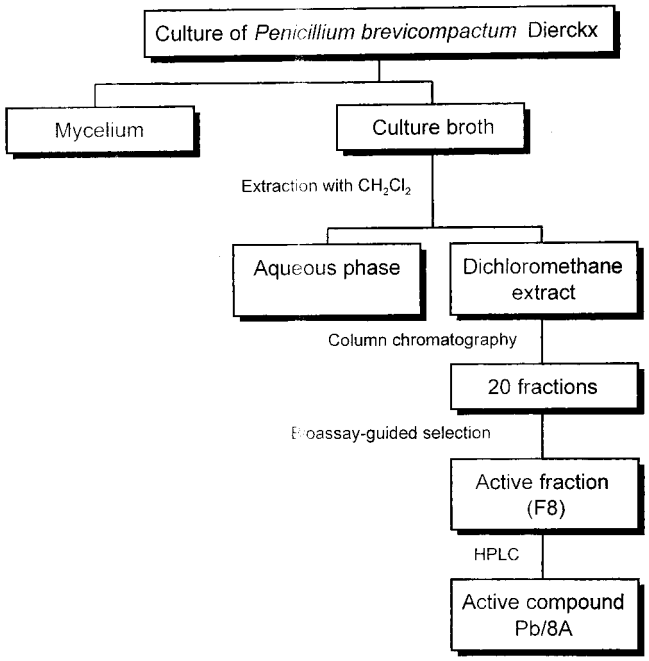
3

Although at first sight the structures of **1** and **2** seem unrelated, chemical studies have shown that **2** can be converted into the bicyclic isomer **3** upon acid catalysis; and because basic skeletons of **1** and **3** are the same, it appears that the new natural product **2** can be biogenetically related to brevioxime.

Results and Discussion

A systematic screening was performed with 118 strains of *Penicillium* isolated from fungal contamination of cereals. The most promising results were obtained with a strain of *P. brevicompactum*. A summary of the procedure followed to isolate the active compound is illustrated in Scheme 1. The dichloromethane extract obtained from the culture medium exhibited the highest entomotoxicity and anti-JH activity to *Oncopeltus fasciatus* (20% mortality and 40% precocious adults at 10 μg/cm^2, following the method of Bowers et al.[22]). In addition, the extract showed an important fungicidal activity against *Colletotrichum gloesporoides*, *Alternaria tenuis*, *Fusarium culmorum*, and *Trichoderma viride* at 500 μg/mL.

Scheme 1. Isolation and Purification of the Anti-JH Compound 2 from *P. brevicompactum*



The entomotoxicity and anti-JH activity bioassay served as a guide for silica gel column separation, which led to a fraction (F8) with remarkable in vivo anti-JH activity. Preparative HPLC of this fraction allowed isolation of the active pure compound Pb/8A. Its structure was tentatively assigned, by means of combined spectral data, as *N*-(2-methyl-3-oxodecanoyl)-2-pyrroline (**2**).

To confirm the structure and to prepare higher amounts of compound for further biological assays, we designed an alternative synthesis based on the approach outlined in Scheme 2. If successful, slight modifications of this approach could allow synthesis of a number of related intermediates and derivatives with enhanced biological activities or both.

The synthetic scheme involves the use of commercial starting materials such as pyrrolidine and octanoyl chloride. The first step was acylation[23,24] of Meldrum's acid[25,26] with octanoyl chloride. This treatment gave an intermediate, which was submitted to aminolysis without previous purification, by reaction with pyrrolidine in refluxing benzene.[27] This led to the β-ketoamide **4** in 61% overall yield from octanoyl chloride.

The next step was introduction of a methyl group[28,29] between both carbonyls. Thus, after using NaH to

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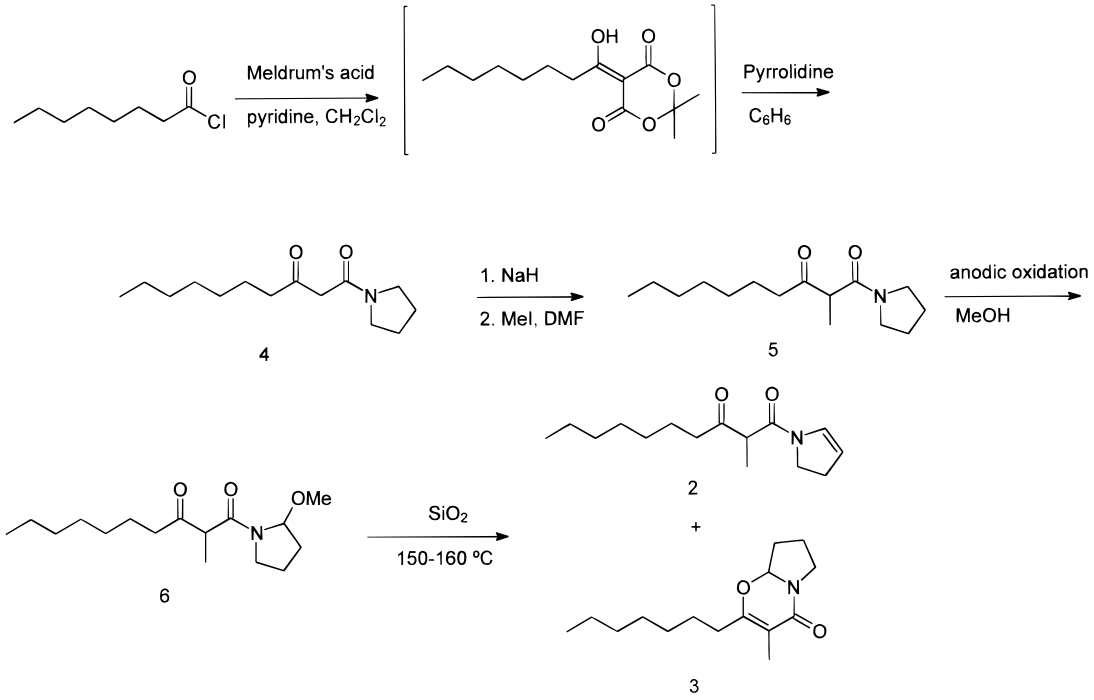
Scheme 2. Synthesis of the Natural Ketoamide 2



generate the carbanion, methylation was achieved by treatment with iodomethane. The desired alkylated β-ketoamide **5** was obtained in 79% yield.

To achieve the pyrrolidine to pyrroline conversion of **5**, a methoxy group was introduced at C_2 by means of anodic oxidation, following the method previously described by Shono.[30−35] A methanolic solution of β-ketoamide **5** was subjected to a constant electric current of 20 mA, in the presence of tetrabutylammonium *p*-toluenesulfonate as a supporting electrolyte, until 3.7 F/mol had passed through the solution. In this way, the methoxylated β-ketoamide (**6**) was obtained in 45% yield, and 50% of the starting material was recovered. The two diasteromers of **6** (**a** and **b**) were resolved by column chromatography. Both of them were present in solution as a mixture of the two possible amide conformations, which gave separate signals in NMR.

Finally, elimination of methanol was carried out by adsorption of **6** on SiO_2 and subsequent heating to 150−160 °C.[31,36−40] Under these conditions, a 1:1 mixture of the desired pyrroline **2** and the isomeric bicyclic product **3** was obtained.

Table 1. Entomotoxic and In Vivo Anti-JH Activity against *O. Fasciatus*

products[a]	dose (μg/cm^2)	toxicity %[b]	anti-JH activity %[c]
2	10	20.0 ± 4.5	71.4 ± 5.4[e]
4	10.0	91.1 ± 3.9	nd[d]
	7.5	56.7 ± 4.7	nd
	5.0	6.7 ± 0.0	nd
5	10.0	100	
	7.5	100	
	5.0	82.2 ± 2.2	nd
	2.5	24.4 ± 4.6	nd
	1.0	13.3 ± 0.0	nd

[a] Products without activity are not reflected in the table. [b] Each value means the average (n = 3) and deviation standard of percentage of mortality, with respect to control. Results were obtained by the contact method. [c] Percentage of precocious adults with respect to surviving nymphs in the entomotoxic test. [d] nd: not detected. [e] Anti-JH activity has been detected by topical application, on newly moulted fourth-instar nymphs, at 10 μg/nymph.

Insecticidal, anti-JH, and antimicrobial activities of the natural product **2**, as well as for the bicyclic isomer **3** and the synthetic intermediates **4**−**6**, have been evaluated. Table 1 contains the relevant data for the compounds showing activity against the milkweed bug *O. fasciatus*. The natural product (**2**) exhibited an important antagonistic JH activity that induced precocious metamorphosis. The effects of this product on treated nymphs were of the same type as those described for the precocenes.[22,41] In addition, **2** has been shown to be a true anti-JH agent according to Staal,[42] because its coadministration with a juvenoid (methoprene) is able to reverse the action. Experiments are under way to determine the mechanism of action of this compound. On the other hand, two intermediates in the synthesis of **2**, compounds **4** and **5**, have shown a strong knockdown toxicity to *O. fasciatus*

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Table 2. Fungicidal Activities of Products 2−6

target phytopathogens	percentage of radial mycelial growth inhibition[a] % (mean ± SD)[b]					
	2	**3**	**4**	**5**	**6a**	**6b**
Fusarium culmorum	0	0	<10	0	<10	<10
Fusarium oxysporium ssp. *gladioli*	0	0	11.4 ± 2.9	0	0	0
Fusarium oxysporium ssp. *niveum*	<10	11.4 ± 1.6	10.2 ± 1.54	0	0	<10
Geotrichum candidum	0	0	14.3 ± 5.8	0	0	<10
Colletotrichum gloesporoides	46.8 ± 4.9	<10	26.3 ± 3.0	17.5 ± 2.50	11.1 ± 2.0	12.9 ± 1.0
Colletotrichum coccodes	31.7 ± 2.9	20.0 ± 1.7	45.9 ± 4.4	20.0 ± 3.30	38.2 ± 4.4	47.8 ± 5.8
Trichothecium roseum	34.7 ± 3.4	18.0 ± 4.4	26.2 ± 2.5	<10	22.0 ± 2.5	33.3 ± 4.9
Alternaria tenuis	26.0 ± 5.8	28.2 ± 4.4	27.1 ± 2.7	31.9 ± 4.6	15.5 ± 1.4	21.7 ± 2.7
Rosellinia necatrix	16.1 ± 7.8	0	0	0	0	0
Verticillium dahliae	18.4 ± 4.0	30.1 ± 7.0	38.3 ± 5.4	17.3 ± 2.0	35.9 ± 4.5	38.5 ± 7.8
Trichoderma viride	17.3 ± 8.3	<10	<10	15.0 ± 1.0	<10	<10
Penicillium italicum	0	20.9 ± 3.6	18.2 ± 3.5	0	<10	<10
Aspergillus parasiticus	0	0	0	16.2 ± 2.7	0	0

[a] Assay concentration: 100 μg/mL. [b] Each value means the average and standard deviation of three replicates.

(Table 1). Compound **5** was more active than **4**, clearly suggesting that introduction of a methyl group in the molecule enhances the entomotoxicity.

Table 2 shows fungicidal activities of the compounds. In all cases, minimum inhibitory concentration (MIC) values were greater than 100 μg/mL, so none of the compounds were strongly effective in inhibiting the growth of the tested microorganisms. However, the selective activity exhibited by compounds **2**, **4**, and **6** on *Colletotrichum* genus could be of some interest. Under certain conditions, a selective fungicide may be very useful for controlling a particular microorganism; thus, these compounds could be used as lead molecules for the synthesis of analogues with improved fungicidal activity against *Colletotrichum*, a very important phytopathogen.

Bactericidal activities have been determined against six selected Gram-positive and Gram-negative bacteria. Only compound **3** showed a moderate activity on *Bacillus cereus*, with an inhibition zone of 11 mm at the dose of 20 μg/mm^2.

Conclusion

A new natural product, **2**, has been isolated from *P. brevicompactum*. Its structure has been tentatively assigned based on spectral data and unambiguously confirmed by synthesis. Compound **2** and its bicyclic isomer **3** arise from a common precursor (**6**) in the synthetic sequence; this reveals that **2** can be biogenetically related to the recently discovered brevioxime (**1**). The biological activities of **2** and **3** and their synthetic intermediates **4**−**6** suggest that these compounds can be useful as lead molecules for the development of new biorational pesticides.

Experimental Section

All chemicals were obtained from commercial suppliers and used without further purification. IR spectra were obtained as liquid films (or KBr plates for the natural product); ν_{max} is given for the main absorption bands. ^{1}H and ^{13}C NMR spectra were recorded at 300 and 75 MHz (or 400 and 100 MHz for the natural product), respectively, in $CDCl_3$ solvent; chemical shifts are reported in δ (ppm) values, using TMS as the internal standard. Mass spectra were obtained under electron impact (or chemical ionization for the natural product); the ratios *m*/*z* and the relative intensities are reported. Isolation and purification were done by flash column chromatography on silica gel 60 (230−400 mesh). Analytical TLC was carried out on precoated plates (silica gel 60 F_{254}), and spots were visualized with UV light and in a I_2 chamber.

Isolation and Characterization of the Active Compound. The procedure was similar to that previously reported for brevioxime.[21] Briefly, the fungus was isolated in our laboratories and classified by The International Mycological Institute (IMI, Surrey, U.K.) as *P. brevicompactum* Dierckx. A sample of the strain is filed in the "Colección de Cultivos de la Cátedra de Microbiología" of the Department of Biotechnology (Universidad Politécnica de Valencia). It is coded as P79 and kept in agar slants with potato dextrose agar (PDA) as the culture medium.

The strain was seeded in Petri dishes with a PDA culture medium and incubated for 7 days at 28 °C. Then, sterile distilled water with Tween 80 (0.05%) was used to obtain a suspension containing ca. 10^6 conidia/mL. This suspension was added to an Erlenmeyer flask with antibiotic test broth (1:9 volume ratio), and the mixture was incubated for 15 days, in the dark, at 28 °C.

After incubation, the culture medium was extracted three times with CH_2Cl_2 (1:3, v/v). The resulting extract was dried over $CaCl_2$, filtered, and evaporated in vacuo. The residue (2.0 g from 20 L of culture) was submitted to column chromatography on silica gel (1:60, w/w) using mixtures of CH_2Cl_2, AcOEt, Me_2CO, and MeOH (stepwise gradient) as eluent. This led to the separation of 20 fractions. Through the use of the method of Bowers et al.[22] (see below), it was possible to localize a significant biological activity in fraction number 8, whose yield was 125.6 mg.

Preparative HPLC of fraction 8 was achieved using the following conditions: column Lichrosorb Si-60, 7 μm (25.0 × 2.5 cm); mobile phase CH_2Cl_2/AcOEt (70:30, v/v); flow 8 mL/min; detection by UV (254 nm) and refraction index, simultaneously. A fraction was obtained (retention time: 24.9 min) consisting in 12.6 mg of the pure active compound, whose structure was tentatively assigned on the basis of spectral data to be **2**: $[\alpha]^{20}_D = 27°$ (*c* 0.07, $CHCl_3$); HRMS *m*/*z* 251.1888 ($C_{15}H_{25}NO_2$ requires 251.1885); IR ν_{max} 2952, 2924, 2855, 1718, 1642, 1610, 1457, 1419; ^{1}H NMR δ_H 6.9, 6.6 (m + m, 1H), 5.3 (m, 1H), 3.9 (m, 2H), 3.6 3.5 (q + q, J = 7 Hz, 1H), 2.8−2.4 (m, 4H), 1.6 (m, 2H), 1.4 (d, J = 7 Hz, 3H), 1.2 (m, 8H), 0.9 (t, J = 7 Hz, 3H); ^{13}C NMR δ_C 207.2, 165.6, 129.3, 128.3, 113.1, 111.6, 53.3, 45.5, 39.3, 31.7, 29.0, 28.1, 23.5, 22.6, 14.1, 13.1; MS *m*/*z* 251 (M^+, 2), 167 (1), 126 (5), 125 (6), 96 (4), 70 (6), 69 (100), 68 (20), 57 (10), 55 (4), 41 (3).

Synthesis of *N*-(2-Methyl-3-oxodecanoyl)-2-pyrroline. *N*-(3-Oxodecanoyl)pyrrolidine (4). To a cooled solution (0 °C) of 2,2-dimethyl-1,3-dioxane-4,6-dione (5.43 g, 36.9 mmol) in dichloromethane (35.0 mL) were added pyridine (6.0 mL, 74.2 mmol) and octanoyl chloride (5.2 mL, 30.7 mmol) via syringe, dropwise, under nitrogen. The solution was stirred at 0 °C for 1 h 25 min, after which it was allowed to warm to room temperature for an additional period of 2 h 10 min. The dichloromethane solution was washed with dilute HCl, water, and brine, dried, and concentrated to give the acylated Meldrum's acid, which was used for the aminolysis without further purification.

A solution of the acylated Meldrum's acid and pyrrolidine (5.7 mL, 68.9 mmol) in benzene (180.0 mL) was refluxed for 12 h. The solvent was evaporated under reduced pressure, and the residue was purified by column chromatography on silica gel (gradient elution with mixtures of EtOAc and hexane, with 30−40% EtOAc) to provide the β-ketoamide **4** (4.50 g, 61%) as a brown oil: HRMS *m/z* 239.1891 ($C_{14}H_{25}NO_2$ requires 239.1885); IR ν_{max} 2940, 2920, 2840, 1710, 1630, 1450, 1420, 1360, 1330, 1295, 1250, 1220, 1190, 1160, 1105, 910, 860, 780, 720; ^{1}H NMR δ_H 3.5 (s, 2H), 3.5−3.4 (m, 4H), 2.6 (t, J = 8 Hz, 2H), 2.0−1.9 (m, 4H), 1.6 (m, 2H), 1.3 (br s, 8H), 0.9 (t, J = 7 Hz, 3H); ^{13}C NMR δ_C 204.6, 171.8, 50.3, 47.0, 45.7, 43.0, 31.5, 28.9, 25.8, 24.2, 23.3, 22.4, 13.9; MS *m/z* 239 (M^+, 23), 222 (3), 210 (5), 197 (10), 196 (5), 182 (6), 168 (53), 155 (67), 140 (32), 127 (4), 113 (85), 112 (100), 98 (80), 85 (43), 72 (41), 71 (56), 70 (96), 69 (31), 57 (27), 56 (28), 55 (50), 43 (45).

***N*-(2-Methyl-3-oxodecanoyl)pyrrolidine (5).** To a stirred slurry of prewashed NaH (60% dispersion oil; 0.79 g, 19.8 mmol) in DMF (30.0 mL) at 0 °C was added a solution of the β-ketoamide **4** (4.50 g, 18.8 mmol) in DMF (5.0 mL), via a double-ended needle, dropwise. After hydrogen evolution had ceased, the mixture was warmed to room temperature, stirred for 1 h 30 min, and then recooled to 0 °C. Iodomethane (1.3 mL, 21.7 mmol) was added. After being stirred at room temperature for 3 h 10 min, the mixture was diluted with water and extracted with CH_2Cl_2. The combined extracts were washed with brine, dried, and concentrated. Chromatography of the residue on silica gel (gradient elution with mixtures of EtOAc and hexane, with 20−30% EtOAc) provided the β-ketoamide **5** (3.74 g, 79%) as a yellow oil: HRMS *m/z* 253.2050 ($C_{15}H_{27}NO_2$ requires 253.2041); IR ν_{max} 2930, 2900, 2840, 1710, 1630, 1445, 1420, 1360, 1330; ^{1}H NMR δ_H 3.6−3.4 (m, 5H), 2.5 (m, 2H), 2.1−1.8 (m, 4H), 1.6 (m, 2H), 1.4 (d, J = 7 Hz, 3H), 1.3 (br s, 8H), 0.9 (t, J = 7 Hz, 3H); ^{13}C NMR δ_C 207.1, 168.4, 52.8, 46.6, 45.8, 39.3, 31.4, 28.8, 25.8, 24.0, 23.2, 22.3, 13.8, 13.0; MS *m/z* 254 (M^+ + 1, 2), 253 (M^+, 8), 207 (1), 182 (6), 169 (6), 154 (1), 127 (100), 126 (48), 112 (6), 99 (10), 98 (27), 71 (17), 70 (51), 57 (42), 55 (39), 43 (39).

2-Methoxy-*N*-(2-methyl-3-oxodecanoyl)pyrrolidine (6). A solution of β-ketoamide **5** (0.39 g, 1.6 mmol) in methanol (60.0 mL) containing tetrabutylammonium *p*-toluenesulfonate (1.84 g, 4.4 mmol) as a supporting electrolyte was placed into an electrolysis cell equipped with carbon electrodes (8.5 cm^2). A constant current (20 mA) were passed through the solution. After 3.7 F/mol of electricity had passed through the solution, the solvent was evaporated under reduced pressure. Water was added to the residue, and the product was extracted with CH_2Cl_2. The combined organic layer was dried over anhydrous sodium sulfate. Thereafter, the drying agent was removed by filtration, the solvent was evaporated to dryness, and the residue was filtered through silica gel using OEtAc as an eluent, to eliminate the supporting electrolyte. The solvent was evaporated under reduced pressure, and the residue was purified by column chromatography on silica gel, using a hexane/EtOAc mixture (75:25) as eluent, to afford two diastereomers of the methoxylated β-ketoamide **6** (0.13 + 0.07 g, 45%) as a yellow oil and the unreacted β-ketoamide **5** (0.20 g, 0.8 mmol).

Spectral data of the first eluted diastereomer **6a**: HRMS *m/z* 283.2140 ($C_{16}H_{29}NO_3$ requires 283.2147); IR ν_{max} 2910, 2840, 1710, 1650, 1450, 1405, 1360, 1170, 1075, 1060, 810; ^{1}H NMR δ_H 5.5 and 5.0 (d + d, J = 4 Hz, 1H), 3.7−3.3 (m, 3H), 3.4 and 3.3 (s + s, 3H), 2.5 (m, 2H), 2.2−1.8 (m, 4H), 1.5 (m, 2H), 1.4 and 1.3 (d + d, J = 7 Hz, 3H), 1.3 (br s, 8H), 0.9 (t, J = 7 Hz, 3H); ^{13}C NMR δ_C 206.9, 170.5, 169.9, 88.5, 87.2, 56.6, 54.0, 53.0, 46.2, 45.9, 39.4, 39.2, 31.5, 31.3, 30.6, 29.0, 28.9, 23.4, 22.7, 22.5, 20.8, 14.0, 13.9, 13.0; MS *m/z* 283 (M^+, 2), 268 (10), 253 (23), 252 (16), 251 (20), 199 (4), 183 (9), 167 (8), 156 (34), 127 (64), 126 (30), 125 (61), 100 (22), 97 (52), 85 (17), 70 (100), 69 (26), 57 (20), 155 (16).

Spectral data for the second eluted diastereomer **6b**: HRMS *m/z* 283.2146 ($C_{16}H_{29}NO_3$ requires 283.2147); IR ν_{max} 2920, 2840, 1710, 1650, 1455, 1405, 1370, 1170, 1090, 1070, 810; ^{1}H NMR: δ_H 5.4 and 4.9 (d + d, J = 4 Hz, 1H, H-2), 3.8−3.2 (m, 3H), 3.3 and 3.2 (s + s, 3H), 2.5 (m, 2H), 2.2−1.8 (m, 4H), 1.5 (m, 2H), 1.4 and 1.3 (d + d, J = 7 Hz, 3H), 1.2 (br s, 8H), 0.9 (t, J = 7 Hz, 3H); ^{13}C NMR δ_C 206.5, 170.7, 88.7, 87.3, 56.5, 53.6, 52.8, 52.2, 46.1, 45.9, 39.8, 39.3, 31.5, 31.2, 30.4, 29.0, 28.9, 23.3, 22.7, 22.4, 20.9, 13.9, 13.8, 14.0; MS *m/z* 283 (M^+, 1), 268 (26), 253 (15), 252 (12), 251 (12), 199 (1), 183 (13), 167 (4), 157 (13), 156 (12), 127 (32), 126 (21), 125 (46), 100 (35), 97 (57), 85 (23), 70 (100), 69 (25), 57 (22), and 55 (17).

***N*-(2-Methyl-3-oxodecanoyl)2-pyrroline (2) and 2-Heptyl-3-methyl-4-oxo-6,7,8,8a-tetrahydro-4*H*-pyrrolo[2,1-*b*]-1,3-oxazine (3).** A mixture of the two diastereomeric α-methoxy amides **6** (13 mg, 0.05 mmol) and silica gel (8 mg, 0.14 mmol) was heated to 150−160 °C in a flask, under reduced pressure and a nitrogen atmosphere. After 2 h 45 min, water was added to the residue, and the slurry was extracted with CH_2Cl_2. The combined organic layer was dried over anhydrous sodium sulfate. Then, the drying agent was removed by filtration, the solvent was evaporated to dryness, and the residue was purified by column chromatography on silica gel (gradient elution with mixtures of EtOAc and hexane, with 15−20% EtOAc) to provide, in order of elution, the enamine **2** (3 mg, 29%), the starting β-ketoamide **6** (4 mg, 0.01 mmol), and the bicyclic enone **3** (4 mg, 37%).

The enamine **2** was a yellow oil which showed spectral data identical to those reported above for the natural product.

The enone **3** was a yellow oil: HRMS *m/z* 251.1883 ($C_{15}H_{25}NO_2$ requires 251.1885); IR ν_{max} 2920, 2840, 1720, 1655, 1425, 1370, 1345, 1080, 760; ^{1}H NMR δ_H 5.2 (dd, J = 6 and 5 Hz, 1H), 3.7 and 3.4 (m + m, 2H), 2.4−1.8 (m, 6H), 1.8 (s, 3H), 1.5 (m, 2H), 1.3 (br s, 8H), 0.9 (t, J = 7 Hz, 3H); ^{13}C NMR δ_C 168.2, 163.8, 106.4, 87.5, 44.3, 31.8, 31.7, 30.6, 29.3, 29.0, 26.8, 22.6, 21.9, 14.1, 10.1; MS *m/z* 251 (M^+, 39), 250 (92), 223 (7), 210 (12), 183 (53), 166 (16) 152 (25), 141 (100), 140 (65), 139 (42), 127 (97), 126 (97), 113 (97), 112 (98), 111 (81), 98 (93), 97 (81), 83 (99), 71 (94).

Biological Assays. Insects. *Oncopeltus fasciatus* Dallas was maintained at 28 ± 1 °C, 50−60% relative humidity, 16 h/8 h (day/night) photoperiod, and a diet based on sunflowers seeds.

Target Microorganisms. Fungicidal activity was measured against 13 agronomically important phytopathogens. These strains were provided by the "Colección Española de Cultivos Tipo" (CECT) or by the "Coleccion de la Catedra de Microbiologia" (CCM) of the Department of Biotechnology (Universidad Politecnica de Valencia). *Aspergillus parasiticus* (CECT 2681), *Geotrichum candidum* (CCM 245), *A. tenuis* (CECT 2662), *C. gloesporoides* (CECT 2859), *Colletotrichum coccodes* (CCM 327), *Fusarium oxysporium* ssp *gladioli* (CCM 233), *F. oxysporum* ssp *niveum* (CCM 259), *F. culmorum* (CCM 172), *Penicillium italicum* (CECT 2294), *T. viride* (CECT 2423), *Trichothecium roseum* (CECT 2410), *Rosellinia necatrix* (CCM 297), *Verticillium dahliae* (CCM 269). Six different bacterial strains were used in order to determine bactericidal activity: *Staphylococcus aureus* (CECT 86), *Enterococcus faecalis* (CCM 12), *Salmonella typhi* (CECT 409), *Erwinia carotovora* (CECT 225), *Escherichia coli* (CECT 405), and *B. cereus* (CECT 148).

Entomotoxicity and Anti-JH Activity. The test was carried out basically according to the contact method of Bowers et al.[22] Briefly, 15 third-instar *O. fasciatus* nymphs were confined to a 9-cm Petri dish coated with 500 μg/cm^2 (100 μg/cm^2 for the fractions) of the extract being tested, with lower doses for higher activities. Products were assayed at 10 μg/cm^2, and, in a parallel way, assays were performed by topical application on newly moulted fourth-instar nymphs of *O. fasciatus*, at 10 μg/nymph. Toxicity effects were considered according to the number of insects dead after 72 h of exposure to the chemicals. All assays were made three times. The surviving nymphs were transferred to a 500-cm^3 glass flask and held at standard conditions. After metamorphosis occurred and the production of viable offsprings signaled successful reproduction, the tests were finished. The tests were considered positive for JH antagonistic activity either when precocious metamorphosis occurred or when sterility of the resulting adults was detected. Controls were run in parallel and received the same amount of acetone as treated insects.

Antifungal Activity. The extract and products at concentrations of 500 and 100 μg/mL, respectively, dissolved in acetone and added to PDA. PDA plates containing only acetone were used as control plates. Seven-day-old cultures of each fungus on PDA plates were used as an inoculum onto the control and test plates. The radial mycelial growth was measured, and the percentage of inhibition was calculated on the basis of growth in control plates after 4 days of incubation at 28 °C, except for *R. necatrix* and *V. dahliae*, which were measured after an incubation period of 6 days. The antifungal activity of each sample was determined three times.

Antibacterial Activity. Disk method: 15 mL of Mueller-Hinton agar on Petri dishes was inoculated with 1 mL of 24-h-old bacterial cultures in nutrient broth. The optical density of the resulting solution was 0.2 at 700 nm. Whatman no. 113 paper disks (5 mm in diameter) were saturated with 100 μg/mm^2 of the fungi extract, and control disks were saturated with acetone. After 24 h of incubation, bactericidal activity was measured and expressed as the width of the clear inhibition zones, in millimeters, including the disk. All assays were made three times.

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Supporting Information Available: ^{1}H-NMR and ^{13}C-NMR spectra of compounds **2**−**6** (12 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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