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

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Two new natural products have been isolated from culture broth of *Penicillium brevicompactum* Dierckx. The structures have been assigned as *N*-(2-methyl-3-oxodecanoyl)pyrrole and *N*-(2-methyl-3-oxodec-8-enoyl)pyrrole on the basis of spectral data. Synthesis of analogues has been carried out by acylation of the pyrrole ring at C_2 with different acylated Meldrum's acids. Two analogues (**6b** and **7b**) have shown interesting insecticidal activities, and three other ones (**6a**, **6c**, and **7a**) have exhibited significant broad-spectrum fungicidal activities. These synthetic products might be considered as a starting point in the search for new pesticides.

Keywords: *Penicillium brevicompactum; fungal metabolites; Meldrum's acid; 2-(3-oxoacyl)pyrrole; insecticide; fungicide*

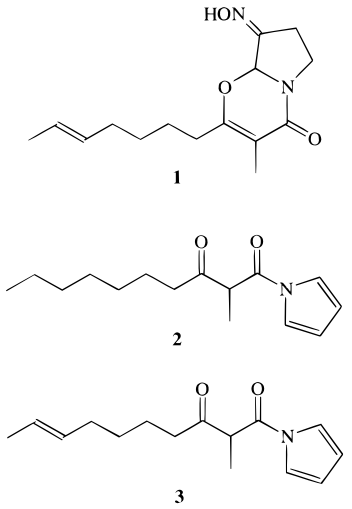
INTRODUCTION

The isolation and identification of fungal metabolites have attracted considerable attention, both for the discovery of new bioactive compounds and for taxonomic purposes.

Particularly, the fungus *Penicillium brevicompactum* Dierckx has been described as one of the most prolific producers of secondary metabolites. These metabolites include mycophenolic acid and related compounds (Birkinshaw et al., 1952), the Raistrick phenols (Oxford and Raistrick, 1932, 1933; Godin, 1955), the pebrolides (McCorkindale et al., 1981), and the *N*-benzoyl derivatives of phenylalanine, phenylalaninol, and their ester, asperphenamate (Doerfler et al., 1981). In addition, the fungus also produces brevigellina (McCorkindale and Baxter, 1981), several piperazine-2,5-dione derivatives, a drimane diterpenoid (Ayer et al., 1990), the brevianamides (Birch and Wright, 1970; Birch and Russell, 1972), and compactin (Brown et al., 1976).

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 juvenile hormone (JH) biosynthesis inhibitor (Moya et al., 1997). Its chemical structure contains an unusual heterobicyclic skeleton and an oxime functionality.

Now we report on the isolation of *N*-(2-methyl-3-oxodecanoyl)pyrrole (**2**) and *N*-(2-methyl-3-oxodec-8-enoyl)pyrrole (**3**), two new pyrrolic metabolites from *P. brevicompactum*. Their chemical structures are new: they were described neither as fungal metabolites nor as synthetic compounds. The fact that compounds **2** and **3** were isolated from the same extract as the active



brevioxime (**1**) (Moya et al., 1997), together with the structural analogies among the three natural products **1**−**3**, prompted us to synthesize a series of related pyrroles and to determine their biological activities. Moreover, other pyrrolic compounds of natural origin have been used as lead molecules for the development of commercial fungicides (Nyfeler and Ackermann, 1992). To increase the stability of the resulting analogues toward hydrolysis, the acyl side chains have been attached as C-substituents at the heterocyclic ring. Some of the pyrroles obtained following this approach exhibit promising insecticidal and fungicidal properties.

MATERIALS AND METHODS

All chemicals were obtained from commercial suppliers and used without further purification. IR spectra were obtained as liquid films (or KBr plates for isolated natural products); 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

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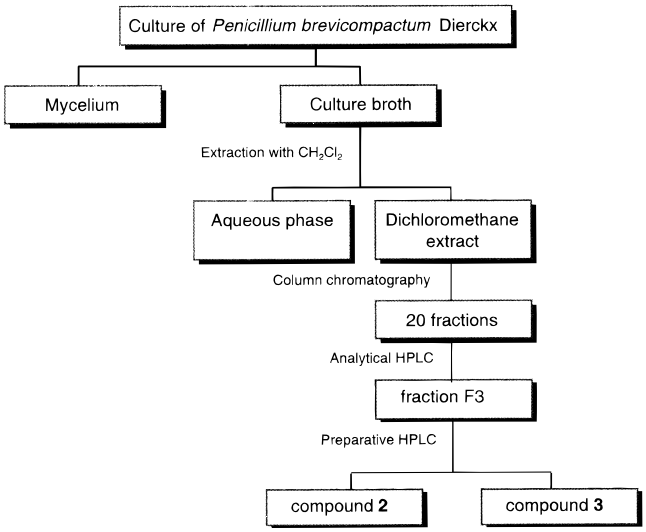
Scheme 1. Isolation and Identification of Two New Natural Products from *P. brevicompactum*



for isolated natural products), respectively, in $CDCl_3$ solvent; chemical shifts are reported in δ (parts per million) values, using tetramethylsilane (TMS) as internal standard. Mass spectra were obtained under electron impact (or chemical ionization for isolated natural products); 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_{254}), and spots were visualized with UV light and in an I_2 chamber.

Isolation and Characterization of the Compounds. The procedure, summarized in Scheme 1, was similar to that previously reported for brevioxime (Moya et al., 1997). 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 codified as P79 and kept in agar slants with potato dextrose agar (PDA) as culture medium.

The strain was seeded in Petri dishes with PDA culture medium and incubated for 7 days at 28 °C. Sterile distilled water with Tween 80 (0.05%) was then used to obtain a suspension containing ~10^6 conidia/mL. This suspension (100 mL) was added to a 5 L Erlenmeyer flask with 1 L of antibiotic test broth (composition: yeast extract, 2.0 g; bacto peptone, 3.0 g; glucose, 2.0 g; sucrose, 30.0 g; corn steep, 5.0 g; $NaNO_3$, 2.0 g; $K_2HPO_4 \cdot 3H_2O$, 1.0 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; KCl, 0.2 g; $FeSO_4 \cdot 7H_2O$, 0.01 g; distilled water, 1000 mL; pH 7) and incubated for 15 days, in the dark, without shaking, 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 contained in 20 flasks) was submitted to column chromatography on silica gel (1:60, w/w) using a stepwise gradient from CH_2Cl_2 to MeOH [CH_2Cl_2; CH_2Cl_2/AcOEt (95:5); CH_2Cl_2/AcOEt (50:50); AcOEt; AcOEt/acetone (90:10); AcOEt/acetone (50:50); acetone; MeOH]; 300 mL of each mobile phase was eluted and collected in aliquots of 30 mL, which were pooled in 20 fractions according to their similarity in TLC. These obtained fractions were systematically studied for the search of new metabolites. Fraction 3 was shown to contain the two new natural products described here.

Preparative HPLC chromatographic resolution of fraction 3 (14.3 mg) was achieved using the following conditions: column, Spherisorb W, 5 μm (25.0 × 0.7 cm); mobile phase, hexane/AcOEt (98:2, v/v); flow, 1.5 mL/min; detection by UV (254 nm) and refraction index, simultaneously. Two pure products were obtained: compound **2** [retention time (t_r) = 24.3 min; 2.8 mg] and compound **3** (t_r = 29.5; 6.6 mg).

Compound **3** was assigned to be *N*-(2-methyl-3-oxodec-8-enoyl)pyrrole on the basis of spectral data: $[\alpha]^{20}_D = 23°$ (*c* 0.20, $CHCl_3$); obtained as an oil; HRMS, *m*/*z* 248.1661 ($M + H^+$, $C_{15}H_{22}NO_2$ requires 248.1650); IR ν_{max} 3150, 2937, 2871, 1727, 1700, 1465, 1404, 1345, 1275, 1129, 1072, 965, 900, 741, and 587; ^{1}H NMR δ_H 7.3 (m, 2H, H-2 + H-5), 6.3 (m, 2H, H-3 + H-4), 5.4 (m, 2H, H-8′ + H-9′), 4.1 (q, J = 7 Hz, 1H, H-2′), 2.5 and 2.4 (m + m, 2H, H-4′), 1.9 (m, 2H, H-7′), 1.6 (d, J = 5 Hz, 3H, CH=CHCH_3), 1.6−1.5 (m, 2H, H-5′), 1.5 (d, J = 7 Hz, CHCH_3), and 1.3 (m, 2H, H-6′); ^{13}C NMR δ_C 131.1 ($C_{8'}$), 125.5 ($C_{9'}$), 119.6 ($C_2 + C_5$), 114.2 ($C_3 + C_4$), 53.6 ($C_{2'}$), 39.8 ($C_{4'}$), 32.2, 28.9, 23.0 ($C_{5'}$-$C_{7'}$), 17.8, and 13.8 (2 × CH_3); MS, *m*/*z* 229 (1), 200 (1), 180 (2), 178 (2), 165 (3), 150 (6), 125 (4), 123 (3), 97 (8), 94 (6), 83 (10), 81 (16), 67 (100), and 55 (30).

Compound **2** was assigned to be *N*-(2-methyl-3-oxodecanoyl)-pyrrole on the basis of its spectral data and by comparison with the spectral data of compound **3**: $[\alpha]^{19}_D = 14°$ (*c* 0.14, $CHCl_3$); obtained as an oil; HRMS, *m*/*z* 278.2121 ($M + C_2H_5^+$, $C_{17}H_{28}NO_2$ requires 278.2120); IR ν_{max} 3150, 2951, 2925, 2855, 1725, 1703, 1466, 1404, 1345, 1275, 1128, 1070, 903, and 742; ^{1}H NMR δ_H 7.3 (m, 2H, H-2 + H-5), 6.3 (m, 2H, H-3 + H-4), 4.1 (q, J = 7 Hz, 1H, H-2′), 2.6 and 2.5 (m + m, 2H, H-4′), 1.6 (d, J = 7 Hz, CHCH_3), 1.3 (m, 8H, (CH_2)$_4$CH_3), and 0.9 (m, 3H, CH_2CH_3); MS, *m*/*z* 249 (2), 183 (1), 154 (1), 150 (1), 127 (11), 123 (2), 109 (2), 98 (2), 94 (5), 83 (6), 67 (100), 57 (18), and 55 (10).

General Synthetic Procedures. *Acylation of Pyrrole.* This reaction was carried out with different acyl side chains according to the following procedure. To a cooled solution (0 °C) of 2,2-dimethyl-1,3-dioxane-4,6-dione (1.12 mmol) in dichloromethane (1.50 mL) were added pyridine (2.47 mmol) and the corresponding acyl chloride (1.23 mmol) via syringe, dropwise, under nitrogen. The solution was stirred at 0 °C for 1 h, after which time it was allowed to warm to room temperature for an additional period of 2 h. The dichloromethane solution was washed with dilute HCl, water, and brine, dried, and concentrated to dryness to give the almost pure acylated Meldrum's acid, which was used for the aminolysis without further purification.

The solution of the acylated Meldrum's acid and pyrrole (2.15 mmol) in benzene (9.00 mL) was refluxed for 14 h. The solvent was evaporated under reduced pressure, and the residue was purified by column chromatography on silica gel to afford the β-diketone.

*2-(3-Oxodecanoyl)pyrrole (**6a**):* 37% yield; mp 45−47 °C (from hexane); HRMS, *m*/*z* 235.1578 ($C_{14}H_{21}NO_2$ requires 235.1572); IR ν_{max} 3280, 3090, 2900, 2820, 1700, 1610, 1550, 1450, 1430, 1390, 1310, 1180, 1110, 1030, 940, 870, 790, and 740; ^{1}H NMR δ_H 10.0 (br s, 1H, N−H), 7.1 (m, 1H, H-5k), 7.0 (m, 1H, H-5e), 6.9 (m, 1H, H-3k), 6.8 (m, 1H, H-3e), 6.3 (m, 1H, H-4), 5.9 (s, 1H, H-2′e), 3.9 (s, 1H, H-2′k), 2.6 (t, J = 7 Hz, 2H, H-4′k), 2.3 (m, 2H, H-4′e), 1.6 (m, 2H, H-5′), 1.3 [br s, 8H, (CH_2)$_4$CH_3], and 0.9 (t, J = 7 Hz, 3H, CH_3); ^{13}C NMR δ_C 209.9 ($C_{3'}$k), 191.4 ($C_{1'}$k), 185.2 and 179.0 ($C_{1'}$e and $C_{3'}$e), 132.5 (C_2k), 130.2 (C_2e), 126.3 (C_5k), 124.5 (C_5e), 118.6 (C_3k), 114.7 (C_3e), 111.3 (C_4k), 111.0 (C_4e), 95.3 ($C_{2'}$e), 53.6 ($C_{2'}$k), 45.2 ($C_{4'}$k), 43.2 ($C_{4'}$e), 36.4, 34.0, 31.7, 31.6, 29.2, 29.0, 26.4, 24.7, 23.5, 22.6 (C_5′-C_9′), and 14.1 (CH_3); MS, *m*/*z* 235 (M^+, 63), 217 (6), 193 (4), 164 (16), 151 (100), 136 (51), 123 (8), 109 (89), 94 (78), 80 (7), 68 (13), and 57 (14). Anal. Calcd for $C_{14}H_{21}NO_2$: C, 71.49%; H, 8.94%; N, 5.96%. Found: C, 71.34%; H, 9.35%; N, 5.54%.

*2-(3-Oxopentanoyl)pyrrole (**6b**):* 28% yield; obtained as an oil; HRMS, *m*/*z* 165.0783 ($C_9H_{11}NO_2$ requires 165.0789); IR ν_{max} 3280, 3100, 2960, 2940, 2860, 1710, 1640, 1540, 1400, 1320, 1220, 1180, 1120, 1080, 1040, 990, 950, 930, 880, 810, 790, and 750; ^{1}H NMR δ_H 9.7 (br s, 1H, N−H), 7.1 (m, 1H, H-5k), 7.0 (m, 1H, H-5e), 6.9 (m, 1H, H-3k), 6.8 (m, 1H, H-3e), 6.3 (m, 1H, H-4), 5.9 (s, 1H, H-2′e), 3.9 (s, 2H, H-2′k), 2.6 (q, J = 7 Hz, 2H, H-4′k), 2.3 (q, J = 7 Hz, 2H, H-4′e), 1.2 (t, J = 7 Hz, 3H, CH_3e), and 1.1 (t, J = 7 Hz, 3H, CH_3k); ^{13}C NMR δ_C 204.8 ($C_{3'}$k), 185.9 ($C_{1'}$k), 183.1 and 182.1 ($C_{1'}$e and $C_{3'}$e), 131.5 (C_2k), 130.1 (C_2e), 126.4 (C_5k), 124.7 (C_5e), 118.6 (C_3k), 114.8 (C_3e), 111.1 (C_4k), 110.9 (C_4e), 94.4 ($C_{2'}$e), 53.1 ($C_{2'}$k), 36.4 ($C_{4'}$k), 29.3 ($C_{4'}$e), 10.4 (CH_3e), and 7.5 (CH_3k); MS, *m*/*z* 165 (M^+, 100), 150 (2), 148 (3), 136 (58), 125 (2), 109 (30), 94 (65), 80 (7) 68 (21),

and 57 (7). Anal. Calcd for $C_9H_{11}NO_2$: C, 65.45%; H, 6.66%; N, 8.48%. Found: C, 64.94%; H, 6.98%; N, 8.39%.

*2-(3-Oxo-4-phenylbutanoyl)pyrrole (**6c**):* 42% yield; mp 115−117 °C (from hexane); HRMS, *m/z* 227.0944 ($C_{14}H_{13}NO_2$ requires 227.0946); IR ν_{max} 3270, 1700, 1620, 1590, 1560, 1530, 1490, 1450, 1430, 1410, 1310, 1130, 1110, 1040, 950, 920, 870, 830, 780, 740, and 690; ^{1}H NMR δ_H 9.5 (br s, 1H, N−H), 7.4−7.3 (m, 5H, Ph), 7.1 (m, 1H, H-5k), 7.0 (m, 1H, H-5e), 6.8 (m, 1H, H-3), 6.2 (m, 1H, H-4), 5.8 (s, 1H, H-2′e), 3.9 and 3.8 (s + s, 2H + 2H, H-2′k + H-4′k), and 3.6 (s, 2H, H-4′e); ^{13}C NMR δ_C 201.7 ($C_{3'}$k), 182.9 ($C_{1'}$k), 182.8 and 181.8 ($C_{1'}$e and $C_{3'}$e), 135.6 ($C_{1''}$), 131.5 (C_2k), 130.0 (C_2e), 129.6 ($C_{3''}$k), 129.2 ($C_{3''}$e), 128.7 ($C_{2''}$k), 128.6 ($C_{2''}$e), 127.2 ($C_{4''}$k), 127.0 ($C_{4''}$e), 126.2 (C_5k), 124.7 (C_5e), 118.4 (C_3k), 115.1 (C_3e), 111.1 (C_4k), 111.0 (C_4e), 95.9, ($C_{2'}$e), 52.1 ($C_{2'}$k), 50.2 ($C_{4'}$k), and 42.8 ($C_{4'}$e); MS, *m/z* 227 (M^+, 53), 136 (100), 123 (5), 118 (2), 109 (40), 94 (93), 91 (26), 86 (7), and 68 (24). Anal. Calcd for $C_{14}H_{13}NO_2$: C, 74.01%; H, 5.73%; N, 6.17%. Found: C, 73.70%; H, 5.78%; N, 6.24%.

*2-(3-Hydroxy-5-phenylpenta-2,4-dienoyl)pyrrole (**6d**):* 40% yield; obtained as an oil; HRMS, *m/z* 239.0950 ($C_{15}H_{13}NO_2$ requires 239.0946); IR ν_{max} 3290, 1630, 1590, 1550, 1520, 1450, 1435, 1380, 1320, 1120, 790, 730, and 690; ^{1}H NMR δ_H 9.6 (br s, 1H, N−H), 7.6 (d, J = 16 Hz, 1h, H-5′), 7.5 (dd, J = 7 and 2 Hz, 2H, H-2″ + H-6″), 7.4 (m, 3H, H-3″−H-5″), 7.1 (m, 1H, H-5), 6.9 (m, 1H, H-3), 6.6 (d, J = 16 Hz, 1H, H-4′), 6.3 (m, 1H, H-4) and 6.1 (s, 1H, H-2′); ^{13}C NMR δ_C 189.5 ($C_{1'}$), 172.8 ($C_{3'}$), 138.0 ($C_{1''}$), 135.4 (C_2), 129.6 ($C_{4''}$), 128.9 ($C_{5'} + C_{3''} + C_{5''}$), 127.8 ($C_{2''} + C_{6''}$), 124.5 ($C_5$), 122.6 ($C_{4'}$), 114.8 ($C_3$), 111.2 ($C_4$), and 98.0 ($C_{2'}$); MS, *m/z* 239 ($M^+$, 41), 221 (7), 211 (7), 210 (6), 179 (5), 169 (13), 149 (16), 131 (27), 126 (15), 115 (9), 111 (25), 101 (20), 97 (37), 91 (62), 83 (100), 71 (35), 69 (42), 57 (62), 55 (50), and 49 (84).

Methylation of β-Diketones 6a and 6b. *General Procedure.* To a stirred slurry of prewashed NaH (60% dispersion oil; 1.64 mmol) in dimethylformamide (DMF) (2.50 mL) at 0 °C was added a solution of the β-diketone **6a** or **6b** (1.26 mmol) in DMF (4.00 mL), via double-ended needle technique, dropwise. After hydrogen evolution had ceased, the mixture was warmed to room temperature, stirred for 2 h, and then recooled to 0 °C. Iodomethane (1.77 mmol) was then added. After being stirred at room temperature for 4 h, the mixture was diluted with water and extracted with CH_2Cl_2. The combined extracts were washed with brine, dried, and concentrated to dryness. Chromatography of the residue on silica gel provided the methylated 2-acylpyrrole **7a** or **7b**.

*2-(2-Methyl-3-oxodecanoyl)pyrrole (**7a**):* 45% yield; obtained as an oil; HRMS, *m/z* 249.1728 ($C_{15}H_{23}NO_2$ requires 249.1728); IR ν_{max} 3280, 3100, 2905, 2840, 1710, 1620, 1540, 1445, 1400, 1140, 1090, 1040, 1000, 900, and 750; ^{1}H NMR δ_H 10.0 (br s, 1H, N−H), 7.1 (m, 1H, H-5), 7.0 (m, 1H, H-3), 6.3 (m, 1H, H-4), 4.2 (q, J = 7 Hz, 1H, H-2′), 2.5 (m, 2H, H-4′), 1.5 (m, 2H, H-5′), 1.4 (d, J = 7 Hz, 3H, CHCH_3), 1.2 (br s, 8H, (CH_2)$_4$CH$_3$), and 0.8 (t, J = 7 Hz, 3H, CH$_2$CH_3); ^{13}C NMR δ_C 206.9 ($C_{3'}$), 186.9 ($C_{1'}$), 131.1 (C_2), 125.9 (C_5), 117.7 (C_4), 111.1 (C_3), 56.2 ($C_{2'}$), 40.7 ($C_{4'}$), 31.6, 28.9, 23.5, 22.6 ($C_{5'}$-$C_{9'}$), 14.0, and 13.7 (2 × CH_3); MS, *m/z* 249 (M^+, 24), 219 (2), 181 (2), 165 (5), 150 (2), 123 (100), 94 (34), 69 (4), 66 (5), and 57 (12).

*2-(2-Methyl-3-oxopentanoyl)pyrrole (**7b**):* 99% yield; obtained as an oil; HRMS, *m/z* 179.0945 ($C_{10}H_{13}NO_2$ requires 179.0946); IR ν_{max} 3280, 3150, 2980, 2920, 2860, 1710, 1620, 1540, 1450, 1420, 1400, 1320, 1150, 1090, 1045, 900, and 750; ^{1}H NMR δ_H 10.1 (br s, 1H, N−H), 7.1 (m, 1H, H-5), 7.0 (m, 1H, H-3), 6.3 (m, 1H, H-4), 4.2 (q, J = 7 Hz, 1H, H-2′), 2.5 (m, 2H, H-4′), 1.4 (d, J = 7 Hz, 3H, CHCH_3), and 1.0 (t, J = 7 Hz, 3H, CH$_2$CH_3); ^{13}C NMR δ_C 207.3 ($C_{3'}$), 187.1 ($C_{1'}$), 131.1 (C_2), 126.3 (C_5), 117.9 (C_3), 111.1 (C_4), 55.8 ($C_{2'}$), 34.1 ($C_{4'}$), 13.8, and 7.7 (2 × CH_3); MS, *m/z* 179 (M^+, 67), 150 (2), 123 (73), 106 (3), 94 (100), 84 (2), 66 (11), and 57 (12).

Biological Assays. *Insects. Oncopeltus fasciatus* Dallas were maintained at 28 ± 1 °C, 50−60% relative humidity, and 16 h/8 h (light/dark) photoperiod and on a diet based on sunflowers 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 gloesporoides* (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). Six different bacterial strains were used 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 *Bacillus cereus* (CECT 148).

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).

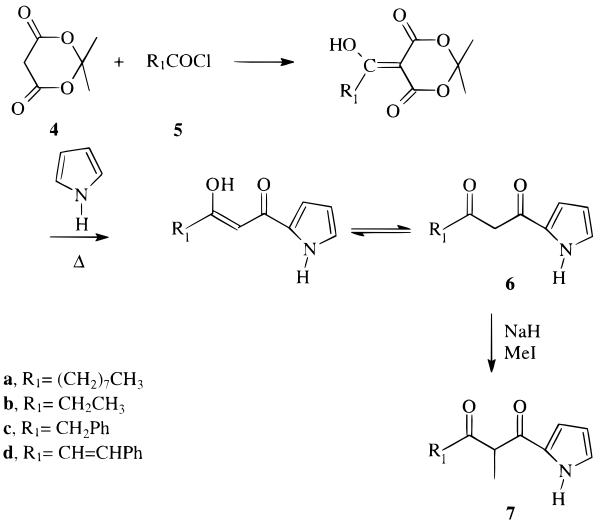
Entomotoxicity and Anti-JH Activity. The test was carried out basically according to the contact method of Bowers et al. (1976). Briefly, 15 third-instar *O. fasciatus* nymphs were confined to a 9 cm Petri dish coated, across the bottom, with 10 μg/cm^2 of the product. Chemicals showing high activity at 10 μg/cm^2 were retested at 7.5, 6, 5, 4, 2.5, and 1 μg/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} values. All assays were made three times. Because of the small isolated quantities, the natural pyrroles were assayed by topical application, at 10 μg/nymph, on newly molted fourth-instar *O. fasciatus* nymphs. 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.

Antifungal Activity. The products, dissolved in acetone, were added to PDA, in a concentration of 100 μg/mL. PDA plates containing only acetone were used as control plates. 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 °C. The antifungal activity of each product was determined three times. Analysis of variance (ANOVA) was performed for fungicidal data (Table 2), and the least significant difference (LSD) test was used to compare means (Statgraphics Plus 2.1).

RESULTS AND DISCUSSION

Two new natural products have been isolated in this work, from *P. brevicompactum*, following the procedure summarized in Scheme 1. The metabolite obtained in higher amount was assigned to possess structure **3** on the following basis. Its molecular formula, $C_{15}H_{21}NO_2$, was established by HRMS. The spectrum showed fragment ions corresponding to cleavage of the amide group (*m/z* 181.1226 [$C_{11}H_{17}O_2$]) and the $C_{2'}$−$C_{3'}$ bond (*m/z* 125.0968 [$C_8H_{13}O$]). The ^{1}H NMR spectrum exhibited two signals (δ 7.3 and 6.3) corresponding to the four protons of a symmetric pyrrole ring and a broad singlet (δ 5.4) attributable to the two protons of a dialkyl-substituted double bond. The corresponding carbons appeared in the ^{13}C NMR spectrum as two sets of signals at δ 119.6 and 114.1 (pyrrol) and δ 131.1 and 125.5 (double bond). Moreover, the ^{1}H NMR spectrum presented a signal at 4.1 ppm corresponding to a methine group connected to a methyl group. The IR spectrum showed two bands at 1727 and 1700 cm^{-1}, suggesting the presence of two carbonyl groups. This

Scheme 2. Synthesis of 2-(3-Oxoacyl)pyrroles



was compatible with a β-keto amide substructure. Comparison of the ^{1}H NMR spectrum of **3** with that of brevioxime (**1**) (Moya et al., 1997) revealed that both compounds share the same side chain. The other metabolite was assigned to possess structure **2**. Its molecular formula was $C_{15}H_{23}NO_2$, as established by HRMS. As in the case of **3**, the mass spectrum showed cleavage of the amide bond. The IR spectrum showed two carbonyl groups (1725 and 1703 cm^{-1}). The ^{1}H NMR spectrum exhibited signals (at 4.1 and 1.6 ppm as a quartet and a doublet, respectively) corresponding to a $CHCH_3$ unit between the two carbonyl groups. The rest of the signals and the absence of olefinic peaks indicated that both natural products differed only in the degree of unsaturation, due to the presence or absence of the double bond in the side chain.

The fact that compounds **2** and **3** were isolated from a different fraction obtained from the same extract as the active brevioxime (**1**) (Moya et al., 1997), together with the presence of a side chain of seven carbons (with or without a double bond) in the three natural products **1**−**3**, prompted us to synthesize other related compounds to explore their biological activities.

However, the relative weakness of the C−N bond between a carbonyl group and the N atom of pyrrole suggested that this type of amide could be easily hydrolyzable. For this reason, the introduction of similar side chains as C-substituents in the pyrrole ring would lead to more stable compounds. Thus, it seemed that this change could be interesting to obtain new active analogues.

To introduce this variation, we decided to start from different acyl chlorides (**5**) and Meldrum's acid (**4**) (Meldrum, 1908; Davidson and Bernhardt, 1948; Oikawa et al., 1978; Pak et al., 1992). As acylation of pyrrole is easier at C_2 than at the nitrogen atom, reaction of the acylated Meldrum's acid intermediate led directly to the desired products **6**. This simple reaction was carried out with different acyl chlorides, giving rise to a variety of substituted derivatives (Scheme 2).

In the NMR spectra it was evident that these compounds exist in solution as an equilibrium mixture of the keto and enol forms, except for compound **6d**, which exhibits only the enol form due to its extended conjugation.

Methylation of **6** was achieved by treatment with iodomethane after basification with NaH (Benetti and Romagnoli, 1995; Abad et al., 1997). This reaction led to **7a**,**b** in a straightforward manner. In this series the keto−enol balance is completely shifted toward the keto form as a consequence of the introduction of the methyl group and the lower flexibility of the side chain.

Table 1. Toxicity of Active Pyrrolic Compounds against *O. fasciatus*[a]

product	slope	intercept	r[b]	LD_{50} (μg/cm^2)
6b	5.0022	1.3934	0.96	5.26
7b	3.1583	2.7731	0.98	5.07

[a] Regression analysis, linear model: $y = ax + b$; log-dose vs probit mortality. [b] correlation coefficient. **6b**, 2-(3-oxopentanoyl)pyrrole; **7b**, 2-(2-methyl-3-oxopentanoyl)pyrrole.

Compound **7a** was a structural isomer of the natural product **2**; however, comparison of their spectral properties revealed their differences. As the symmetry of the pyrrole ring disappears in **7a**, the NMR spectrum of this compound showed different peaks for each proton or carbon nuclei. On the other hand, the β-ketoamide to β-diketone transformation was accompanied by characteristic changes in the IR frequencies of the carbonyl groups and in their ^{13}C NMR chemical sifts.

Biological Activities. There are a number of both naturally occurring and synthetic pyrroles that possess plant protection properties. In particular, most of these compounds are effective against fungi (Nyfeler and Ackermann, 1992; Knuppel et al., 1992; Artico et al., 1995) and insects (Addor et al., 1992; Kuhn et al., 1992; Kameswaran et al., 1992; Black et al., 1994). For this reason, the natural pyrrolic products, **2** and **3**, were tested for entomotoxicity and/or fungicidal activity. None of them showed toxicity against *O. fasciatus* when assayed by topical application on newly molted fourth-instar nymphs at the dose of 10 μg/nymph. Compound **3** did not show fungicidal activity either. A possible reason for this lack of activity might, in principle, be the weakness of the amide bond, which could be easily hydrolyzed by the enzymes of the test insects and fungi. Hence, structural modifications were introduced to increase the stability of these molecules. The most simple modification was to attach the side chain at one of the carbons of the heterocyclic ring. Thus, it seemed that this amide-to-ketone isomerization could be interesting to obtain stable, potentially active isomers.

Noteworthy, two of the synthetic products (**6b** and **7b**) have significant insecticidal activity. Acute LD_{50} values for third-instar nymphs exposed to these products by the contact method were 5.26 and 5.07 μg/cm^2, respectively. Although the two products have similar LD_{50} values, it seems that methylation between the two carbonyl groups, yielding compound **7b**, is associated with an important decrease in the slope of the dose−response curve (Table 1). Thus, compound **6b** produced 100% mortality at 7.5 μg/cm^2, whereas **7b** showed ∼73.3% of toxicity at the same dose. The most potent insecticidal N-substituted pyrroles, typified by the experimental AC-303,630, the original model of which is the natural product dioxapyrrolomycin, are highly toxic to insect (LC_{50} of 1−3 ppm by feeding and 1−10 μg/g fo body weight by injection) and appear as novel broad spectrum insecticides and acaricides (Black et al., 1994). Although, comparatively, **6a** and **7a** do not reach the level of activity of the N-substituted pyrroles, results were good enough to consider further studies to improve the entomotoxicity and to appraise the spectrum of insecticidal activity. On the other hand, insects were

Table 2. Fungicidal Activities of Active Products

target phytopathogen	percentage of radial mycelial growth inhibition,[a] % (mean ± SD)[b]				
	6a[c]	**6b**	**6c**	**6d**	**7a**
F. culmorum	0^{A}	0^{A}	$<20^{B}$	0^{A}	$<20^{B}$
F. oxysporum ssp. *gladioli*	24.8 ± 0.3^{B}	0^{A}	31.0 ± 3.4^{C}	0^{A}	60.7 ± 1.2^{D}
F. oxysporum ssp. *niveum*	27.7 ± 0.9^{B}	0^{A}	51.7 ± 4.3^{C}	0^{A}	47.7 ± 5.2^{C}
G. candidum	$<20^{B}$	0^{A}	26.4 ± 0.4^{C}	0^{A}	36.7 ± 3.4^{D}
C. gloesporoides	29.3 ± 3.5^{B}	$<20^{A}$	56.4 ± 1.0^{C}	$<20^{A}$	40.9 ± 2.1^{D}
C. coccodes	25.7 ± 3.8^{A}	33.8 ± 1.3^{B}	62.5 ± 2.3^{C}	54.3 ± 2.7^{D}	31.8 ± 1.5^{B}
T. roseum	20.2 ± 2.3^{B}	30.9 ± 1.3^{C}	82.2 ± 3.6^{D}	0^{A}	30.8 ± 2.3^{C}
A. tenuis	34.8 ± 3.0^{B}	0^{A}	50.5 ± 0.5^{C}	0^{A}	56.9 ± 2.3^{D}
R. necatrix	$<20^{B}$	0^{A}	$<20^{B}$	0^{A}	28.2 ± 2.0^{C}
V. dahliae	27.8 ± 3.6^{C}	$<20^{B}$	55.1 ± 4.8^{D}	0^{A}	30.8 ± 2.3^{C}
T. viride	$<20^{B}$	0^{A}	23.0 ± 2.0^{C}	0^{A}	43.0 ± 1.0^{D}
P. italicum	24.1 ± 0.1^{B}	0^{A}	$<20^{C}$	0^{A}	31.2 ± 0.1^{D}
A. parasiticus	24.6 ± 0.1^{B}	0^{A}	25.7 ± 1.4^{B}	0^{A}	43.3 ± 0.1^{C}

[a] Assays concentration: 100 μg/mL. [b] Each value represents the average and standard deviation of three independent experiments. Within each line, mean values labeled with the same superscript (A−D) do not present statistically significant differences ($P > 0.05$).[c] **6a**, 2-(3-oxodecanoyl)pyrrole; **6b**, 2-(3-oxopentanoyl)pyrrole; **6c**, 2-(3-oxo-4-phenylbutanoyl)pyrrole; **6d**, 2-(3-hydroxy-5-phenylpenta-2,4-dienoyl)pyrrole; **7a**, 2-(2-methyl-3-oxodecanoyl)pyrrole.

unaffected, at test levels, by all of the other synthetic products, showing that the introduction of longer chains or additional aromatic rings produces an adverse effect on the entomotoxicity. None of the compounds showed in vivo JH antagonistic activity.

The fungicidal activities of the synthesized products are summarized in Table 2. Compounds **7a** and **6c**, together with **6a**, appear to be broad-spectrum toxicants; their fungitoxic activity was determined against fungi belonging to 10 different genera, and mycelial growth was inhibited in all cases, albeit to different extents. On the other hand, apart from a few exceptions, *Colletotrichum* species and *T. roseum* were more sensitive to the products, whereas *F. culmorum* and *R. necatrix* showed lesser sensitivity.

The fungicidal activity of compound **7a** was ~2-fold that of **6a**. It seems that the introduction of a methyl group between the two carbonyls has beneficial effects with regard to the inhibition of fungal growth. In this context, it is interesting to note that methylation increases the rigidity of the molecule and shifts the keto−enol equilibrium toward the keto form. In the nonmethylated compounds (such as **6a**), the enolic structure predominates. This could be related to the observed differences in the biological activities.

In addition, the chain length appears to be very important, conferring fungicidal activity to the products. Contrary to what was observed for insecticidal activity, pyrroles with longer chains were the most active.

Compound **6c** showed the highest fungicidal activities, with growth inhibitions >50% in almost half of the fungi assayed. Other pyrroles containing phenyl substituents have also shown important fungicidal activities. This is the case of the natural pyrrolnitrin, a secondary metabolite produced by different *Pseudomonas* species. Two of its analogues have been selected for commercial fungicide development (Nyfeler and Ackermann, 1992).

On the other hand, compound **6d**, structurally related to **6c**, was selectively effective against the *Colletotrichum* genus, particularly against *C. coccodes*. In this case, the loss of activity, as indicated by the reduced range of phytopathogens affected, could be related to the high level of conjugation, which moves the balance to the enol form. Again, as discussed above for compound **6a**, it seems that the enol forms of this series are less active.

In summary, a significant activity increase over the original lead molecules has been achieved by structural modification; however, improvements are still required to obtain new products able to achieve a more effective control of insects and fungi. In this context, the structures here presented are simple enough to warrant consideration as a starting point for further synthetic modifications.

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