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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 (II).

Ángel Cantín, Pilar Moya, Miguel A. Miranda, Jaime Primo & Eduardo Primo-Yúfera*

Instituto de Tecnología Química UPV-CSIC and Departamento de Química, Universidad Politécnica de Valencia, 46022-Valencia, Spain

Running title: Active analogues from metabolites of *Penicillium brevicompactum*.

*Correspondence: E. Primo-Yúfera, Instituto de Tecnología Química UPV-CSIC, Universidad Politécnica de Valencia, Avenida de los Naranjos s/n, Apartado 22012, 46022-Valencia, SpainFax: 34-6-3877809. Phone: 34-6-3877807. E-mail: eprimo@itq.upv.es

ABSTRACT

Chemical synthesis of new analogues of the bioactive enamides isolated from *P. brevicompactum* (**2-3)**, has been carried out in order to improve their biological activities. Two types of structural modifications have been introduced: replacement of the eigth carbons side chain present in the natural products (**1-4**) by other substituents frecuently found in already described active compounds and, use of other nitrogenated five-member rings with different degrees of functionalization. In this way, insecticidal and fungicidal activities have been improved in relation to those showed by the natural products. Thus, compound **9**, possessing 3-pyrroline ring, exhibited important insecticidal activity against third instar nymphs of *Oncopeltus fasciatus* Dallas (100% mortality at 7.5 μg/cm^2). Remarkable fungicidal activity was also found and preliminary structure-activity relationship could be established.

Keyword: *Penicillium brevicompactum*, fungal metabolites, β-ketoamide, analogues, insecticide, fungicide.

INTRODUCTION

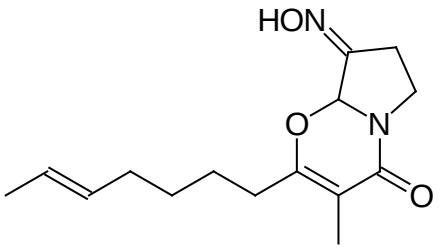
The synthesis of bioactive natural products is a powerful tool to confirm the structures and activities associated with metabolites which are usually isolated in minimal quantities. This type of work also leads to a series of potentially active synthesic intermediates, chemically related to the natural compounds. Thus, active natural products can be used as lead molecules in order to obtain different analogues with common substructures and/or functionalities, sometimes with enhanced activities as compared to the reference compounds.

Recently, we have reported on the isolation and identification of bioactive metabolites from fungal origin. The study of the culture broth of *Penicillium brevicompactum* Dierckx led to the isolation and identification of a new family of compounds with important biological activities. One of the most interesting compounds, brevioxime (**1**), exhibits a very high activity as juvenile hormone (JH) biosynthesis inhibitor (Moya et al., 1997; Castillo et al., 1998). Other metabolites possessing in vivo anti-JH activity, *N*-(2-methyl-3-oxodecanoyl)-2-pyrroline (**2a**), *N*-(2-methyl-3-oxodec-8-enoyl)-2-pyrroline (**2b**) and insecticidal activity, 2-hept-5-enyl-3-methyl-4-oxo-6,7,8,8a,-tetrahydro-*4H*-pyrrolo[2,1-b]-1,3-oxazine (**3**), were synthesized following a common route, which diverged only in the last step; this confirmed the chemical and likely biosynthetic relationship between both natural products (**1-3**) (Moya et al., 1998; Cantín et al., 1998).

Because of the importance of the biological activities of some members of this family and because the structures were simple enough to warrant consideration as a starting point for synthetic modification, we designed a programme aimed at producing analogues with improved biological activity.

The first approach was to use two isolated pyrrolic metabolites, which did not show activity, as lead molecules to obtain related compounds with fungicidal and insecticidal activities (Cantín et al., 1998). More recently, we have reported on a new series of analogues derived from already active enamides with improved activities as compared to the natural products (Moya et al., *in press*).

As an extension of this work, we wish now to report on the synthesis and biological activities of a new series of analogues, where the structural modifications involve important deviations from the parent compounds.



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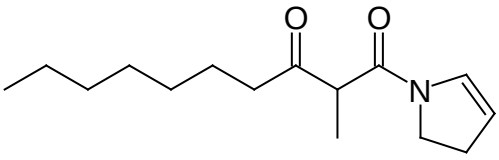

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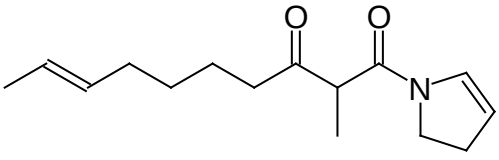

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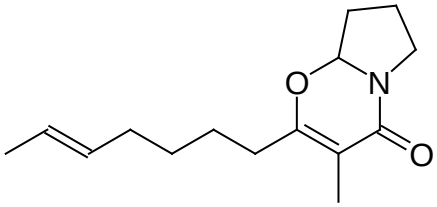


3

MATERIALS AND METHODS

All chemicals were obtained from commercial suppliers and used without further purification. IR spectra were obtained as liquid films; ν_{max} is given for the main absorption bands. ^{1}H and ^{13}C NMR spectra were recorded at 300 and 75 MHz, respectively, in $CDCl_3$ solvent; chemical shifts are reported in δ (ppm) values, using TMS as internal standard. The assignment of ^{13}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 TLC was carried out on precoated plates (silica gel 60 F_{254}); spots were visualized with UV light and in a I_2 chamber.

General Synthetic Procedures

Synthesis of β-Oxoamides. The following procedure was employed with different acyl side chains: to a cooled solution (0 ºC) of 2,2-dimethyl-1,3-dioxane-4,6-dione (1.1 mmol) in dichloromethane (1.5 mL), were added pyridine (2.2 mmol) and the corresponding acyl chloride (0.9 mmol) *via* syringe, dropwise, under nitrogen. The solution was stirred at 0 ºC for 1 h, after which it was allowed to warm up 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 acylated Meldrum's acid and pyrrolidine (2.1 mmol) were refluxed in benzene (9.0 mL) 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-oxoamide.

***N*-(3-Cyclopropyl-3-oxopropanoyl)pyrrolidine (4c).** 19 % yield; obtained as an oil; HRMS (EI): m/z 181.1099 ($C_{10}H_{15}NO_2$ requires 181.1102); IR ν_{max} 2980, 2960, 2890, 1670, 1600, 1390, 1350, 1310, 1230, 1180, 1170, 1140, 1070, 1035, 990, 970 940, 900, 880, 860, 840, 800, 730 and 720; ^{1}H NMR: δ_H 3.6 (s, 2H, H-2'), 3.5 and 3.4 (t+t, *J*= 7 Hz, 4H, H-2+H-5), 2.1 (m, 1H, H-1''), 2.0-1.8 (m, 4H, H-3+H-4), 1.1 and 0.9 (m+m, 4H, H-2''+H-3''); ^{13}C NMR: δ_C 203.8 ($C_{3'}$), 164.6 ($C_{1'}$), 50.4 ($C_{2'}$), 46.5 (C_2), 45.2 (C_5), 25.3 (C_3), 23.7 (C_4), 20.1 ($C_{4''}$), and 10.8 ($C_{2''}$+$C_{3''}$); MS m/z 181 (M^+, 46), 166 (11), 153 (13), 140 (5), 138 (6), 124 (3), 112 (35), 98 (14), 96 (5), 84 (11), 70 (100), 69 (26), 55 (18), 43 (10) and 41 (10).

Methylation of β-Oxoamides. A solution of β-oxoamide (0.9 mmol) in DMF (3.0 mL) was added dropwise to a suspension of NaH (60 % dispersion oil; 1.1 mmol) (pre-washed with pentane) in DMF (1.5 mL) at 0 ºC, after which the mixture was warmed to room temperature and stirred for 2 h 30 min. It was then re-cooled to 0 ºC and treated with iodomethane (1.9 mmol). After being stirred at room temperature for 5 h 15 min the mixture was diluted with water and extracted with CH_2Cl_2. The combined extracts were washed with brine, dried and concentrated to dryness, providing the methylated β-oxoamide.

***N*-(3-Cyclopropyl-2-methyl-3-oxopropanoyl)pyrrolidine (5c).** 82 % yield; obtained as an oil; HRMS (EI): m/z 195.1268 ($C_{11}H_{17}NO_2$ requires 195.1259); IR: ν_{max} 2960, 2860, 1700, 1630, 1420, 1380, 1330, 1300, 1250, 1220, 1190, 1160, 1130, 1100, 1040, 1010, 940, 910, 870 and 810; ^{1}H NMR: δ_H 3.7 (q, *J*= 7 Hz, 1H, H-2'), 3.5-3.4 (m, 4H, H-2+H-5), 2.1 (m, 1H, H-1''), 2.0 (m, 4H, H-3 + H-4), 1.4 (d, *J*= 7 Hz, 3H, CH_3), 1.1-0.9 (m, 4H, H-2''H-3''); ^{13}C NMR: δ_C 207.0 ($C_{3'}$), 168.0 ($C_{1'}$), 53.2 ($C_{2'}$), 46.2 (C_2), 45.6 (C_5), 25.6 (C_3), 23.7 (C_4), 17.8 ($C_{1''}$), 12.9 (CH_3), 11.1 and 10.9 ($C_{2''}$+$C_{3''}$); MS

m/z 195 (M^+, 52), 180 (5), 167 (23), 152 (4), 138 (6), 127 (64), 126 (63), 110 (8), 99 (12), 98 (31), 84 (7), 70 (100), 69 (61) and 55 (25).

***N*-(3-Cyclopropyl-2,2-dimethyl-3-oxopropanoyl)pyrrolidine (7c).** 79 % yield from **4a**; obtained as an oil; HRMS (CI): m/z 210.1491 ($M+H^+$, $C_{12}H_{20}NO_2$ requires 210.1494); IR: ν_{max} 2980, 2940, 2860, 1690, 1620, 1460, 1410, 1370, 1330, 1250, 1220, 1170, 1160,1090, 1050, 1010, 1000, 960, 910, 890, 870, 810 and 720; ^{1}H NMR: δ_H 3.5 and 3.2 (t+t, *J*= 7 Hz, 4H, H-2+H-5), 2.0 (m, 1H, H-1''), 1.9 (m, 4H, H-3+H-4), 1.4 (s, 6H, 2xCH_3), 1.0 and 0.9 (m+m, 4H, H-2''+H-3''); ^{13}C NMR: δ_C 210.5 ($C_{3'}$), 170.8 ($C_{1'}$), 56.2 ($C_{2'}$), 47.1 (C_2), 46.3 (C_5), 26.4 (C_3), 23.2 (C_4), 22.0 (2xCH_3), 17.5 ($C_{1''}$) and 11.4 ($C_{2''}$+$C_{3''}$); MS (CI) m/z 210 ($M+H^+$, 100), 209 (M^+, 15), 196 (3), 181 (3), 166 (2), 150 (2), 141 (27), 140 (17), 139 (13), 124 (3) and 111 (4).

***N*-[2-Methyl-4-(3-phenoxyphenyl)-3-oxopentanoyl]pyrrolidine (5d).** Two diastereomers. Combined yield: 71 %; obtained as oils. HRMS (EI): m/z 351.1830 ($C_{22}H_{25}NO_3$ requires 351.1834); IR: ν_{max} 3040, 2960, 2910, 2865, 1705, 1625, 1570, 1475, 1430, 1360, 1320, 1240, 1150, 1060, 970, 910, 745 and 680; MS: m/z 351 (M^+, 75), 295 (1), 280 (1), 224 (4), 197 (23), 181 (6), 167 (7), 154 (39), 127 (89), 103 (10), 98 (100), 91 (18), 77 (10) and 55 (2).

The first eluted diastereomer exhibited the following NMR: ^{1}H NMR: δ_H 7.3 (m, 2H, H-3'''+H-5'''), 7.2 (m, 1H, H-5''), 7.1 (tt, *J*= 8 and 1 Hz, 1H, H-4'''), 7.0-6.8 (m, 5H, H-2''+H-4''+H-6''+H-2'''+H-6'''), 4.0 (q, *J*= 7 Hz, 1H, H-4'), 3.4 (q, *J*= 7 Hz, 1H, H-2'), 3.3, 3.2 and 2.7 (m+m+m, 4H, H-2+H-5), 1.8 (m, 4H, H-3+H-4), 1.4 and 1.3 (d+d, *J*= 7 Hz, 6H, 2xCH_3); ^{13}C NMR: δ_C 206.8 ($C_{3'}$), 169.2 ($C_{1'}$), 157.6, 156.6, 142.7 ($C_{1''}$, $C_{3''}$, $C_{1'''}$), 129.9, 129.8, 123.5, 122.7, 118.9, 118.5, 117.0 ($C_{2''}$, $C_{4''}$-$C_{6''}$, $C_{2'''}$-$C_{6'''}$), 50.9 ($C_{4'}$), 49.8 ($C_{2'}$), 46.6 (C_2), 45.8 (C_5), 25.8 (C_3), 24.1 (C_4), 18.3 and 13.0 (2xCH_3).

The second eluted diastereomer exhibited the following NMR: ^{1}H NMR: δ_H 7.3 (m, 2H, H-3’’’+H-5’’’), 7.2 (t, *J*= 8 Hz, 1H, H-5’’), 7.1 (tt, *J*= 8 and 1 Hz, 1H, H-4’’’), 7.1-6.9 (m, 4H, H-2’’+H-6’’+H-2’’’+H-6’’’), 6.8 (ddd, *J*= 8, 3 and 1 Hz, 1H, H-4’’), 4.0 (q, *J*= 7 Hz, 1H, H-4’), 3.6 (q, *J*= 7 Hz, 1H, H-2’), 3.4, 3.0 and 2.9 (m+m+m, 4H, H-2+H-5), 1.8 (m, 4H, H-3+H-4), 1.4 and 1.3 (d+d, *J*= 7 Hz, 6H, 2xC*H*$_3$); ^{13}C NMR: δ_C 206.8 ($C_{3'}$), 167.8 ($C_{1'}$), 157.0, 156.6, 141.9 ($C_{1''}$, $C_{3''}$, $C_{1'''}$), 129.7, 129.6, 123.3, 122.9, 118.7, 118.4, 117.2 ($C_{2''}$, $C_{4''}$-$C_{6''}$, $C_{2'''}$-$C_{6'''}$), 51.6 ($C_{4'}$), 50.0 ($C_{2'}$), 46.0 (C_2), 45.8 (C_5), 26.0 and 25.8 (C_3), 23.9 (C_4), 18.2 and 13.4 (2xCH_3).

***N*-[2,2-Dimethyl-4-(3-phenoxyphenyl)-3-oxopentanoyl]pyrrolidine (7d).** 11 % yield as byproduct together with **5d**; obtained as an oil; HRMS (EI): m/z 365.1992 ($C_{23}H_{27}NO_3$ requires 365.1991); IR: ν_{max} 3040, 2960, 2920, 2860, 1700, 1620, 1570, 1480, 1435, 1400, 1240, 1205 and 690; ^{1}H NMR: δ_H 7.3 (m, 2H, H-3’’’+H-5’’’), 7.2 (t, *J*= 8 Hz, 1H, H-5’’), 7.1 (tt, *J*= 8 and 1 Hz, 1H, H-4’’’), 7.0-6.9 (m, 4H, H-2’’+H-6’’+H-2’’’+H-6’’’), 6.8 (ddd, *J*= 8, 3 and 1 Hz, 1H, H-4’’), 4.1 (q, *J*= 9 Hz, 1H, H-4’), 3.4, 3.2, 3.0 and 2.6 (m+m+m+m, 4H, H-2+H-5), 1.6 (m, 4H, H-3+H-4), 1.5 (s, 3H, (C*H*$_3$)$_2$C), 1.4 (d, *J*= 7 Hz, 3H, CHC*H*$_3$) and 1.3 (s, 3H, (C*H*$_3$)$_2$C); ^{13}C NMR: δ_C 210.4 ($C_{3'}$), 169.4 ($C_{1'}$), 157.0, 156.8, 142.6 ($C_{1''}$, $C_{3''}$, $C_{1'''}$), 129.7, 129.6, 123.4, 122.7, 118.8, 118.2, 117.2 ($C_{2''}$, $C_{4''}$-$C_{6''}$, $C_{2'''}$-$C_{6'''}$), 57.2 ($C_{2'}$), 47.4 (C_2), 47.2 ($C_{4'}$), 46.8 (C_5), 25.9 (C_3), 23.8 (CH_3), 22.9 (C_4), 21.8 and 20.2 (2xCH_3); MS: m/z 365 (M^+, 41), 224 (7), 197 (28), 168 (24), 141 (100), 112 (19), 104 (9), 98 (62), 91 (7), 77 (7) and 55 (13).

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^2). A constant current (20 mA) was passed through the solution. After

4.0 F/mol of electricity were passed, the solvent was evaporated under reduced pressure. Water was added to the residue and the product was extracted with CH_2Cl_2. The combined organic layer was dried over anhydrous sodium sulfate. Thereafter, the drying agent was removed by filtration, the solvent was evaporated to dryness and the residue was filtered through silica gel using ethyl acetate as eluent, in order 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*-(3-cyclopropyl-2-methyl-3-oxopropanoyl)pyrrolidine (6c). Two diastereomers. Combined yield: 40 %; obtained as oils.

Spectral data of the first eluted diasteromer **6c1**: HRMS (CI): m/z 226.1441 ($M+H^+$, $C_{12}H_{20}NO_3$ requires 226.1443); IR ν_{max} 2920, 2880, 1690, 1635, 1380, 1155, 1140, 1050, 1000 and 810; ^1H NMR: δ_H 5.6 and 5.0 (d+d, *J*= 4 Hz, 1H, H-2), 3.8 (q, *J*= 7 Hz, 1H, H-2'), 3.7 (m, 2H, H-5), 3.4 and 3.3 (s+s, 3H, OMe), 2.1 (m, 2H, H-3), 1.9 (m, 2H, H-4), 1.8 (m, 1H, H-1''), 1.5 and 1.4 (d+d, *J*= 7 Hz, 3H, CHC*H*$_3$) and 1.0 and 0.9 (m+m, 4H, H-2''+H-3''); ^{13}C NMR: δ_C 208.5 and 207.2 ($C_{3'}$), 170.2 and 169.7 ($C_{1'}$), 88.4 and 87.1 (C_2), 56.4 and 54.0 ($C_{2'}$), 54.2 and 53.5 (OMe), 46.0 and 45.9 (C_5), 31.4 and 30.7 (C_3), 22.9 and 20.8 (C_4), 18.0 and 17.7 ($C_{1''}$), 14.1 and 13.2 (CH_3), 11.9, 11.8, 11.7 and 11.5 ($C_{2''}$+$C_{3''}$); MS (CI) m/z 226 ($M+H^+$, 59), 225 (M^+, 14), 210 (38), 195 (100), 193 (89), 185 (5), 166 (7), 156 (12), 140 (4), 128 (41) and 125 (23).

Spectral data of the second eluted diasteromer **6c2**: HRMS (EI): m/z 225.1375 ($C_{12}H_{19}NO_3$ requires 225.1365); IR ν_{max} 2920, 2880, 2820, 1690, 1640, 1380, 1155, 1130, 1050, 990, 930, 910, 855 and 810; ^1H NMR: δ_H 5.5 and 5.0 (d+d, *J*= 4 Hz, 1H, H-2), 3.7 (m, 3H, H-5+H-2'), 3.4 and 3.2 (s+s, 3H, OMe), 2.1 (m, 2H, H-3), 1.9 (m, 2H, H-4), 1.8 (m, 1H, H-1''), 1.5 and 1.4 (d+d, *J*= 7 Hz, 3H, CHC*H*$_3$) and 1.0 and 0.9

(m+m, 4H, H-2''+H-3''); ^{13}C NMR: δ_C 207.4 and 206.5 ($C_{3'}$), 170.5 and 170.4 ($C_{1'}$), 88.8 and 87.5 (C_2), 56.6 and 53.8 ($C_{2'}$), 53.7 and 51.1 (OMe), 46.0 and 45.9 (C_5), 31.4 and 30.5 (C_3), 22.8 and 21.0 (C_4), 18.3 and 18.1 ($C_{1''}$), 13.9 and 13.8 (CH_3), 11.6 and 11.1 ($C_{2''}$+$C_{3''}$); MS m/z 225 (M^+, 14), 210 (4), 193 (73), 167 (6), 156 (3), 126 (16), 100 (30), 97 (23), 85 (19), 83 (26), 70 (100) and 55 (13).

Synthesis of Enamides. The corresponding methoxy derivative (0.05 mmol) and silica gel (0.05 mmol) were heated at 150-160 ºC in a flask, under reduced pressure and nitrogen atmosphere. After 2 h 45 min, water was added to the residue and the slurry was extracted with CH_2Cl_2. The combined organic layer was dried over anhydrous sodium sulfate. Then, the drying agent was removed by filtration, the solvent was evaporated to dryness and the residue was purified by column chromatography on silica gel. Under those conditions enamides were obtained; when the reaction was carried out with β-oxoamides, bicyclic oxazines were also formed.

***N*-(3-Cyclopropyl-2-methyl-3-oxopropanoyl)-2-pyrroline (2c) and 3-Methyl-2-cyclopropyl-4-oxo-6,7,8,8a-tetrahydro-4H-pyrrolo[2,1-b]-1,3-oxazine (3c).**

The enamide **2c** was an oil obtained in 13 % yield; HRMS (EI): m/z 193.1095 ($C_{11}H_{15}NO_2$ requires 193.1102); IR ν_{max} 3080, 2980, 2860, 1690, 1630, 1410, 1360, 1040, 1000, 900 and 720; ^{1}H NMR: δ_H 7.0 and 6.5 (m, 1H, H-2), 5.3 (m, 1H, H-3), 3.9 (t, *J*= 9 Hz, 2H, H-5), 3.7 (q, *J*= 7Hz, 1H, H-2'), 2.7 and 2.6 (m, 2H, H-4), 2.0 (m, 1H, H-1''), 1.48 and 1.47 (d+d, *J*= 3 Hz, 3H, CH_3), 1.1 and 0.9 (m+m, 4H, H-2''+H-3''); ^{13}C NMR: δ_C 207.3 ($C_{3'}$), 165.5 ($C_{1'}$), 129.4 and 128.3 (C_2), 112.9 and 111.5 (C_3), 54.0 ($C_{2'}$), 45.5 (C_5), 28.1 (C_4), 18.0 ($C_{1''}$), 13.2 (CH_3), 11.8 and 11.6 ($C_{2''}$+$C_{3''}$); MS m/z 193 (M^+, 20), 125 (3), 124 (2), 96 (7), 69 (100), 68 (47), 55 (7), 53 (4) and 41 (50).

The oxazine **3c** was obtained in 36 % yield as a yellow oil. HRMS (EI): m/z 193.1104 ($C_{11}H_{15}NO_2$ requires 193.1102); IR: ν_{max} 3070, 2960, 2920, 2860, 1700, 1640,

1430, 1350, 1250, 1180, 1080, 950, 920, 900, 880, 810, 760 and 640; ^{1}H NMR: δ_H 5.1 (dd, *J*= 8 and 4 Hz, 1H, H-8a), 3.7 and 3.4 (m+m, 2H, H-6), 2.4-1.6 (m, 5H, H-7+H-8+H-1'), 1.9 (s, 3H, C*H*$_3$), 1.1, 0.9 and 0.7 (m+m+m, 4H, H-2'+H-3'); ^{13}C NMR: δ_C 166.4 (C_4), 163.1 (C_2), 105.6 (C_3), 87.6 (C_{8a}), 44.3 (C_6), 31.6 (C_8), 21.9 (C_7), 10.5 ($C_{1''}$), 9.7 ($C_{1'}$), 7.8 and 4.4 ($C_{2'}$+$C_{3'}$); MS m/z 193 (M^+, 72), 165 (7), 156 (9), 142 (10), 100 (23), 97 (12), 83 (32), 70 (100), 69 (74) and 55 (10).

***N*-Octanoyl-3-pyrroline (9).** To a mixture of 3-pyrroline (14.1 mmol) with 1.7 M KOH (9.0 mL) was added a solution of octanoyl chloride (14.0 mmol) in CH_2Cl_2 (9.0 mL) dropwise (10 min). After being stirred at room temperature for 5 h 30 min, the mixture was extracted with CH_2Cl_2; the resulting organic extracts were washed with brine, dried over Na_2SO_4 and concentrated to dryness to give the *N*-octanoyl-3-pyrroline in a straightforward manner as an oil. 85 % yield HRMS (EI): m/z 195.1619 ($C_{12}H_{21}NO$ requires 195.1623); IR: ν_{max} 3020, 2900, 2820, 1710, 1630, 1605, 1440, 1345, 1320, 1260, 1190, 1100, 1060, 990, 940, 910, 800, 730, 710 and 660; ^{1}H NMR: δ_H 5.9 (m, 2H, H-3+H-4), 4.2 (m, 4H, H-2+H-5),2.3 (t, *J*= 8 Hz, 2H, H-2'), 1.7 (m, 2H, H-3'), 1.3 (m, 8H, (C*H*$_2$)$_4$CH$_3$) and 0.9 (t, *J*= 6 Hz, 3H, C*H*$_3$); ^{13}C NMR: δ_C 171.6 ($C_{1'}$), 126.3 (C_3), 124.8 (C_4), 53.2 (C_2), 53.0 (C_5), 34.3 ($C_{2'}$), 31.6, 29.3, 29.0, 24.7, 22.4 ($C_{3'}$-$C_{7'}$) and 14.0 (CH_3); MS: m/z 195 (M^+, 75), 180 (1), 169 (6), 166 (6), 153 (22), 143 (3), 138 (12), 127 (11), 124 (29), 111 (100), 110 (57), 96 (19), 84 (19), 69 (92), 68 (99), 57 (39) and 55 (15).

5-[1-(2,5-Dihydro-1*H*-pyrrolyl)octylydene]-2,2-dimethyl-1,3-dioxane-4,6-dione (8). 28 % yield from octanoyl chloride; obtained as an oil; HRMS (EI): m/z 321.1950 ($C_{18}H_{27}NO_4$ requires 321.1940); IR: ν_{max} 3080, 2920, 2840, 1740, 1705, 1660,

1640, 1410, 1390, 1330, 1290, 1200, 1150, 1040, 950, 930, 790, 730 and 660; ^{1}H NMR: δ_H 6.0 and 5.9 (m+m, 2H, CH=CH), 4.6 and 4.5 (br s+ br s, 4H, 2xNCH_2), 3.2 (t, *J*= 8 Hz, 2H, H-2'), 1.7 (s, 6H, C(CH_3)$_2$), 1.6 (m, 2H, H-3'), 1.3 (m, 8H, (CH_2)$_4$CH_3) and 0.9 (t, *J*= 7 Hz, 3H, CH_3); ^{13}C NMR: δ_C 180.3 ($C_{1'}$), 162.2 (C_4+C_6), 126.1 and 122.3 (*C*H=*C*H), 101.8 (C_2), 82.0 (C_5), 60.7 and 57.8 (2xNCH_2), 34.9, 31.4, 29.5, 28.7, 26.9, 26.0, 22.4 ($C_{3'}$-C_9, C(*C*H_3)$_2$) and 13.9 (CH_3); MS: m/z 321 (M^+, 2), 320 (9), 319 (8), 305 (6), 265 (30), 246 (16), 238 (18), 235 (17), 220 (10), 203 (10), 191 (52), 177 (100), 160 (69), 148 (50), 134 (62), 121 (45), 118 (45), 106 (60), 92 (38) and 81 (46); Anal. Calcd. for $C_{18}H_{27}NO_4$ 65.75, C; 8.49, H. Found 65.42, C; 8.58, H.

***N*-(3-Oxodecanoyl)-2-pyrrolidinone (10).** Obtained as an oil following the previously described procedure for β-oxoamides in 70 % yield; HRMS (EI): m/z 253.1678 ($C_{14}H_{23}NO_3$ requires 253.1677); IR: ν_{max} 2910, 2840, 1735, 1690, 1610, 1450, 1400, 1360, 1320, 1190, 1160, 1070, 1010, 930, 880, 830, 800 and 720; ^{1}H NMR: δ_H 4.0 (s, 2H, H-2'), 3.9 (t, *J*= 7 Hz, 2H, H-5), 2.6 (m, 4H, H-3+H-4'), 2.1 (m, 2H, H-4), 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_2CH_3); ^{13}C NMR: δ_C 203.7 ($C_{3'}$), 175.6 (C_2), 167.2 ($C_{1'}$), 51.4 ($C_{2'}$), 45.1 ($C_{5'}$), 42.9 ($C_{4'}$), 33.8, 33.1, 31.5, 28.9, 23.2, 22.5 (C_3, $C_{5'}$-$C_{9'}$), 16.8 (C_4) and 13.9 (CH_3); MS: m/z 253 (M^+, 6), 235 (1), 211 (4), 182 (6), 169 (100), 154 (42), 150 (9), 127 (40), 112 (12), 99 (19), 86 (99), 69 (6) and 57 (40).

***N*-(2-Methyl-3-oxodecanoyl)-2-pyrrolidinone (11).** Obtained as an oil in 70 % yield following the same procedure described previously for β-oxoamides; HRMS (EI): m/z 267.1833 ($C_{15}H_{25}NO_3$ requires 267.1834); IR: ν_{max} 2920, 2850, 1730, 1685, 1450, 1400, 1350, 1240, 1120, 1010, 910, 830 and 715; ^{1}H NMR: δ_H 4.5 (q, *J*= 7 Hz, 1H, H-

2'), 3.9 (m, 2H, H-5), 2.7 (m, 2H, H-3), 2.6 (t, *J*= 8 Hz, 2H, H-4'), 2.1 (m, 2H, H-4), 1.6 (m, 2H, H-5'), 1.4 (d, *J*= 7 Hz, 3H, C*H*$_3$CH), 1.3 (br s, 8H, (C*H*$_2$)$_4$CH$_3$) and 0.9 (t, *J*= 7 Hz, 3H, CH$_2$C*H*$_3$); ^{13}C NMR: δ_C 207.7 (C$_{3'}$), 175.6 (C$_2$), 170.8 (C$_{1'}$), 53.8 (C$_{2'}$), 45.6 (C$_5$), 40.8 (C$_{4'}$), 33.5, 31.6, 29.1, 23.3, 22.5 (C$_3$, C$_{5'}$-C$_{9'}$), 17.0 (C$_4$), 14.0 and 12.6 (2xCH$_3$); MS: m/z 267 (M$^+$, 4), 196 (1), 183 (59), 168 (20), 141 (100), 127 (39), 113 (57), 86 (87), 83 (17), 69 (10), 57 (79) and 55 (14).

***N*-(2-Methyl-3-hydroxydecanoyl)-2-pyrrolidinone (12).** A solution of the ketoimide **11** (170 mg, 0.6 mmol) in CH_2Cl_2 (35.0 mL) was cooled at –30 ºC and $Zn(BH_4)_2$ (0.14 M in diethyl ether; 4.5 mL, 0.6 mmol) was added to it. After the mixture had been stirred for 1 h 15 min, getting to –20 ºC, it was treated with acetone and then allowed to warm to room temperature. The mixture was diluted with CH_2Cl_2 and washed with water and brine, dried and concentrated to give an oily residue wich was purified by column chromatography on silica gel using hexane/EtOAc (8:2) as a eluent to afford the β-hydroxyimide **12** (82 mg, 49 %) as a yellow oil. HRMS (EI): m/z 270.2067 (M+H$^+$, $C_{15}H_{28}NO_3$ requires 270.2069); IR: ν_{max} 3450, 2920, 2840, 1740, 1690, 1450, 1350, 1250, 1220, 1090, 1020, 970, 930, 890 and 830; ^{1}H NMR: δ_H 3.9 (m, 1H, H-3'), 3.8 (td, *J*= 7 and 2 Hz, 2H, H-5), 3.7 (m, 1H, H-2'), 3.0 (d, *J*= 3 Hz, 1H, OH), 2.6 (t, *J*= 8 Hz, 2H, H-3), 2.0 (m, 2H, H-4), 1.3 (br s, 8H, (C*H*$_2$)$_4$CH$_3$), 1.2 (d, *J*= 7 Hz, 3H, CHC*H*$_3$) and 0.9 (t, *J*= 7 Hz, 3H, CH$_2$C*H*$_3$); ^{13}C NMR: δ_C 178.7 (C$_{1'}$), 175.3 (C$_2$), 71.5 (C$_{3'}$), 45.7 (C$_5$), 42.9 (C$_{2'}$), 33.9, 33.8, 31.8, 29.6, 29.2, 26.0, 22.6 (C$_3$, C$_{4'}$-C$_{9'}$), 17.0 (C$_4$), 14.1 and 10.0 (2xCH$_3$); MS: m/z 270 (M+H$^+$, 3), 251 (9), 183 (4), 170 (27), 166 (13), 141 (100), 113 (59), 98 (13), 86 (99), 69 (10), 57 (14) and 55 (14).

Biological activity.

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 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).

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 20 µg/cm^2 of the product. Toxicity effects were considered according to the number of insects dead after 72 h of exposure to the chemicals. 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 100 µg/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 µg/mL was performed in order to

appraise the level of activity of the synthesized compounds. Spores from seven day-old cultures of each fungus on PDA plates were used as an inoculum onto the control and test plates. The radial mycelial growth was measured and the percentage of inhibition was calculated on the basis of growth in control plates, after 4 days of incubation (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 1) and the least significant difference (LSD) test was used to compare means (Statgraphics Plus 4.0).

RESULTS AND DISCUSSION

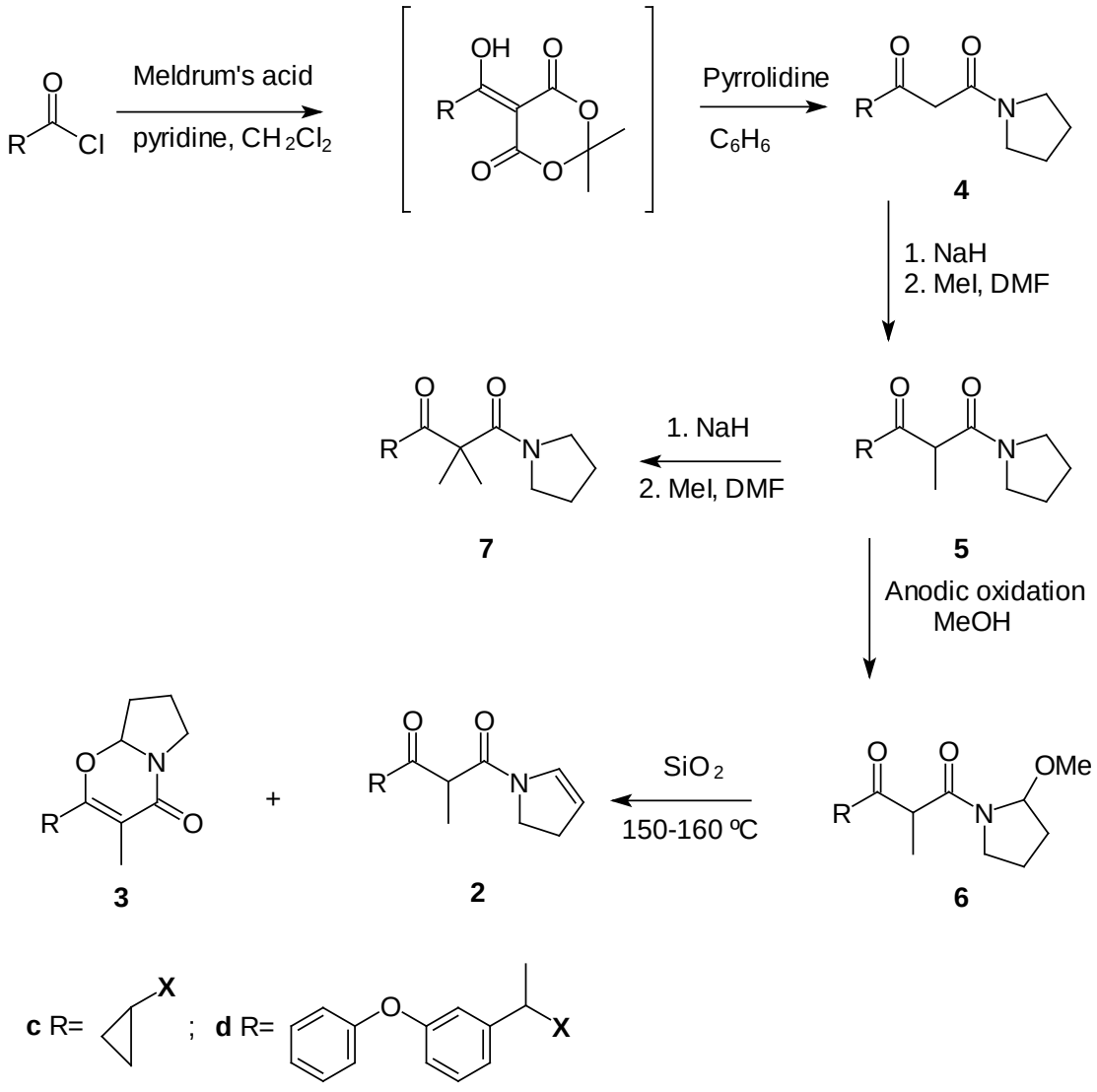
Recently, we have reported on the chemical synthesis of some biologically active natural products previously isolated in our laboratories (**2a**, **2b**, **3**). In the course of these studies we obtained a series of intermediates which showed interesting activities, improving in some cases the activities found for the natural products.

These results encouraged us to introduce certain modifications in the synthetic sequences which could lead to related active analogues; the ultimate goal would be to improve the activities of the natural products. Thus we decided to introduce two types of changes: (a) replacement of the eight carbons side chain present in the natural products by other substituents frecuently found in already described active compounds and (b) use of other nitrogenated five member rings with different degrees of functionalization.

In connection with the first approach, some functional groups present in known active compounds were considered taking into account their compatibility with the required reaction conditions and their availability. Hence, cyclopropyl (present in

360 synthetic pyretroids) and phenoxyphenyl (very common in pesticides) were selected for this work.

The chlorides of cyclopropanecarboxylic acid and fenoprofen (2-[3-phenoxyphenyl]propionic acid) were used as starting materials (Scheme 1).



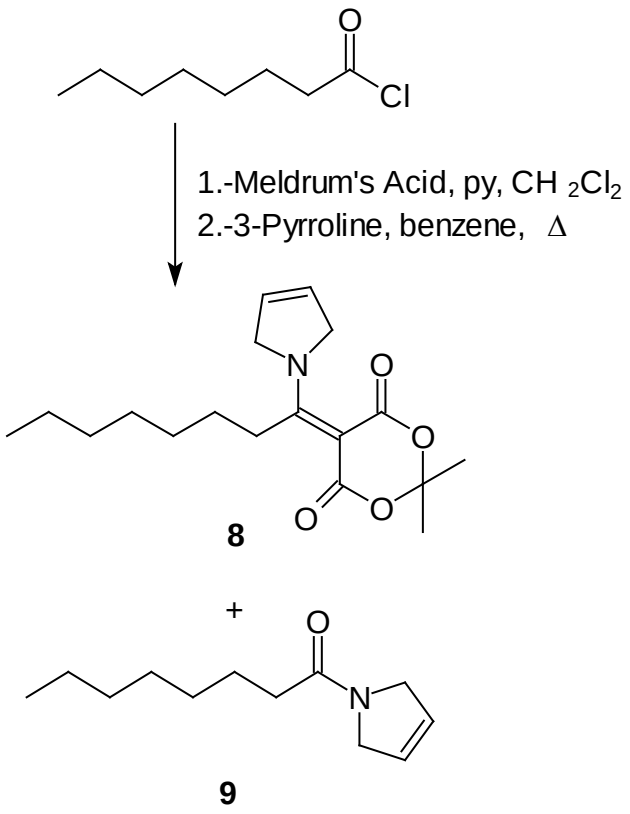
Scheme 1. Synthetic sequence for obtaining analogues.

365

Briefly summarized, the reaction sequence implied formation of acylated Meldrum's acid (Meldrum, 1908; Davidson and Bernhardt, 1948; Oikawa et al., 1978) as first step in the construction of enamide ring. Subsequent aminolysis (Pak et al.,

1992) with pyrrolidine and alkylation (Benetti and Romagnoli, 1995; Abad et al., 1997) provided the β-ketoamide system. In the case of the cyclopropane derivative, anodic oxidation (Shono, 1984; Shono et al., 1982; Shono et al., 1982; Shono et al.,1981; Shono et al., 1981) followed by elimination of MeOH heating at 150-160 ºC (Slomczynska et al., 1996; Cornille et al., 1995; Cornille et al., 1994; Moeller et al., 1994; Moeller et al., 1992) afforded the β-ketoenamide **2c**, along with the bicyclic isomer **3c**.

However, when fenoprofen was used as starting product, the anodic oxidation step led to a complex reaction mixture including some products arising from oxidation of the phenoxyphenyl group; in view of this result, no attemps were done to isolate the desired methoxylated products.



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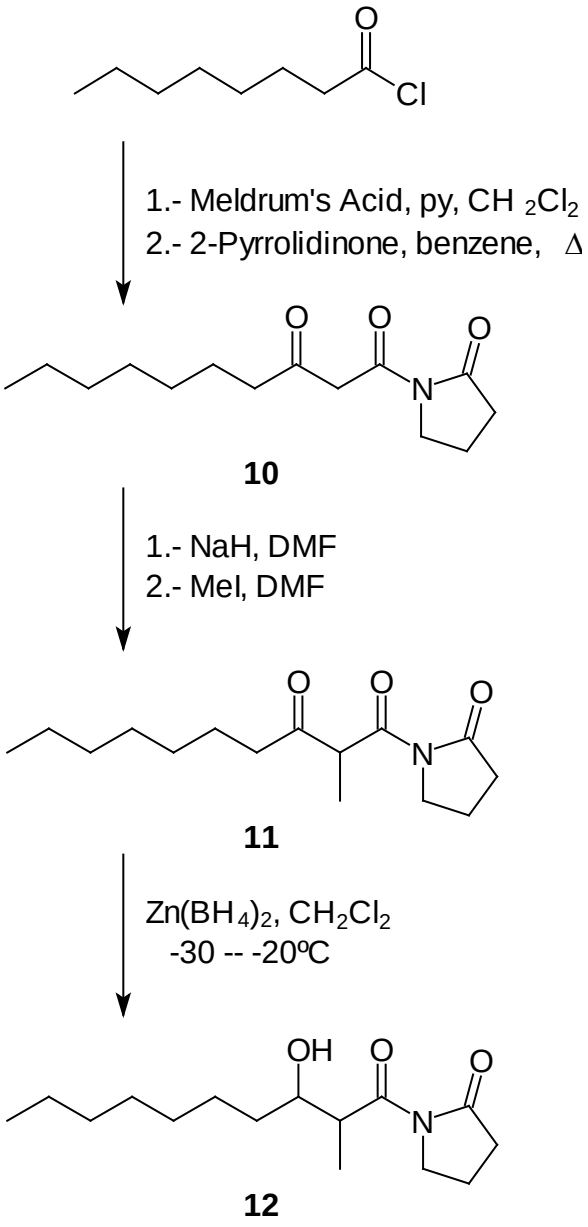
In the second approach, the moditications affected the nature of the 5-member ring. Thus, 2-pyrrolidinone and 3-pyrroline were used to carry out the aminolysis of the Meldrum's acid derivative. With 3-pyrroline, the enamine product **8** was obtained as

side-product, together with the monocarbonylic amide **9** formed by direct reaction of unreacted octanoyl chloride with 3-pyrroline. The structure of this byproduct was proved by direct synthesis by means of a Schotten-Baumann reaction.



When 2-pyrrolidinone was employed the expected imide was obtained in good yield; the same was true with the subsequent alkylation of activated position. Finally, reduction of the ketone group (Evans and DiMare 1986; Evans et al., 1984; Nakata et al., 1982; Nakata and Oishi 1980; Saksena and Mangiaracina 1983; Eguchi et al., 1996)

was carried out using $Zn(BH_4)_2$ (Gensler et al., 1960; Wiberg 1953). Although cyclization of this compound with formation of a hemiketal could give rise to the heterobicyclic system present in **3**, with a different functionalization, such process was not observed with compound **12**.

Biological activities.

Fungicidal activity. Table 1 shows the fungicidal activity, expressed as the percentage of growth inhibition against different agronomically important plant pathogens, of the new analogues.

At first sight, it is interesting to note that although none of the analogues were strongly effective inhibiting the growth of tested microorganisms [comparatively, the levels of activity are clearly lower than those of a conventional fungicide as benomyl (Table 1)], the data obtained in this report, together with those recently reported (Moya et al., *in press*) allowed us to establish preliminary structure-activity relationships.

Regarding the first approach, compound **7d** possessing the phenoxyphenyl substituent yielded the best fungicidal activity, as it showed growth inhibitions >50% for *C. gloesporoides, T. roseum* and *A. tenuis;* in addition, substantial inhibitions of other five fungal species were also obtained. The second phenoxyphenyl substituted product (**5d**), although considerably active against *C. gloesporoides* and *T. roseum*, did not exhibit percentages of inhibition >50%. This fact suggests that the double methylation in the β-ketoamide system enhances the fungicidal activity.

On the other hand, introduction of the cyclopropyl group resulted in an adverse effect on the activity; only compound **3c** showed an important activity against *V. dahliae*, which was still remarkably against *F. culmorum*, *C. coccodes* and *P. citrophthora*.

The second synthetic approach gave higher but more selective growth inhibitions. Compound **9**, possessing 3-pyrroline instead of pyrrolidine, was highly active against *C. coccodes* (aprox. 75%), showing moderate activity against other nine fungi.

Products obtained when pyrrolidine was substituted by 2-pyrrolidinone yielded different levels of activity. The best one, as regards the spectrum of affected fungi, was found with compound **11**. However, product **12** was particularly active against *C. coccodes*, showing significant differences with the latter compound. Thus, it seems that reduction of the ketone group selectively increases the activity against this fungal species. Finally, the lack of a methyl group between the carbonyls in these structures (compound **10**) produced a decreased fungicidal activity in all cases, suggesting that the methyl group, which likely provides rigidity to the molecule, is important confering activity to the products.

Insecticidal activity.

Only compounds possessing 3-pyrroline ring showed insecticidal activity. Product **9** was highly active against *O. fasciatus* exhibiting 100% mortality at a dose of 7.5 μg/cm^2; at lower doses the toxicity decresed considerably exhibiting 20% mortality at 5.0 μg/cm^2. Compound **8** was less active showing a percentage of mortality of 40% at the dose of 10 μg/cm^2.

The rest of compounds did not show activity in our assay conditions.

As mentioned above, important improvements in biological activities have been achieved either in this or in our previous paper (Moya et al., in press) based also on the synthesis of analogues using the active pyrroline natural products as starting points. Thus, the reported success of this approach, combined with the growing need to develop

new products for ecologically acceptable programs of pest control, makes this kind of work an attractive option for the biorational pesticide design.

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Table 1. Analogues Showing Fungicidal Activity.

	Radial Mycelial Growth Inhibition % (mean±SD)[a]												
Product	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*
3c	38.9±0.7^{A}	24.1±0.9^{A}	26.2±1.6^{A}	0	11.8±0.3^{A}	37.8±2.0^{A}	0	23.9±3.0^{AE}	65.4±1.2^{A}	37.5±3.3^{A}	0	23.6±4.2^{A}	11.3±1.6^{A}
5d	23.5±3.9^{CB}	23.1±1.6^{A}	17.4±2.7^{B}	12.3±2.5^{A}	41.4±1.3^{B}	37.6±1.5^{A}	46.6±3.4^{A}	39.5±0.8^{B}	19.0±3.3^{B}	25.9±4.2^{B}	30.7±1.2^{A}	22.2±3.9^{AB}	27.9±2.7^{B}
7d	16.3±3.7^{D}	34.8±3.2^{BC}	36.9±2.7^{C}	11.7±2.9^{A}	62.2±5.9^{C}	55.7±1.8^{B}	53.2±2.0^{B}	55.2±3.3^{C}	22.7±2.8^{B}	48.5±1.3^{C}	38.7±3.1^{B}	17.8±3.9^{B}	37.9±2.3^{C}
8	39.7±2.0^{A}	26.0±1.8^{A}	28.0±1.0^{AD}	23.9±0.5^{B}	29.9±2.6^{B}	18.8±2.8^{C}	38.6±3.0^{C}	21.1±4.1^{A}	13.9±2.5^{C}	18.3±2.5^{D}	54.0±4.0^{C}	29.9±2.0^{CD}	39.3±1.2^{C}
9	24.5±4.2^{C}	38.5±1.8^{C}	30.5±3.3^{DE}	0	35.4±2.4^{B}	76.7±5.4^{D}	34.3±1.7^{D}	51.4±7.5^{CD}	39.6±3.6^{D}	48.3±2.9^{C}	38.0±2.0^{B}	31.2±3.2^{CD}	19.3±1.2^{D}
10	8.2±1.0^{E}	35.5±5.1^{B}	27.6±1.7^{AD}	0	34.5±3.7^{B}	48.3±3.5^{E}	0	30.2±4.3^{E}	49.8±0.3^{F}	26.7±2.7^{B}	22.0±2.0^{D}	26.4±2.0^{AC}	21.3±2.3^{D}
11	18.1±1.9^{BD}	26.8±4.7^{A}	21.0±1.8^{B}	24.4±0.9^{B}	35.6±2.3^{B}	52.0±1.8^{BE}	49.5±1.8^{AB}	47.5±4.9^{D}	56.2±3.0^{E}	27.6±2.5^{B}	21.1±1.8^{D}	8.6±0.7^{E}	31.7±1.5^{E}
12	24.3±4.4^{C}	36.5±1.7^{BC}	33.3±1.7^{EC}	0	36.6±3.9^{B}	69.0±3.5^{F}	19.6±3.4^{E}	38.1±1.7^{B}	45.9±3.6^{F}	36.7±2.9^{A}	38.0±3.5^{B}	33.3±2.1^{D}	31.5±1.3^{E}
Benomyl	87.0±1.4	11.1±0.0	100	0	100	100	100	0	100	100	100	100	100

[a] values represent means ± standard deviations of growth inhibitions from three independent experiments. Assays concentration of analogues: 100 µg/mL; Benomyl concentration: 2.5 µg/mL. Within each column, mean values showing the same superscripts (A-F) are not significantly different (P>0.05). Target Plant Pathogens: *1*, *F. culmorum*; *2*, *F. oxysporun ssp gladioli*; *3*, *F. oxysporum ssp niveum*; *4*, *G. candidum*; *5*, *C. gloesporioides*; *6*, *C. coccodes*; *7*, *T. roseum*; *8*, *A. tenuis*; *9*, *V. dahliae*; *10*, *P. citrophthora*; *11*, *T. viride*; *12*, *P. italicum*; *13*, *A. parasiticus*.