

# Synthesis and Biological Evaluation of New Analogues of the Active Fungal Metabolites *N*-(2-Methyl-3-oxodecanoyl)-2-pyrroline and *N*-(2-Methyl-3-oxodec-8-enoyl)-2-pyrroline

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To evaluate the effect of simplifying the  $\beta$ -ketoamide system present in active isolated metabolites from *Penicillium brevicompactum* (**2** and **3**) on the activity, new analogues with a monocarbonylic amide functionality have been obtained. In this way, the insecticidal and fungicidal activities have been improved in relation to the natural products taken as lead molecules. Thus, two of the synthetic analogues (**5a** and **5b**) showed very important insecticidal activities against third-instar nymphs of *Oncopeltus fasciatus* Dallas, with acute LD<sub>50</sub> values of 3.0 and 1.5  $\mu\text{g}/\text{cm}^2$ , respectively. Moreover, some analogues showed good levels of fungicidal activity against a wide range of commercially important and taxonomically diverse fungi; remarkably, compound **7c** has proved to be highly active against *Colletotrichum gloeosporoides* and *Colletotrichum coccodes*, with ED<sub>50</sub> values of 2.04 and 11.7  $\mu\text{g}/\text{mL}$ , respectively.

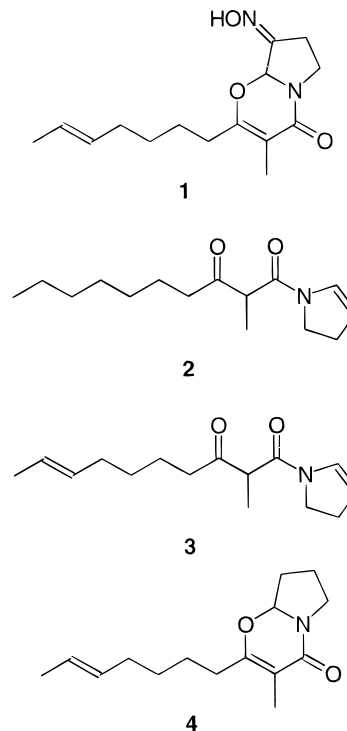
**Keywords:** Amide; fungicidal; insecticidal

## INTRODUCTION

The independent synthesis of bioactive natural products is often necessary to confirm their structures and activities as these compounds are secondary metabolites usually isolated in minimal quantities. Such work also leads to a series of synthetic intermediates, chemically related to the natural compounds, which are potentially active; sometimes these analogues are even more active than the isolated compounds. Thus, active natural products can be used as lead molecules to design different derivatives with similar functionalities but with improved biological activities.

Recently, we have achieved the isolation and identification of a new family of bioactive metabolites from fungal extracts. Thus, brevioxime (**1**), isolated from *Penicillium brevicompactum*, exhibits a very high activity as a juvenile hormone (JH) biosynthesis inhibitor (Moya et al., 1997; Castillo et al., 1998). The related compounds, *N*-(2-methyl-3-oxodecanoyl)-2-pyrroline (**2**), *N*-(2-methyl-3-oxodec-6-enoyl)-2-pyrroline (**3**), and 2-hept-5-enyl-3-methyl-4-oxo-6,7,8,8a,-tetrahydro-4*H*-pyrrolo-[2,1-*b*]-1,3-oxazine (**4**), isolated from the same source, show in vivo JH antagonistic and insecticidal activity (Moya et al., 1998; Cantín et al., 1999). Two inactive pyrrolic metabolites, presumably belonging to the same biosynthetic pathway, were used as starting points to obtain active analogues, upon introduction of simple structural changes (Cantín et al., 1998).

Now we wish to report the synthesis and biological activities of several derivatives of the enamides **2** and **3**, which were prepared as part of a program aimed at



improving the activities exhibited by these natural products.

## MATERIALS AND METHODS

All chemicals were obtained from commercial suppliers and used without further purification. IR spectra were obtained as liquid films;  $\nu_{\text{max}}$  is given for the main absorption bands. <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded at 300 and 75 MHz, respectively, in CDCl<sub>3</sub> solvent; chemical shifts are reported in

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$\delta$  (parts per million) values, using tetramethylsilane (TMS) as internal standard. The assignment of  $^{13}\text{C}$  signals is supported by DEPT experiments. Mass spectra were obtained under electron impact or chemical ionization; 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 thin layer chromatography (TLC) was carried out on precoated plates (silica gel 60 F<sub>254</sub>), and spots were visualized with ultraviolet (UV) light and in an  $\text{I}_2$  chamber.

**General Synthetic Procedures.** *Synthesis of N-Acylpyrrolidines.* To a mixture of pyrrolidine (14.1 mmol) with 1.7 M KOH (9.0 mL) was added a solution of acyl chloride (14.0 mmol) in  $\text{CH}_2\text{Cl}_2$  (9.0 mL) dropwise (10 min). After being stirred at room temperature for 5.5 h, the mixture was extracted with  $\text{CH}_2\text{Cl}_2$ ; the resulting organic extracts were washed with brine, dried over  $\text{Na}_2\text{SO}_4$ , and concentrated to dryness to give the *N*-acylpyrrolidine in a straightforward manner as oils.

*N-Octanoylpyrrolidine (5a):* 91% yield; obtained as an oil; HRMS (EI)  $m/z$  197.1774 ( $\text{C}_{12}\text{H}_{23}\text{NO}$  requires 197.1779); IR  $\nu_{\text{max}}$  2900, 2860, 2840, 1605, 1410, 1330, 1230, 1160, 1090, 1030, 905, and 830;  $^1\text{H}$  NMR  $\delta_{\text{H}}$  3.4 (m, 4H, H-2 + H-5), 2.2 (t,  $J = 7$  Hz, 2H, H-2'), 2.0–1.8 (m, 4H, H-3 + H-4), 1.6 (m, 2H, H-3'), 1.3 [br s, 8H,  $(\text{CH}_2)_4\text{CH}_3$ ], and 0.9 (t,  $J = 7$  Hz, 3H,  $\text{CH}_3$ );  $^{13}\text{C}$  NMR  $\delta_{\text{C}}$  171.8 ( $\text{C}_1$ ), 46.5 ( $\text{C}_2$ ), 45.5 ( $\text{C}_5$ ), 34.8 ( $\text{C}_2'$ ), 31.6, 29.4, 29.0, 26.0, 24.9, 24.3, 22.5 ( $\text{C}_3$ ,  $\text{C}_4$ ,  $\text{C}_3' - \text{C}_7'$ ), and 14.0 ( $\text{CH}_3$ ); MS  $m/z$  197 ( $\text{M}^+$ , 11), 168 (12), 154 (9), 140 (13), 126 (73), 113 (100), 98 (46), 85 (56), 71 (72), 70 (80), 57 (30), and 55 (65).

*N-Oct-6-enoylpyrrolidine (5b):* 85% yield; obtained as an oil; HRMS (EI)  $m/z$  195.1627 ( $\text{C}_{12}\text{H}_{21}\text{NO}$  requires 195.1623); IR  $\nu_{\text{max}}$  2910, 2845, 1640, 1430, 1330, 1250, 1220, 1190, 1160, and 960;  $^1\text{H}$  NMR  $\delta_{\text{H}}$  5.4 (m, 2H, H-6' + H-7'), 3.4 (m, 4H, H-2 + H-5), 2.2 (t,  $J = 7$  Hz, 2H, H-2'), 2.0–1.7 (m, 6H, H-3 + H-4 + H-5'), 1.6 (m, 5H, H-3' + H-8'), and 1.4 (m, 2H, H-4');  $^{13}\text{C}$  NMR  $\delta_{\text{C}}$  171.5 ( $\text{C}_1$ ), 130.9 ( $\text{C}_6$ ), 124.7 ( $\text{C}_7$ ), 46.4 ( $\text{C}_2$ ), 45.4 ( $\text{C}_5$ ), 34.5, 32.2, 29.2, 25.9, 24.2 ( $\text{C}_3$ ,  $\text{C}_4$ ,  $\text{C}_2' - \text{C}_5'$ ), and 17.7 ( $\text{CH}_3$ ); MS  $m/z$  195 ( $\text{M}^+$ , 87), 180 (6), 166 (12), 152 (7), 140 (30), 127 (95), 126 (57), 113 (58), 99 (36), 98 (65), 85 (42), 70 (100), and 55 (85).

*N-[2-(3-Phenoxyphenyl)propionyl]pyrrolidine (5c):* 83% yield; mp 80–83 °C (from hexane); HRMS (EI)  $m/z$  295.1577 ( $\text{C}_{19}\text{H}_{21}\text{NO}_2$  requires 295.1572); IR  $\nu_{\text{max}}$  3040, 2960, 2860, 1700, 1630, 1575, 1480, 1420, 1360, 1330, 1240, 1160, 1060, 1020, 950, 920, 750, and 695;  $^1\text{H}$  NMR  $\delta_{\text{H}}$  7.3 (m, 2H, H-3''' + H-5'''), 7.2 (t,  $J = 8$  Hz, 1H, H-5''), 7.1 and 6.9 (m, 5H, H-2'' + H-6'' + H-2''' + H-4''' + H-6'''), 6.8 (ddd,  $J = 8, 3$ , and 1 Hz, 1H, H-4''), 3.7 (q,  $J = 7$  Hz, 1H, H-2'), 3.5 and 3.4 (m + m, 4H, H-2 + H-5), 1.8 (m, 4H, H-3 + H-4), and 1.4 (d,  $J = 7$  Hz, 3H,  $\text{CH}_3$ );  $^{13}\text{C}$  NMR  $\delta_{\text{C}}$  171.5 ( $\text{C}_1$ ), 157.1, 156.8, 143.4 ( $\text{C}_1''$ ,  $\text{C}_3''$ ,  $\text{C}_1'''$ ), 129.7, 129.4, 122.9, 122.0, 118.4, 117.9, 116.6 ( $\text{C}_2''$ ,  $\text{C}_4'' - \text{C}_6''$ ,  $\text{C}_2''' - \text{C}_6'''$ ), 46.0 ( $\text{C}_2$ ), 45.7 ( $\text{C}_5$ ), 44.4 ( $\text{C}_2'$ ), 25.7 ( $\text{C}_3$ ), 23.8 ( $\text{C}_4$ ), and 19.8 ( $\text{CH}_3$ ); MS  $m/z$  295 ( $\text{M}^+$ , 81), 242 (5), 224 (3), 197 (18), 181 (4), 104 (8), 103 (7), 98 (100), 91 (7), 77 (10), and 55 (33).

**Anodic Oxidation of N-Acylpyrrolidines.** A solution of amide (1.6 mmol) in methanol (60.0 mL) containing tetrabutylammonium *p*-toluenesulfonate (4.4 mmol) as a supporting electrolyte was placed into an electrolysis cell equipped with carbon electrodes (8.5 cm<sup>2</sup>). A constant current (20 mA) was passed through the solution. After 4.0 F/mol of electricity had been passed, the solvent was evaporated under reduced pressure. Water was added to the residue, and the product was extracted with  $\text{CH}_2\text{Cl}_2$ . 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 ethyl acetate as eluent, to eliminate the supporting electrolyte. The solvent was evaporated under reduced pressure, and the residue was purified by column chromatography on silica gel, to afford the methoxylated amide.

*2-Methoxy-N-octanoylpyrrolidine (6a):* 45% yield; obtained as an oil; HRMS (CI)  $m/z$  228.1965 ( $\text{M} + \text{H}^+$ ,  $\text{C}_{13}\text{H}_{26}\text{NO}_2$  requires 228.1963); IR  $\nu_{\text{max}}$  2910, 2840, 1650, 1410, 1350, 1330,

1240, 1160, 1110, 1040, 990, 910, and 830;  $^1\text{H}$  NMR  $\delta_{\text{H}}$  5.4 and 5.0 (d + d,  $J = 4$  Hz, 1H, H-2), 3.6 (m, 2H, H-5), 3.4 and 3.3 (s + s, 3H, OMe), 2.3 (m, 2H, H-2'), 2.2–1.8 (m, 4H, H-3 + H-4), 1.6 (m, 2H, H-3'), 1.3 [br s, 8H,  $(\text{CH}_2)_4\text{CH}_3$ ], and 0.9 (t,  $J = 7$  Hz, 3H,  $\text{CH}_3$ );  $^{13}\text{C}$  NMR  $\delta_{\text{C}}$  173.5 ( $\text{C}_1$ ), 88.7 and 86.9 ( $\text{C}_2$ ), 56.4 and 53.9 (OMe), 46.1 and 45.4 ( $\text{C}_5$ ), 34.6, 34.0, 31.6, 31.3, 30.9, 29.4, 29.3, 29.0, 25.1, 24.5, 22.9, 22.5, 21.0 ( $\text{C}_3$ ,  $\text{C}_4$ ,  $\text{C}_2' - \text{C}_7'$ ), and 14.0 ( $\text{CH}_3$ ); MS (CI)  $m/z$  228 ( $\text{M} + \text{H}^+$ , 61), 214 (74), 195 (73), 184 (8), 180 (7), 173 (9), 142 (12), 129 (100), 113 (29), and 111 (85).

*2-Methoxy-N-oct-6-enoylpyrrolidine (6b):* 25% yield; obtained as an oil; HRMS (CI)  $m/z$  226.1813 ( $\text{M} + \text{H}^+$ ,  $\text{C}_{13}\text{H}_{24}\text{NO}_2$  requires 226.1807); IR  $\nu_{\text{max}}$  2920, 2860, 1645, 1400, 1350, 1310, 1230, 1170, 1090, 1070, 1060, 960, 910, 810, and 720;  $^1\text{H}$  NMR  $\delta_{\text{H}}$  5.4 (m, 2H, H-6' + H-7'), 5.0 (d,  $J = 4$  Hz, 1H, H-2), 3.6 (m, 2H, H-5), 3.4 and 3.3 (s + s, 3H, OMe), 2.3 (m, 2H, H-2'), 2.1–1.8 (m, 6H, H-3 + H-4 + H-5'), 1.6 (m, 5H, H-3' + H-8'), and 1.4 (m, 2H, H-4');  $^{13}\text{C}$  NMR  $\delta_{\text{C}}$  173.1 and 173.0 ( $\text{C}_1$ ), 131.0 and 130.9 ( $\text{C}_6$ ), 124.9 and 124.8 ( $\text{C}_7$ ), 88.6 and 86.8 ( $\text{C}_2$ ), 56.3 and 53.8 (OMe), 46.0 and 45.3 ( $\text{C}_5$ ), 34.4, 33.8, 32.2, 31.3, 30.8, 29.2, 29.1, 24.5, 23.9, 22.8, 20.9 ( $\text{C}_3$ ,  $\text{C}_4$ ,  $\text{C}_2' - \text{C}_5'$ ), and 17.8 ( $\text{CH}_3$ ); MS (CI)  $m/z$  226 ( $\text{M} + \text{H}^+$ , 9), 212 (22), 194 (100), 165 (5), 142 (10), 129 (16), 124 (9), and 111 (10).

*2-Methoxy-N-[2-(3-phenoxyphenyl)propionyl]pyrrolidine (6c):* two diastereomers; combined yield 27%; obtained as oils.

*Spectral data of the first eluted diastereomer 6c1:* HRMS (EI)  $m/z$  325.1689 ( $\text{C}_{20}\text{H}_{23}\text{NO}_3$  requires 325.1678); IR  $\nu_{\text{max}}$  3060, 2975, 2931, 2888, 1658, 1581, 1488, 1403, 1238, 1163, 1083, 917, and 694;  $^1\text{H}$  NMR  $\delta_{\text{H}}$  7.4–6.8 (m, 9H, Ar-H), 5.5 and 4.8 (d + d,  $J = 5$  Hz, 1H, H-2), 3.9 (q,  $J = 7$  Hz, 1H, H-2'), 3.6–3.3 (m, 2H, H-5), 3.3 and 3.2 (s + s, 3H, OMe), 2.1–1.7 (m, 4H, H-3 + H-4), and 1.4 (m, 3H,  $\text{CH}_3$ );  $^{13}\text{C}$  NMR  $\delta_{\text{C}}$  173.8 and 173.3 ( $\text{C}_1$ ), 157.5, 156.9, 143.9 ( $\text{C}_1''$ ,  $\text{C}_3''$ ,  $\text{C}_1'''$ ), 130.1, 130.0, 123.5, 122.2, 118.8, 117.1, 117.0 ( $\text{C}_2''$ ,  $\text{C}_4'' - \text{C}_6''$ ,  $\text{C}_2''' - \text{C}_6'''$ ), 88.0, 87.4 ( $\text{C}_2$ ), 56.5, 55.9 (OMe), 45.9, 45.7 ( $\text{C}_5$ ), 44.7, 44.0 ( $\text{C}_2'$ ), 31.3, 30.6 ( $\text{C}_3$ ), 22.9 ( $\text{C}_4$ ), 20.4, and 19.8 ( $\text{CH}_3$ ); MS  $m/z$  325 ( $\text{M}^+$ , 23), 310 (41), 294 (72), 224 (15), 197 (46), 181 (10), 128 (100), 103 (18), 91 (20), 85 (84), 77 (30), 70 (32), and 55 (29).

*Spectral data of the second eluted diastereomer 6c2:* HRMS (EI)  $m/z$  325.1672 ( $\text{C}_{20}\text{H}_{23}\text{NO}_3$  requires 325.1677); IR  $\nu_{\text{max}}$  3060, 2973, 2931, 2884, 1658, 1579, 1489, 1442, 1400, 1242, 1084, 926, and 694;  $^1\text{H}$  NMR  $\delta_{\text{H}}$  7.4–6.8 (m, 9H, Ar-H), 5.4 (d + d,  $J = 5$  Hz, 1H, H-2), 3.9 (q,  $J = 7$  Hz, 1H, H-2'), 3.8–3.5 (m, 2H, H-5), 3.4 and 3.1 (s, 3H, OMe), 2.2–1.7 (m, 4H, H-3 + H-4), and 1.4 (m, 3H,  $\text{CH}_3$ );  $^{13}\text{C}$  NMR  $\delta_{\text{C}}$  173.5 ( $\text{C}_1$ ), 157.4, 144.1, 143.6 ( $\text{C}_1''$ ,  $\text{C}_3''$ ,  $\text{C}_1'''$ ), 130.0, 129.6, 123.2, 122.3, 118.6, 118.2, 117.1 ( $\text{C}_2''$ ,  $\text{C}_4'' - \text{C}_6''$ ,  $\text{C}_2''' - \text{C}_6'''$ ), 88.5, 87.6 ( $\text{C}_2$ ), 56.9 (OMe), 45.7 ( $\text{C}_5$ ), 44.9 ( $\text{C}_2'$ ), 31.1 ( $\text{C}_3$ ), 22.7 ( $\text{C}_4$ ), 20.7, and 19.8 ( $\text{CH}_3$ ); MS  $m/z$  325 ( $\text{M}^+$ , 34), 310 (15), 294 (10), 224 (19), 197 (30), 181 (7), 128 (100), 103 (10), 91 (10), 85 (50), 77 (12), 70 (13), and 55 (8).

**Synthesis of Enamides.** The corresponding methoxy derivative (0.05 mmol) and silica gel (0.05 mmol) were heated at 150–160 °C in a flask, under reduced pressure and nitrogen atmosphere. After 2.75 h, water was added to the residue and the slurry was extracted with  $\text{CH}_2\text{Cl}_2$ . The combined organic layer was dried over anhydrous sodium sulfate. The drying agent was then removed by filtration, the solvent was evaporated to dryness, and the residue was purified by column chromatography on silica gel. Under those conditions enamides were obtained; when the reaction was carried out with  $\beta$ -oxoamides, bicyclic oxazines were also formed.

*N-Octanoyl-2-pyrroline (7a):* 30% yield; obtained as an oil; HRMS (EI)  $m/z$  195.1619 ( $\text{C}_{12}\text{H}_{21}\text{NO}$  requires 195.1623); IR  $\nu_{\text{max}}$  2960, 2920, 2860, 1630, 1550, 1410, 1350, 1160, 1110, 1050, and 840;  $^1\text{H}$  NMR  $\delta_{\text{H}}$  7.0 and 6.5 (m + m, 1H, H-2), 5.2 (m, 1H, H-3), 3.8 (dd,  $J = 9$  Hz, 2H, H-5), 2.7 and 2.6 (m + m, 2H, H-4), 2.3 and 2.2 (t + t,  $J = 7$  Hz, 2H, H-2'), 1.6 (m, 2H, H-3'), 1.3 [m, 8H,  $(\text{CH}_2)_4\text{CH}_3$ ], and 0.9 (t,  $J = 7$  Hz, 3H,  $\text{CH}_3$ );  $^{13}\text{C}$  NMR  $\delta_{\text{C}}$  169.1 ( $\text{C}_1$ ), 129.3 and 128.9 ( $\text{C}_2$ ), 111.3 and 111.0 ( $\text{C}_3$ ), 45.5 and 44.7 ( $\text{C}_5$ ), 34.5, 34.2 ( $\text{C}_4$ ), 31.6, 29.3, 29.0, 25.0, 22.5 ( $\text{C}_4$ ,  $\text{C}_2' - \text{C}_7'$ ), and 14.0 ( $\text{CH}_3$ ); MS  $m/z$  195 ( $\text{M}^+$ , 12), 156 (5), 145 (33), 141 (98), 129 (48), 127 (52), 111 (39), 98 (26), 86 (45), 70 (73), 69 (64), 57 (100), and 55 (37).

*N-Oct-6-enoyl-2-pyrroline (7b)*: 28% yield; obtained as an oil; HRMS (EI)  $m/z$  194.1463 ( $C_{12}H_{19}NO$  requires 193.1466); IR  $\nu_{\max}$  2920, 2850, 1647, 1224, 1333, 1106, 964, 734, and 612;  $^1H$  NMR  $\delta_H$  7.0 and 6.5 (m + m, 1H, H-2), 5.4 (m, 2H, H-6' - H-7'), 5.2 (m, 1H, H-3), 3.8 (dd,  $J$  = 9 Hz, 2H, H-5), 2.7 and 2.6 (m + m, 2H, H-4), 2.3 and 2.2 (t + t,  $J$  = 7 Hz, 2H, H-2'), 2.0 (m, 4H, H-4 + H-5'), 1.6 (m, 5H, H-3' + H-8'), and 1.4 (m, 2H, H-4');  $^{13}C$  NMR  $\delta_C$  169.0 ( $C_1$ ), 130.9 ( $C_6$ ), 130.9 and 129.2 ( $C_2$ ), 125.0 ( $C_7$ ), 111.4 and 110.1 ( $C_3$ ), 45.5 and 44.8 ( $C_5$ ), 34.1- ( $C_4$ ), 32.2, 30.1, 29.2, 24.4 ( $C_2'$ - $C_5'$ ), and 17.9 ( $CH_3$ ); MS  $m/z$  193 ( $M^+$ , 37), 138 (5), 124 (21), 111 (17), 97 (11), 96 (11), 84 (18), 81 (35), 69 (72), 68 (100), and 55 (98).

*N-[2-(3-Phenoxyphenyl)propionyl]-2-pyrroline (7c)*: 29% yield; obtained as an oil; HRMS (EI)  $m/z$  293.1416 ( $C_{19}H_{19}NO_2$  requires 293.1416); IR  $\nu_{\max}$  3060, 2931, 2888, 1647, 1573, 1489, 1420, 1258, 1110, and 610;  $^1H$  NMR  $\delta_H$  7.4-6.8 (m, 9H, Ar-H), 6.5 (m, 1H, H-2), 5.2 and 5.1 (m + m, 2H, H-3), 4.0-3.5 (m, 3H, H-5 + H-2'), 2.8-2.5 (m, 2H, H-4), and 1.5 (d,  $J$  = 7 Hz, 3H,  $CH_3$ );  $^{13}C$  NMR  $\delta_C$  169.1 ( $C_1$ ), 157.4, 157.3, 143.9 ( $C_1'$ ,  $C_3'$ ,  $C_1''$ ), 130.1, 129.7, 128.4, 123.2, 122.0, 118.7, 117.2, 111.8, 110.4 ( $C_2$ ,  $C_3$ ,  $C_2'$ ,  $C_4'$ - $C_6'$ ,  $C_2''$ - $C_6''$ ), 45.2 ( $C_5$ ), 44.9 ( $C_2'$ ), 27.9 ( $C_4$ ), 19.9, and 19.7 ( $CH_3$ ); MS  $m/z$  293 ( $M^+$ , 52), 250 (2), 197 (72), 104 (14), 91 (17), 77 (20), and 69 (100).

**Synthesis of Imides.** A mixture of 2-pyrrolidinone (2.7 mmol), acyl chloride (1.8 mmol), and  $Et_3N$  (2.0 mmol) in benzene (20 mL) was refluxed for 8 h. The reaction mixture was extracted with  $CH_2Cl_2$ ; the resulting organic extracts were washed with brine, dried over  $Na_2SO_4$ , and concentrated to dryness, providing a residue that was purified by column chromatography on silica gel to afford the corresponding imide.

*N-Octanoyl-2-pyrrolidinone (8)*: 68% yield; obtained as an oil; HRMS (EI)  $m/z$  211.1570 ( $C_{12}H_{21}NO_2$  requires 211.1572); IR  $\nu_{\max}$  2952, 2922, 2853, 1738, 1694, 1460, 1360, 1324, 1250, and 593;  $^1H$  NMR  $\delta_H$  3.8 (t,  $J$  = 7 Hz, 2H, H-5), 2.9 (t,  $J$  = 7 Hz, 2H, H-3), 2.6 (t,  $J$  = 8 Hz, 2H, H-2'), 2.0 (m, 2H, H-4), 1.6 (m, 2H, H-3'), 1.3 [br s, 8H, ( $CH_2$ )<sub>4</sub>CH<sub>3</sub>], and 0.9 (t,  $J$  = 7 Hz, 3H,  $CH_3$ );  $^{13}C$  NMR  $\delta_C$  175.4 ( $C_2$ ), 174.4 ( $C_1$ ), 45.4 ( $C_5$ ), 36.7, 33.7, 31.6, 29.1, 29.0, 24.1, 22.5, 17.1 ( $C_3$ ,  $C_4$ ,  $C_2'$ - $C_7'$ ), and 14.0 ( $CH_3$ ); MS  $m/z$  211 ( $M^+$ , 35), 182 (7), 154 (23), 140 (98), 127 (100), 112 (14), 99 (88), 86 (53), 69 (26), 57 (91), and 55 (58).

**Biological Activity.** *Insects.* *Oncopeltus fasciatus* Dallas were maintained at  $28 \pm 1^\circ C$ , 50-60% relative humidity, and 16 h/8 h (light/dark) photoperiod on a diet based on sunflower seeds.

**Target Microorganisms.** Fungicidal activity was measured against 13 agronomically important phytopathogens: *Aspergillus parasiticus* (CECT 2681), *Geotrichum candidum* (CCM 245), *Alternaria tenuis* (CECT 2662), *Colletotrichum gloeosporoides* (CECT 2859), *Colletotrichum coccodes* (CCM 327), *Fusarium oxysporum* ssp. *gladioli* (CCM 233), *Fusarium oxysporum* ssp. *niveum* (CCM 259), *Fusarium culmorum* (CCM 172), *Penicillium italicum* (CECT 2294), *Trichoderma viride* (CECT 2423), *Trichothecium roseum* (CECT 2410), *Rosellinia necatrix* (CCM 297), and *Verticillium dahliae* (CCM 269).

The strains were provided by the "Colección Española de Cultivos Tipo" (CECT) or by the "Colección de la Cátedra de Microbiología" (CCM) of the Department of Biotechnology (Universidad Politécnica de Valencia).

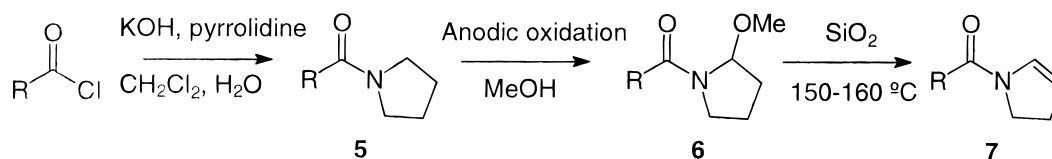
**Entomotoxicity and Anti-JH Activity.** The test was carried out basically according to the contact method of Bowers et al. (1976). Briefly, 15 third-instar *O. fasciatus* nymphs were confined to a 9 cm Petri dish coated, across the bottom, with 10  $\mu g/cm^2$  of the product. Chemicals showing high activity at 10  $\mu g/cm^2$  were retested at 7.5, 6, 5, 4, 2.5, and 1  $\mu g/cm^2$ . Toxicity effects were considered according to the number of insects dead after 72 h of exposure to the chemicals, and probit analysis (Finney, 1971) was applied to determine the  $LD_{50}$ . The surviving nymphs were transferred to a 500  $cm^3$  glass flask and held at standard conditions. After metamorphosis occurred and reproduction was successful with the production of viable offsprings, the tests were finished. The tests were considered positive for JH antagonistic activity when precocious metamorphosis occurred. Controls were run in parallel and received the same amount of acetone as treated insects.

Table 1. Analogues Showing Fungicidal Activity

product	1	2	3	4	5	6	7	8	9	10	11	12	13
5a	0	20.6 $\pm$ 3.3 <sup>AB</sup>	21.6 $\pm$ 0.1 <sup>A</sup>	13.0 $\pm$ 0.5 <sup>A</sup>	36.3 $\pm$ 3.4 <sup>AE</sup>	67.9 $\pm$ 6.6 <sup>A</sup>	34.9 $\pm$ 6.9 <sup>AFG</sup>	26.4 $\pm$ 1.6 <sup>A</sup>	44.6 $\pm$ 4.8 <sup>AE</sup>	41.5 $\pm$ 3.2 <sup>A</sup>	23.3 $\pm$ 2.3 <sup>A</sup>	15.8 $\pm$ 2.8 <sup>AD</sup>	21.9 $\pm$ 1.7 <sup>A</sup>
5b	0	21.3 $\pm$ 2.6 <sup>B</sup>	24.7 $\pm$ 3.4 <sup>A</sup>	0	25.3 $\pm$ 1.8 <sup>B</sup>	50.2 $\pm$ 2.3 <sup>BC</sup>	48.8 $\pm$ 5.3 <sup>B</sup>	22.1 $\pm$ 3.4 <sup>AB</sup>	27.3 $\pm$ 1.5 <sup>BG</sup>	13.6 $\pm$ 4.5 <sup>B</sup>	9.9 $\pm$ 0.1 <sup>B</sup>	0	15.5 $\pm$ 2.8 <sup>B</sup>
5c	48.8 $\pm$ 2.5 <sup>A</sup>	52.0 $\pm$ 1.0 <sup>DE</sup>	52.2 $\pm$ 2.2 <sup>B</sup>	27.5 $\pm$ 2.5 <sup>B</sup>	61.4 $\pm$ 1.7 <sup>C</sup>	70.5 $\pm$ 6.3 <sup>A</sup>	77.4 $\pm$ 10.4 <sup>C</sup>	74.3 $\pm$ 2.9 <sup>C</sup>	76.1 $\pm$ 5.4 <sup>C</sup>	62.0 $\pm$ 2.8 <sup>C</sup>	48.0 $\pm$ 2.0 <sup>C</sup>	48.9 $\pm$ 3.8 <sup>B</sup>	50.9 $\pm$ 1.3 <sup>C</sup>
6a	0	0	0	0	11.7 $\pm$ 2.1 <sup>D</sup>	44.7 $\pm$ 1.8 <sup>B</sup>	14.1 $\pm$ 2.3 <sup>D</sup>	19.9 $\pm$ 3.0 <sup>B</sup>	17.3 $\pm$ 3.7 <sup>D</sup>	15.6 $\pm$ 4.6 <sup>B</sup>	0	0	12.5 $\pm$ 0.8 <sup>B</sup>
6b	0	0	0	0	0	45.8 $\pm$ 2.2 <sup>B</sup>	24.6 $\pm$ 0.7 <sup>A</sup>	10.7 $\pm$ 1.9 <sup>D</sup>	19.5 $\pm$ 2.2 <sup>BD</sup>	0	0	0	7.6 $\pm$ 1.0 <sup>D</sup>
6c1	30.9 $\pm$ 3.6 <sup>B</sup>	47.3 $\pm$ 1.3 <sup>D</sup>	48.0 $\pm$ 1.9 <sup>BC</sup>	15.8 $\pm$ 3.8 <sup>A</sup>	55.7 $\pm$ 2.1 <sup>C</sup>	55.2 $\pm$ 3.4 <sup>CD</sup>	78.6 $\pm$ 1.5 <sup>C</sup>	61.1 $\pm$ 3.5 <sup>F</sup>	48.2 $\pm$ 6.9 <sup>E</sup>	60.2 $\pm$ 6.6 <sup>C</sup>	33.3 $\pm$ 1.0 <sup>D</sup>	24.0 $\pm$ 3.5 <sup>C</sup>	47.0 $\pm$ 3.2 <sup>EE</sup>
6c2	30.3 $\pm$ 5.3 <sup>BC</sup>	33.9 $\pm$ 1.8 <sup>C</sup>	31.6 $\pm$ 5.2 <sup>D</sup>	16.7 $\pm$ 2.9 <sup>A</sup>	43.1 $\pm$ 4.2 <sup>A</sup>	44.5 $\pm$ 0.6 <sup>B</sup>	63.6 $\pm$ 2.7 <sup>E</sup>	44.4 $\pm$ 2.7 <sup>E</sup>	37.5 $\pm$ 5.4 <sup>AF</sup>	50.6 $\pm$ 2.9 <sup>D</sup>	18.2 $\pm$ 0.0 <sup>E</sup>	18.9 $\pm$ 1.3 <sup>D</sup>	23.2 $\pm$ 3.0 <sup>A</sup>
7a	0	15.8 $\pm$ 0.3 <sup>A</sup>	13.5 $\pm$ 4.3 <sup>E</sup>	0	34.1 $\pm$ 4.6 <sup>E</sup>	47.8 $\pm$ 3.9 <sup>B</sup>	42.0 $\pm$ 7.3 <sup>BF</sup>	38.9 $\pm$ 0.6 <sup>G</sup>	19.9 $\pm$ 3.2 <sup>BD</sup>	18.0 $\pm$ 3.4 <sup>B</sup>	8.7 $\pm$ 2.3 <sup>B</sup>	9.0 $\pm$ 2.0 <sup>E</sup>	15.0 $\pm$ 2.5 <sup>B</sup>
7b	27.6 $\pm$ 3.4 <sup>B</sup>	17.9 $\pm$ 2.0 <sup>AB</sup>	21.3 $\pm$ 4.1 <sup>A</sup>	0	84.0 $\pm$ 5.4 <sup>F</sup>	61.3 $\pm$ 1.7 <sup>D</sup>	38.9 $\pm$ 6.6 <sup>BFG</sup>	26.2 $\pm$ 3.7 <sup>A</sup>	34.1 $\pm$ 9.3 <sup>FG</sup>	26.4 $\pm$ 4.2 <sup>E</sup>	0	12.2 $\pm$ 1.9 <sup>AE</sup>	21.9 $\pm$ 4.0 <sup>A</sup>
7c	46.7 $\pm$ 2.1 <sup>A</sup>	38.3 $\pm$ 2.9 <sup>C</sup>	46.1 $\pm$ 2.5 <sup>C</sup>	26.7 $\pm$ 2.9 <sup>B</sup>	100 <sup>G</sup>	100 <sup>E</sup>	84.8 $\pm$ 0.9 <sup>C</sup>	70.0 $\pm$ 2.9 <sup>C</sup>	57.1 $\pm$ 5.5 <sup>H</sup>	78.2 $\pm$ 4.4 <sup>F</sup>	33.1 $\pm$ 3.1 <sup>D</sup>	23.6 $\pm$ 1.4 <sup>C</sup>	43.6 $\pm$ 3.3 <sup>E</sup>
8	23.0 $\pm$ 5.1 <sup>C</sup>	56.0 $\pm$ 8.2 <sup>E</sup>	36.9 $\pm$ 5.4 <sup>D</sup>	40.7 $\pm$ 1.6 <sup>C</sup>	77.2 $\pm$ 6.8 <sup>F</sup>	68.3 $\pm$ 6.0 <sup>A</sup>	34.3 $\pm$ 4.3 <sup>AG</sup>	89.0 $\pm$ 5.2 <sup>H</sup>	36.8 $\pm$ 3.5 <sup>AF</sup>	33.6 $\pm$ 2.0 <sup>G</sup>	65.3 $\pm$ 1.2 <sup>F</sup>	38.9 $\pm$ 1.9 <sup>F</sup>	48.3 $\pm$ 1.5 <sup>C</sup>
benomyl	87.0 $\pm$ 1.4	11.1 $\pm$ 0.0	100	0	100	100	100	0	100	100	100	100	100

<sup>a</sup> Values represent means  $\pm$  standard deviations of growth inhibitions from three independent experiments. Assay concentration of analogues = 100  $\mu g/mL$ ; benomyl concentration = 2.5  $\mu g/mL$ . Within each column, mean values showing the same superscripts (A-H) are not significantly different ( $P > 0.05$ ). Target plant pathogens: 1, *F. culmorum*; 2, *F. oxysporum* ssp. *gladioli*; 3, *F. oxysporum* ssp. *niveum*; 4, *G. candidum*; 5, *C. gloeosporioides*; 6, *C. coccodes*; 7, *T. roseum*; 8, *A. tenuis*; 9, *V. dahliae*; 10, *P. citrophthora*; 11, *T. viride*; 12, *P. italicum*; 13, *A. parasiticus*.



**Scheme 1. Synthetic Sequence with Monocarbonylic Side Chains**a R=  $\text{CH}_3(\text{CH}_2)_6$ b R=  $\text{CH}_3\text{CH}=\text{CH}(\text{CH}_2)_4$ 

c R=

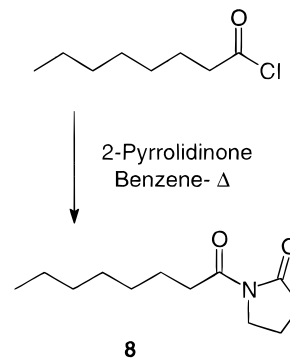
**Antifungal Activity.** The products, dissolved in acetone, were added to PDA, in a concentration 100  $\mu\text{g/mL}$ . PDA plates containing only acetone were used as control plates, and a positive control with benomyl [methyl-1-(butylcarbamoyl)-2-benzimidazolecarbamate; Sigma, Germany] at 2.5  $\mu\text{g/mL}$  was performed to appraise the level of activity of the synthesized compounds. Spores from 7-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 (6 days for *R. necatrix* and *V. dahliae*), at 28  $^\circ\text{C}$ . The antifungal activity of each product was determined three times. When minimum inhibitory concentration (MIC) values were  $<100\text{ }\mu\text{g/mL}$ , the effective dose to inhibit 50% ( $\text{ED}_{50}$ ) of the mycelial growth was estimated by linear regression analysis of the percentage of inhibition versus log of compound concentration. Analysis of variance (ANOVA) was performed for fungicidal data (Table 1), and the least significant difference (LSD) test was used to compare means (Statgraphics Plus 2.1).

**RESULTS AND DISCUSSION**

The sequence of reactions previously used to synthesize **2–4** was modified to prepare the related compound **7**, to ascertain whether the  $\beta$ -ketoamide functionality is essential for the activity. Thus, the side chain present in the natural products was maintained in the monocarbonylic analogues. A phenoxyphenyl group was introduced because it is very common in pesticides.

As shown in Scheme 1, the employed synthetic sequence implied formation of amides by means of a Schotten–Baumann reaction, taking pyrrolidine and the corresponding acid chloride as starting materials in the first step. Subsequent anodic oxidation of the pyrrolidine ring using the method described by Shono (Shono, 1984; Shono et al., 1981a,b, 1982a,b) followed by elimination of MeOH upon acid catalysis and heating at 150–160  $^\circ\text{C}$  (Slomczynska et al., 1996; Cornille et al., 1994, 1995; Moeller et al., 1992, 1994) afforded the corresponding enamides. This worked in a satisfactory way in the case of **7a** and **7b**; however, obtention of **7c** implied in the anodic oxidation a diastereomeric mixture, which appeared joined with a series of oxidation products of aromatic rings. This mixture of diastereomers was resolved by chromatographic column. Both diastereomers were used in the elimination reaction affording the desired enamide **7c**.

Finally, to obtain further analogues, 2-pyrrolidinone was introduced as a five-membered ring instead of pyrrolidine. This was achieved by simple heating of octanoyl chloride with 2-pyrrolidinone and triethylamine in refluxing benzene (Ostrovskaya et al., 1993; Sasaki et al., 1991).



**Biological Activities. Insecticidal and Anti-JH Activity.** Two of the products (**5a** and **5b**) showed potent insecticidal activity against *O. fasciatus*. In the case of **5a**, acute  $\text{LD}_{50}$  and  $\text{LD}_{90}$  values for third-instar nymphs, exposed to the chemical by the contact method, were 3.0 and 5.0  $\mu\text{g/cm}^2$ , respectively. The corresponding values for compound **5b** were 1.5 and 2.0  $\mu\text{g/cm}^2$ . Thus, the latest compound was 2-fold more active than **5a**; these toxicity data unambiguously demonstrate that introduction of an unsaturation in the side chain is associated with an improved entomotoxicity. Insects were unaffected, at test levels, by the other synthetic analogues. Thus, deeper modifications of the side chain or the five-membered ring produce an adverse effect on the toxicity.

Insecticidal activity has been shown to be closely associated with the pyrrolidine moiety. In our previous work on the isolation, synthesis, and biological activity of compound **2** (Moya et al., 1998), the study of the insecticidal activity of its synthetic precursors showed that the presence of the pyrrolidine ring was essential for the activity. Particularly, *N*-(3-oxodecanoyl)pyrrolidine and *N*-(2-methyl-3-oxodecanoyl)pyrrolidine, both with the  $\beta$ -ketoamide functionality, showed  $\text{LD}_{50}$  values of 7.0 and 3.75  $\mu\text{g/cm}^2$ , respectively. As mentioned above, our current results show the same tendency, but in this case there has been a significant improvement of entomotoxicity.

Although natural enamides **2** and **3** show an important in vivo anti-JH activity, neither their previously reported synthetic precursors nor the new analogues assayed in the present work proved to retain such activity; no precocious metamorphosis or even slighter symptoms of JH deficiency, as delayed growth or altered fertility, were detected. It seems that this activity has a very specific structural requirement.

**Fungicidal Activity.** At first sight, it is interesting to note that the introduction of an amide group, instead

of the  $\beta$ -ketoamide functionality present in the natural products and their synthetic precursors, resulted in an important increase of the fungicidal activity [for comparative purposes see Moya et al. (1998) and Cantin et al. (1998)]. However, comparatively, the levels of activity are clearly lower than those of a conventional fungicide such as benomyl (Table 1).

Fungicidal activity of the new analogues, expressed as the percentage of growth inhibition against 13 agronomically important plant pathogens, is shown in Table 1.

In general, the products possessing the phenoxyphenyl substituent showed the best fungicidal activity with regard to the percentage of growth inhibition and the number of affected species; compound **7c** has proved to be highly active against *C. gloeosporoides* [ $ED_{50}$  = 2.04  $\mu$ g/mL; 95% confidence interval of (1.26, 4.12)] and *C. coccodes* [ $ED_{50}$  = 11.7  $\mu$ g/mL with a 95% confidence interval of (7.3, 20.7)], although these results still compare unfavorably with those found for benomyl [ $ED_{50}$  = 0.05  $\mu$ g/mL; 95% confidence interval of (0.04, 0.08)] against *C. gloeosporoides* and  $ED_{50}$  = 0.13  $\mu$ g/mL with a 95% confidence interval of (0.12, 0.17) against *C. coccodes*. Besides, compound **7c** strongly affected the growth of *T. roseum* and *P. citrophthora* and, very interestingly, *A. tenuis*, one of the benomyl-resistant species; all of the other fungi were also inhibited to some extent. The improvement obtained with this product appears to be important and warrants further work. It will be used as a starting point in the search for more active analogues and also for studies on its mechanism of action.

The remaining analogues exhibited minimum inhibitory concentration values >100  $\mu$ g/mL, so none of them were strongly effective against the tested microorganisms. In some cases, however, the obtained data have been useful to appraise the influence of the different chemical transformations on the ability to control fungi.

Among the products possessing the pyrrolidine ring, compound **5c**, with the phenoxyphenyl substituent, showed the best fungicidal activity. Compounds **5a** and **5b** exhibited lesser levels of activity; **5a** was significantly ( $P > 0.05$ ) more active than **5b** against all microorganisms, except for both subspecies of *F. oxysporum* and *A. tenuis*. This fact suggested that the presence of an unsaturation in the side chain of the molecule had adverse effects on fungal growth, contrary to that observed for insecticidal activity.

Introduction of a methoxy group in the pyrrolidine ring to give the corresponding derivatives (**6a**, **6b**, and the diastereomers **6c1** and **6c2**) was associated with a decreased activity. Thus, **6a** and **6b** were ~2-fold less active than **5a** and **5b**, respectively; this loss of activity could be observed not only at the level of activity but also in the number of affected fungi. Compounds **6c1** and **6c2**, the methoxy byproducts of **5c**, were also adversely but not dramatically affected.

Molecules containing a pyrroline ring showed a good fungicidal activity especially against the *Colletotrichum* genus. Contrary to the observed trend with the above products, the unsaturation on the side chain of this enamide (**7b**) appears to enhance the fungitoxicity.

Finally, important fungicidal activities were obtained when a 2-pyrrolidinone ring was introduced in the structure, instead of pyrrolidine. The activities were similar to, or even higher than, those shown by com-

pound **5c** for *Colletotrichum*, *A. tenuis*, and *T. viride*, but the other fungi were less affected. Previously, a similar 2-pyrrolidone derivative with antibiotic activity was described (Takeuchi and Yonehara, 1964). The product, named variotin, was isolated from the culture broth of the fungus *Paecilomyces varioti* and exhibits activity against different kinds of fungi with MIC values in the range 10–160  $\mu$ g/mL. It is interesting to note that, as in our case, variotin is especially effective against various species of the *Colletotrichum* genera, with MIC values from 0.2 to 2.0  $\mu$ g/mL (Yonehara et al., 1959; Takeuchi et al., 1959; Abe et al., 1959).

In summary, good insecticidal and fungicidal activities have been achieved and preliminary structure–activity relationships have been established. In this context, the improvements obtained so far on the biological activities and the simplicity of structures are encouraging to pursue the search of new, more effective, analogues as promising pesticidal candidates.

#### LITERATURE CITED

- Abe, S.; Takeuchi, S.; Yonehara, H. Studies on variotin, a new antifungal antibiotic. II. Taxonomical studies on variotin-producing strain. *J. Antibiot.* **1959**, *12* (5), 201–202.
- Bowers, W. S.; Ohta, T.; Cleere, J. S.; Marsella, P. A. Discovery of insect anti-juvenile hormones in plants. *Science* **1976**, *193*, 542–547.
- Cantín, A.; Moya, P.; Miranda, M. A.; Primo, J.; Primo-Yúfera, E. Isolation of *N*-(2-methyl-3-oxodecanoyl)pyrrole and *N*-(2-methyl-3-oxodec-8-enoyl)pyrrole, two new natural products from *Penicillium brevicompactum*, and synthesis of analogues with insecticidal and fungicidal activity. *J. Agric. Food Chem.* **1998**, *46*, 4748–4753.
- Cantín, A.; Moya, P.; Castillo, M. A.; Primo, J.; Miranda, M. A.; Primo-Yúfera, E. Isolation and synthesis of *N*-(2-Methyl-3-oxodec-8-enoyl)-2-pyrroline and 2-(Hept-5-enyl)-3-methyl-4-oxo-6,7,8,8a-tetrahydro-4H-pyrrolo[2,1-*b*]-13-oxazine—two new fungal metabolites with in vivo anti-juvenile hormone and insecticidal activity. *Eur. J. Org. Chem.* **1999**, 221–226.
- Castillo, M.; Moya, P.; Couillaud, F.; Garcerá, M. D.; Martínez-Pardo, R. A heterocyclic oxime from a fungus with anti-juvenile hormone activity. *Arch. Insect. Biochem. Physiol.* **1998**, *37*, 287–294.
- Cornille, F.; Fobian, Y. M.; Slomczynska, U.; Beusen, D. D.; Marshall, G. R.; Moeller, K. D. Anodic amide oxidations: conformationally restricted peptide building blocks from the direct oxidation of dipeptides. *Tetrahedron Lett.* **1994**, *35*, 6889–6992.
- Cornille, F.; Slomczynska, U.; Smythe, M. L.; Beusen, D. D.; Marshall, G. R.; Moeller, K. D. Electrochemical cyclization of dipeptides toward novel bicyclic, reverse-turn peptidomimetics. Synthesis and conformational analysis of 7,5-bicyclic systems. *J. Am. Chem. Soc.* **1995**, *117*, 909–917.
- Finney, D. J. *Probit Analysis*; Cambridge University Press: Cambridge, U.K., 1971.
- Moeller, K. D.; Rutledge, L. D. Anodic amide oxidations: The synthesis of two spirocyclic L-pyrroglutamide building blocks. *J. Org. Chem.* **1992**, *57*, 6360–6363.
- Moeller, K. D.; Hanau, C. E.; Avignon, A. The use of HMQC-TOCSY experiments for elucidating the structures of bicyclic lactams: uncovering a surprise rearrangement in the synthesis of a key PRO–PHE building block. *Tetrahedron Lett.* **1994**, *35*, 825–828.
- Moya, P.; Castillo, M.; Primo-Yúfera, E.; Couillaud, F.; Martínez-Mañez, R.; Garcerá, M. D.; Miranda, M. A.; Primo, J.; Martínez-Pardo, R. Brevioxime, a new juvenile hormone biosynthesis inhibitor isolated from *Penicillium brevicompactum*. *J. Org. Chem.* **1997**, *62*, 8544–8545.
- Moya, P.; Cantín, A.; Castillo, M. A.; Primo, J.; Miranda, M. A.; Primo-Yúfera, E. 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. *J. Org. Chem.* **1998**, *63*, 8530–8535.
- Ostrovskaya, R. U.; Trofimov, S. S.; Burov, Y. V.; Kikhoshershtov, A. M.; Koralev, G. I.; Rayevsky, K. S.; Skoldinov, A. P.; Wunderlich, G.; Zenker, L. Synthesis and neurotropic activity of some N-di-(n-propyl)acetyl lactams. *Khim.-Farm. Zh.* **1993**, *27*, 13–16.
- Sasaki, H.; Mori, Y.; Nakamura, J.; Shibasaki, J. Synthesis and anticonvulsant activity of 1-acyl-2-pyrrolidinone derivatives. *J. Med. Chem.* **1991**, *34*, 628–33.
- Shono, T. Electroorganic chemistry in organic synthesis. *Tetrahedron Lett.* **1984**, *40*, 811–850.
- Shono, T.; Matsumura, Y.; Tsubata, K. Electroorganic chemistry. A new carbon–carbon bond forming reaction at the  $\alpha$ -position of amines utilizing anodic oxidation as a key step. *J. Am. Chem. Soc.* **1981a**, *103*, 1172–1176.
- Shono, T.; Matsumura, Y.; Tsubata, K. A new synthetic method of  $\alpha$ -amino acids from  $\alpha$ -methoxyurethanes. *Tetrahedron Lett.* **1981b**, *22*, 2411–2412.
- Shono, T.; Matsumura, Y.; Tsubata, K.; Sugihara, Y.; Yamane, S.; Kanazawa, T.; Aoki, T. Electroorganic Chemistry. Electroorganic synthesis of enamides and enecarbamates and their utilization in organic synthesis. *J. Am. Chem. Soc.* **1982a**, *104*, 6697–6703.
- Shono, T.; Matsumura, Y.; Tsubata, K.; Sugihara, Y. A new method of acylation at  $\beta$ -position of aliphatic amines. *Tetrahedron Lett.* **1982b**, *23*, 1201–1204.
- Slomczynska, U.; Chalmers, D. K.; Cornille, F.; Smythe, M. L.; Beusen, D. D.; Moeller, K. D.; Marshall, G. R. Electrochemical cyclization of dipeptides to form novel bicyclic, reverse-turn peptidomimetics. Synthesis and conformational analysis of 6,5-bicyclic systems. *J. Org. Chem.* **1996**, *61*, 1198–1204.
- Takeuchi, S.; Yonehara, H. Crystallized variotin and its revised chemical structure. *J. Antibiot.* **1964**, *17* (6), 267–268.
- Takeuchi, S.; Yonehara, S.; Umezawa, H. Studies on variotin, a new antifungal antibiotic. I. Preparation and properties of variotin. *J. Antibiot.* **1959**, *12* (5), 195–200.
- Yonehara, H.; Takeuchi, S.; Umezawa, H. Variotin, a new antifungal antibiotic, produced by *Paecilomyces varioti* Bainier var. *Antibioticus*. *J. Antibiot.* **1959**, *12* (3), 109–110.

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