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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INTRODUCTION

The isolation and identification of fungal metabolites has 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 include mycophenolic acid and related compounds (Birkinshaw et al., 1952), the Raistrick phenols (Oxford and Raistrick, 1932; Oxford and Raistrick, 1933; Godin, 1955), the pebrolides (McCorkindale et al., 1981) or 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 JH biosynthesis inhibitor (Moya et al., 1997). Its chemical structure contains an unusual heterobicyclic skeleton and an oxime functionality.

Now we wish to 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 *Penicillium brevicompactum*. Their chemical structures are new: they were neither described 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 between the three natural products **1**-**3**, prompted us

to synthesize a series of related pyrroles and to determine their biological activities.

45   Moreover, other pyrrolic compounds of natural origin have been used as lead molecules for

the development of commercial fungicides (Nyfeler and Ackermann, 1992). In order to

increase the stability of the resulting analogues towards 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.

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2

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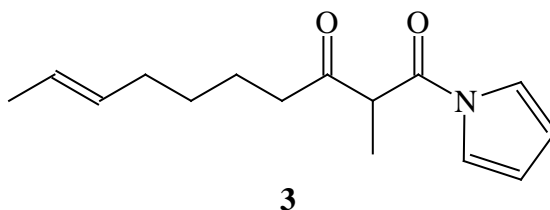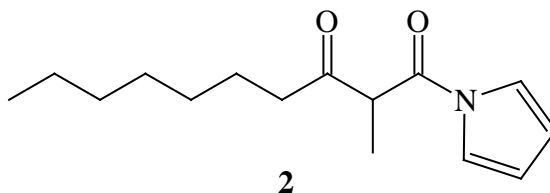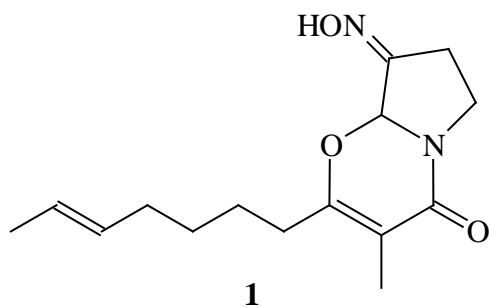
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MATERIALS AND METHODS

All chemicals were obtained from commercial suppliers and used without further

55   purification. IR spectra were obtanined as liquid films (or KBr plates for isolated natural

products); ν_{max} is given for the main absorption bands. ^1H and ^{13}C NMR spectra were recorded at 300 and 75 MHz (or 400 and 100 MHz for isolated natural products), respectively, in $CDCl_3$ solvent; chemical shifts are reported in δ (ppm) values, using TMS as internal standard. Mass spectra were obtained under electron impact (or chemical

60   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 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 compounds. The procedure was similar to

65   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, UK) as *Penicillium 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

70   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. 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

75   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

80   eluent. This led to the separation of 20 fractions which 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 x 0.7 cm); mobile phase

85   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** (rt: 24.3 min; 2.8

mg) and compound **3** (rt: 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. $[\alpha]^{20}_D = 23^o$ (c 0.20, $CHCl_3$); obtained as an oil; HRMS: m/z 248.1661

90   ($M+H^+$, $C_{15}H_{22}NO_2$ 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=CHC*H*$_3$), 1.6-1.5 (m, 2H, H-

5'), 1.5 (d, J= 7 Hz, CHC*H*$_3$) and 1.3 (m, 2H, H-6'); ^{13}C-NMR: δ_C 131.1 ($C_{8'}$), 125.5 ($C_{9'}$),

95   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 ($2xCH_3$); 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** . $[\alpha]^{19}_D = 14^o$ (c

100   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$_2$CH_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 sotution (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 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 almost pure the 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; m.p. 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-

125   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$_2$k), 130.2 (C$_2$e), 126.3 (C$_5$k), 124.5 (C$_5$e), 118.6 (C$_3$k), 114.7 (C$_3$e), 111.3 (C$_4$k), 111.0

(C$_4$e), 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),

130   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$_9$H$_{11}$NO$_2$ requires 165.0789); IR: ν_{max} 3280, 3100, 2960, 2940, 2860, 1710,

135   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$_3$e) and 1.1 (t, J= 7 Hz,

3H, CH$_3$k); ^{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

140   (C$_2$k), 130.1 (C$_2$e), 126.4 (C$_5$k), 124.7 (C$_5$e), 118.6 (C$_3$k), 114.8 (C$_3$e), 111.1 (C$_4$k), 110.9

(C$_4$e), 94.4 (C$_{2'}$e), 53.1 (C$_{2'}$k), 36.4 (C$_{4'}$k), 29.3 (C$_{4'}$e), 10.4 (CH$_3$e) and 7.5 (CH$_3$k); 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$_9$H$_{11}$NO$_2$: C, 65.45%; H, 6.66%; N, 8.48%; Found: C, 64.94%; H,

6.98%; N, 8.39%.

145   **2-(3-Oxo-4-phenylbutanoyl)pyrrole (6c).** 42 % yield; m.p. 115-117º 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$_2$k), 130.0 (C$_2$e), 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$_5$k), 124.7 (C$_5$e),

118.4 (C$_3$k), 115.1 (C$_3$e), 111.1 (C$_4$k), 111.0 (C$_4$e), 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

pre-washed NaH (60 % dispersion oil; 1.64 mmol) in DMF (2.50 mL) at 0 ºC was added a

solution of the β-diketone **6a** or **6b** (1.26 mmol) in DMF (4.00 mL), *via* double ended

needle tecnique, dropwise. After hydrogen evolution had ceased, the mixture was warmed

to room temperature, stirred for 2 h and then re-cooled to 0 ºC. Then iodomethane (1.77 mmol) was 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

175 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

180 (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 (2xCH$_3$); 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).

185 **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,

190 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 (2xCH$_3$); MS: m/z 179 (M$^+$, 67), 150 (2), 123 (73), 106 (3), 94 (100), 84 (2), 66 (11) and 57 (12).

Biological assays.

195   **Insects.** *Oncopeltus fasciatus* Dallas were maintained at 28 ± 1 °C, 50-60 % relative humidity, 16h/8h (light/dark) photoperiod and a diet based on sunflowers seeds.

       Target Microorganisms. Fungicidal activity was measured against thirteen agronomically important phytopathogens: *Aspergillus parasiticus* (CECT 2681), *Geotrichum candidum* (CCM 245), *Alternaria tenuis* (CECT 2662), *Colletotrichum*

200   *gloesporoides* (CECT 2859), *Colletotrichum coccodes* (CCM 327), *Fusarium oxysporium* 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), *Verticillium dahliae* (CCM 269). Six different bacterial strains were used in order to determine

205   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

210   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 with 10 µg/cm^2 of the product, being tested lower dosis for high activities. Toxicity effects were considered according to the number of

215   insects dead after 72 h of exposure to the chemicals and probit analysis (Finney, 1971) was used to determine the LD$_{50}$. 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 reproduction was successful with the production of viable offsprings, the tests

were finished. The tests were considered positive for JH antagonistic activity when

220   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 100 µg/mL. PDA plates containing only acetone were used as control plates.

Seven days-old cultures of each fungus on PDA plates were used as an inoculum onto the

225   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.

230   RESULTS AND DISCUSSION

   Two new natural products have been isolated in this work, from *Penicillium*

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

235   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 ppm) corresponding to the four protons of a symmetric pyrrole ring and a broad

singlet (δ= 5.4 ppm) attributable to the two protons of a dialkyl-substituted double bond.

240   The corresponding carbons appeared in the ^{13}C-NMR spectrum as two sets of signals at δ= 119.6 and 114.1 ppm (pyrrole), and δ= 131.1 and 125.5 ppm (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 was compatible with a β-keto amide

245   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

250   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 only differed 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

255   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 in order to explore their biological activities.

        However, relative weakness of the C-N bond between a carbonyl group and the N

260   atom of pyrrole, suggested that this type of amides 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 in order to obtain new active analogues.

To introduce this variation we decided to start from different acyl chlorides (**5**) and

265   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.

270   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 **7 a-b** in a

275   straightforward manner. In this series the keto-enol balance is completely shifted towards the keto form as a consequence of the introduction of the methyl group and the lower flexibility of the side chain.

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

280   pyrrole ring dissapears in **7a**, the NMR spectra 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.

285   **Biological activities.** There are a number of both naturally occurring and synthetic pyrroles which 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

290   entomotoxicity and/or fungicidal activity. None of them showed toxicity against *O. fasciatus*, when assayed by topical application on newly moulted 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,

295   structural modifications were introduced in order 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

300   activity. Acute LD_{50} for third-instar milkweed bug nymphs exposed to these products by the contact method were 5.26 and 5.07 $\mu g/cm^2$, respectively. Although the two products have similar LD_{50}, 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 $\mu g/cm^2$ whilst **7b**

305   showed aprox. 73.3 % of toxicity at the same dose. Insects were unaffected, at test levels, by all 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.

310　Compounds **7a** and **6c**, together with **6a**, appear to be broad-spectrum toxicants; their fungitoxic activity was determined against fungi belonging to ten different genera, and mycelial growth was inhibited in all cases, albeit to a different extent. On the other hand, apart from a few exceptions, *Colletotrichum* species and *T. roseum* were more sensitive to the products whilst *F. culmorum and R. necatrix* showed lesser sensitivity.

315　The fungicidal activity of compound **7a** was ca. 2-fold that of **6a**. It seems that the introduction of a methyl group between the two carbonyls has beneficial effects as regards 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 towards the keto form. In the non-methylated compounds (such as **6a**), the enolic structure predominates.

320　This could be related to the observed differences in the biological activities.

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

Compound **6c** showed the highest fungicidal activities, with growth inhibitions over

325　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

330　against *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 above discussed 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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