Isolation and Synthesis of *N*-(2-Methyl-3-oxodec-8-enoyl)-2-pyrroline and 2-(Hept-5-enyl)-3-methyl-4-oxo-6,7,8,8a-tetrahydro-4*H*-pyrrolo[2,1-*b*]-1,3-oxazine – Two New Fungal Metabolites with in vivo Anti-Juvenile-Hormone and Insecticidal Activity

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Two new natural products, *N*-(2-methyl-3-oxodec-8-enoyl)-2-pyrroline (**2**) and 2-(hept-5-enyl)-3-methyl-4-oxo-6,7,8,8a-tetrahydro-4*H*-pyrrolo[2,1-*b*]-1,3-oxazine (**3**), have been isolated from *Penicillium brevicompactum* Dierckx. Compound **2** has shown an important in vivo anti-juvenile-hormone (anti-JH) activity while compound **3** has exhibited insecticidal activity against *Oncopeltus fasciatus* Dallas. Both products have been synthesized starting from 1,4-hexadiene, by means of a sequence of reactions which includes the preparation of 6-octenoic acid and its transformation into the corresponding acid chloride, in order to acylate Meldrum's acid. Subsequent aminolysis with pyrrolidine, followed by methylation at the activated position of the β-oxo amide with iodomethane, introduction of a methoxy group at the pyrrolidine ring by anodic oxidation and final elimination of methanol on SiO_2 led to **2** and **3**. The fact that both metabolites can be prepared by the same sequence indicates that they must be biogenetically related. Based on structural similarities, compounds **2** and **3** are also closely related to the recently discovered brevioxime (**1**).

Nowadays, the problems concerning environmental and human damage are important issues in pesticide research. There is a need to develop new methods of controlling plagues involving no harm to the enviroment and which are accepted as safe by the general public.[1] In this context, natural products are an important source of new substances with fungicidal, bactericidal or insecticidal activities. If their properties allow, and if sufficient quantities can be obtained (by isolation or by independent synthesis), such compounds may be used as agricultural chemicals, constituting an interesting alternative to the usual method for plague treatment. On the other hand, the new active metabolites can be taken as lead molecules for the synthesis of analogues with improved biological properties.[2]

Recently, different research groups have demonstrated that fungi are one of the most important sources of bioactive compounds. Thus, secondary metabolites of fungal origin exhibit a wide range of potentially useful biological activities.[3]

The *Penicillium*, *Aspergillus* and *Fusarium* genera are among the fungi with a high capability to produce metabolites toxic to insects.[4] In particular, *Penicillium brevicompactum* Dierckx has been shown to be one of the most prolific producers of secondary metabolites. These include mycophenolic acid and related compounds,[5] the Raistrick phenols,[6] the pebrolides,[7] or the *N*-benzoyl derivatives of phenylalanine, phenylalaninol and their ester, asperphenamate.[8] In addition, the fungus also produces brevigellina,[9] several piperazine-2,5-dione derivatives, a drimane diterpenoid,[10] the brevianamides[11] and compactin.[12] The latter is a reported hypocholesterolaemic agent that was shown to be a reversible, competitive inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase). Previous work on the effects of this product on insects has shown that it is able to produce a potent in vitro JH biosynthesis inhibition, with IC_{50} of the order of $10^{-7}-10^{-9}$ M in lepidoptera[13] and dictioptera.[14] However, as a general rule, the morphogenetic effects of compactin are rare. The last reported active metabolites obtained from *Penicillium brevicompactum* have been the adenophostins A and B; they have a potent action as agonists of the inositol 1,4,5-triphosphate receptor.[15]

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.[16] The structure of this compound presents an unusual heterobicyclic skeleton and an oxime functionality.

Now, we wish to report on the isolation, identification and alternative synthesis of two new natural products (**2** and **3**) from *P. brevicompactum*, with high in vivo anti-JH and insecticidal activities. Compound **2** can be converted into the bicyclic isomer **3** upon acid catalysis. As the basic skeletons of **1** and **3** are the same, it appears that the new natural products, **2** and **3**, can be biogenetically related to brevioxime.

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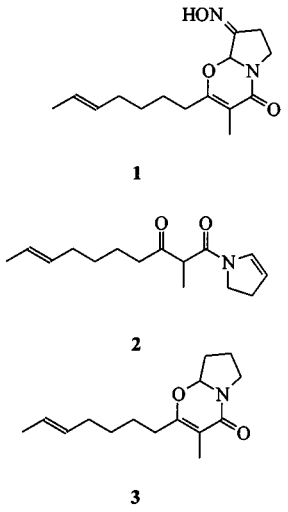
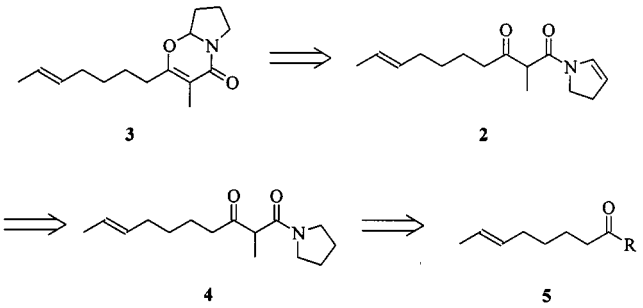


Results and Discussion

In order to confirm the structural assignment of the new natural products and to prepare higher amounts of both compounds for further biological assays, an alternative synthesis for these metabolites was planned.

As both compounds are metabolites isolated from the same fungus extract, and share the same molecular formula, it was considered reasonable to assume that metabolite **2** can undergo an intramolecular cyclization through the enolic form of the ketone group, providing its structural isomer **3**. On this basis we attempted the synthesis of both



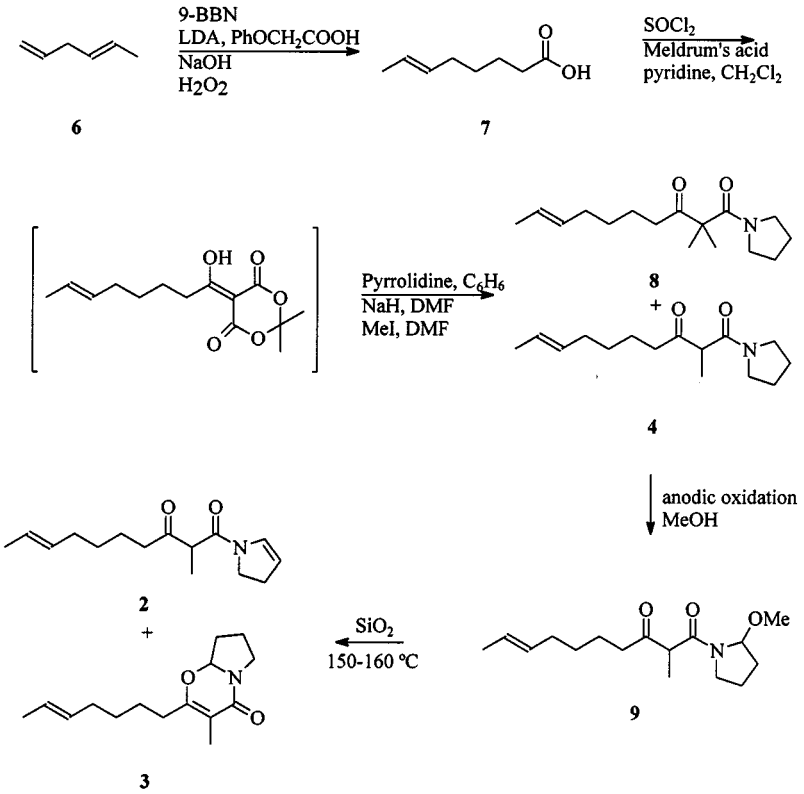
Scheme 1. Retrosynthetic analysis for the natural products **2** and **3**

metabolites using the same sequence of reactions. The designed retrosynthetic analysis is shown in Scheme 1.

This scheme involves initially elaboration of the required C_8 side chain synthon, which is used in the subsequent steps to build up the β-oxo amide system. Final oxidation of the pyrrolidine ring (eventually followed by cyclization) should afford the desired metabolites.

Thus, 6-octenoic acid was taken as a suitable starting material, because it contains a carbonyl group and an unsaturation at the adequate position. 6-Octenoic acid is not a commercial material, but it can be prepared according to procedures already described in the literature.[17]

Hara's method[17b] starts from 1,4-hexadiene which is transformed into the corresponding organoborane by treatment with 9-borabicyclo[3.3.1]nonane (9-BBN), a reagent which is highly regioselective for terminal bonds in the hydroboration of dienes.[18] Subsequent reaction with the



Scheme 2. Synthesis of enamide **2** and oxazine **3**

dianion of phenoxyacetic acid, heating at 66°C, basification with NaOH and final oxidation with H_2O_2 give the desired 6-octenoic acid.

As shown in Scheme 2, the next step was construction of the dicarbonylic system, which was achieved by conversion of the acid into 6-octenoyl chloride followed by reaction with Meldrum's acid.[19] The acylated Meldrum's acid intermediate, without further purification, was then submitted to aminolysis by reaction with pyrrolidine in refluxing benzene.[20] The resulting product was methylated by treatment with NaH and subsequent addition of iodomethane.[21] The major product was the monomethylated oxo amide **4** but the dimethylated analogue **8** was obtained as a by-product.

In order to dehydrogenate the pyrrolidine ring to a 2-pyrroline, anodic oxidation of the heterocyclic compound was carried out, using methanol as solvent. In this manner, a methoxy group was introduced at C-2.[22] A solution of β-oxo amide **4** was submitted to a constant electric current of 20 mA, using tetraethylammonium *p*-toluenesulfonate as a supporting electrolyte, until 4.0 F/mol had passed through the solution. Under these conditions, the methoxylated β-oxo amide **9** was obtained in a yield of 20%, and 67% of the starting β-oxo amide was recovered. The two diasteromers of **9** (**a** and **b**) were resolved by column chromatography. In solution they were present as a mixture of the two possible amide conformers, which gave separate signals in the NMR spectra.

Finally, elimination of methanol was achieved by adsorption of **9** on SiO_2 and subsequent heating at 150−160°C.[23] As anticipated, a mixture of the two isomeric natural products, **2** and **3** was obtained.

The synthetic natural products were assayed for insecticidal and anti-JH activities confirming those showed by the isolated products; a detailed biological study of these products, as well as their synthetic precursors, will be reported on shortly.

Conclusions

N-(2-Methyl-3-oxodec-8-enoyl)-2-pyrroline (**2**) and 2-(hept-5-enyl)-3-methyl-4-oxo-6,7,8,8a-tetrahydro-4*H*-pyrrolo-[2,1-*b*]-1,3-oxazine (**3**) are two new natural products isolated from *Penicillium brevicompactum*. Their structures have been assigned based on spectral data and unambiguously confirmed by alternative syntheses. Compound **2** and its bicyclic isomer **3** arise from the common methoxylated precursor **9** in the synthetic sequence; this reveals that **2** and **3** can be biogenetically related to each other and to recently discovered brevioxime (**1**). The biological activities of **2** and **3** and their synthetic intermediates suggest that these compounds can be useful as lead molecules for the development of new biorational pesticides.

Experimental Section

General: All chemicals were obtained from commercial suppliers and used without further purification. − IR: As liquid films with a Perkin-Elmer 781 spectrometer (or as KBr discs for the natural products with a Nicolet 710 FT-IR spectrometer); ν_{max} is given for the main absorption bands. − ^1H and ^{13}C NMR: Recorded in $CDCl_3$ with a Varian Gemini spectrometer at 300 and 75 MHz (or with a Varian Unity spectrometer at 400 and 100 MHz for the natural products); chemical shifts are reported in δ (ppm) values, using TMS as internal standard. − Mass spectra: Under electron impact with a Hewlett−Packard 8988A spectrometer; the ratios *m/z* and the relative intensities are reported. − HRMS: Recorded with a Fisons VG Autospec spectrometer (GC 8000). − Isolation and purification: Flash column chromatography on silica gel 60 (230−400 mesh). − Analytical TLC: Precoated plates (silica gel 60 F_{254}), spots were visualized with UV light and in an I_2 chamber.

Isolation and Characterization of the Active Compound: The procedure was similar to that previously reported for brevioxime.[16a] 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 slants with potato dextrose agar (PDA) as culture medium. The strain was seeded in Petri dishes with PDA culture medium and incubated for 7 d 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 d 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 with $CaCl_2$, filtered and concentrated in vacuo. The residue (2.0 g from 20 L of culture) was submitted to column chromatography on silica-gel (1:60, w/w) using mixtures of CH_2Cl_2, AcOEt, Me_2CO and MeOH (stepwise gradient) as eluent. This led to the separation of 20 fractions. Using the method of Bowers et al[24] it was possible to localize a significant biological activity in fractions number 8 (in vivo anti-JH activity) and 9 (insecticidal activity).

***N*-(2-Methyl-3-oxodec-8-enoyl)-2-pyrroline (2):** Preparative HPLC chromatographic resolution of fraction 8 was achieved using the following conditions: Lichrosorb Si-60, 7 μm (25.0 × 2.5 cm) column; mobile phase hexane/AcOEt (70:30, v/v); flow 8 mL/min; detection by UV (254 nm) and refraction index, simultaneously. A fraction was obtained (R_t = 25.9 min) consisting of 10.3 mg of the pure active compound, whose structure was tentatively assigned to be *N*-(2-methyl-3-oxodec-8-enoyl)-2-pyrroline (**2**) on the basis of spectral data. − HRMS (CI); *m/z* (%): 250.1807 ($C_{15}H_{23}NO_2$ requires 250.1807). − IR (KBr): $\tilde{\nu}_{max}$ = 2997 cm^{-1}, 2850, 1716 (C=O), 1638 (C=O), 1620, 1455, 1420, 1370, 1276, 1122, 1067, 962, 833, 708, 457. − ^1H NMR (400 MHz, $CDCl_3$. 25°C, TMS): δ_H = 6.9 and 6.5 (m + m, 1 H, 2-H), 5.4 (m, 2 H, 8′-H + 9′-H), 5.3 (m, 1 H, 3-H), 3.9 (m, 2 H, 5-H), 3.6 and 3.5 (q + q, $^3J_{H,H}$ = 7 Hz, 1 H, 2′-H), 2.8 and 2.6 (m + m, 2 H, 4′-H), 2.5 (m, 2 H, 3-H), 2.0 (m, 2 H, 7′-H), 1.7 and 1.6 (m + m, 3 H, CH=CHC*H*$_3$), 1.5 (m, 2 H, 5′-H), 1.4 (d + d, $^3J_{H,H}$ = 7 Hz, 3 H, CHC*H*$_3$), 1.3 (m, 2 H, 6′-H). − ^{13}C NMR (100 MHz, $CDCl_3$. 25°C, TMS): δ_C = 207.2 (C-3′), 165.9 (C-1′), 131.0 (C-8′), 129.5 and 128.5 (C-2), 125.2 (C-9′), 113.1, 111.6 (C-3), 53.4 (C-2′), 45.5 (C-5), 39.2 (C-4′), 32.3, 29.0, 28.2, 23.1 (C-4, C-5′−C-7′), 17.9, 13.2 (2 × CH_3). − MS (70 eV, EI); *m/z* (%): 249 (1) [M^+], 180 (2), 167 (1), 125 (6), 96 (3), 81 (5), 69 (100), 68 (23), 55 (22), 41 (11). − The compound showed a significant anti-JH activity, with a dose required of 2.0 μg/nymph for induction of precocious metamorphosis in 90% of newly molted fourth-instar nymphs of *Oncopeltus fasciatus*, when applied topically.

2-(Hept-5-enyl)-3-methyl-4-oxo-6,7,8,8a-tetrahydro-4*H*-pyrrolo[2,1-*b*]-1,3-oxazine (3): Preparative HPLC chromatographic resolution of fraction 9 was achieved using the following conditions: Spherisorb W, 5 μm (25.0 × 0.8 cm) column; mobile phase hexane/AcOEt (45:55, v/v); flow 1.4 mL/min; detection by UV (254 nm). A fraction was obtained (R_t = 33.4 min) consisting of 18.1 mg of the pure active compound, whose structure was tentatively assigned to be 2-(hept-5-enyl)-3-methyl-4-oxo-6,7,8,8a-tetrahydro-4*H*-pyrrolo[2,1-*b*]-1,3-oxazine (**3**) on the basis of spectral data. − HRMS (CI); *m/z* (%): 250.1789 ($C_{15}H_{23}NO_2$ requires 250.1793). − IR (KBr): $\tilde{\nu}_{max}$ = 2929 cm^{-1}, 2880, 2858, 1654 (C=O), 1436, 1372, 1344, 957, 759. − ^1H NMR (400 MHz, $CDCl_3$, 25°C, TMS): δ_H = 5.4 (m, 2 H, 5′-H + 6′-H), 5.2 (dd, $^3J_{H,H}$ = 6 and 5 Hz; 1 H, 8a-H), 3.7 and 3.4 (m + m, 2 H, 6-H), 2.3 (m, 2 H, 1′-H), 2.2 (m, 2 H, 8-H), 2.0 (m, 2 H, 4′-H), 1.9 (m, 2 H, 7-H), 1.8 (s, 3 H, CH_3), 1.6 (m, 3 H, CH=CHCH_3), 1.5 (m, 2 H, 2′-H), 1.4 (m, 2 H, 3′-H). − ^{13}C NMR (100 MHz, $CDCl_3$, 25°C, TMS): δ_C = 163.9 (C-2), 163.8 (C-4), 131.2 (C-5′), 125.3 (C-6′), 106.7 (C-3), 87.7 (C-8a), 44.3 (C-6), 32.2, 31.8, 30.5, 29.2, 26.3, 21.8 (C-7, C-8, C-1′−C-4′), 17.8, 10.0 (2 × CH_3). − MS (70eV, EI); *m/z* (%): 249 (1) [M^+], 221 (1), 206 (1), 180 (2), 167 (6) 164 (6), 152 (6), 138 (6), 137 (6), 126 (3), 125 (6), 111 (13), 98 (15), 83 (49), 70 (100), 69 (11), 55 (23), 41 (22). − The product exhibited a 50% mortality against *O. fasciatus* when assayed, by topical application, on newly molted fourth-instar nymphs, at 20 μg/nymph.

Synthesis of *N*-(2-Methyl-3-oxodec-8-enoyl)2-pyrroline and 2-Hept-5-enyl-3-methyl-4-oxo-6,7,8,8a-tetrahydro-4*H*-pyrrolo[2,1-*b*]-1,3-oxazine

6-Octenoic Acid (7)

Preparation of the Organoborane Compound: A flame-dried, N_2-flushed, round-bottomed flask equipped with a magnetic stirring bar was charged with dry THF (100.0 mL) at 0°C, and then 1,4-hexadiene (10.0 g, 121.7 mmol) was added. Thereafter, a 0.5 M solution of 9-BBN in dry THF (250.0 mL, 125.0 mmol) was added slowly by double-ended needle technique. The mixture was then stirred at room temp. for 6 h 30 min.

Preparation of the Dianion of Phenoxyacetic Acid: A flask equipped with a magnetic stirring bar and reflux condenser was flame-dried and cooled to 0°C under nitrogen and then charged with a 2.0 M solution of LDA in THF/heptane/ethylbenzene (47:43:10) (250.0 mL, 500.0 mmol). The solution was cooled to 0°C and then phenoxyacetic acid (37.0 g, 243.5 mmol) in THF (200.0 mL) was added by double-ended needle technique. The reaction mixture was warmed to room temp. and stirred for 4 h.

Reaction of the Organoborane with the Dianion of Phenoxyacetic Acid: The organoborane was added to the dianion of phenoxyacetic acid by double-ended needle technique at 0°C, and then the reaction mixture was stirred at 66°C for 14 h 30 min. After this period, the mixture was re-cooled to 0°C and 3 M NaOH (125.0 mL) followed by 35% H_2O_2 (110.0 mL) was slowly added. The reaction mixture was stirred for 3 h at 0°C and then washed with diethyl ether. The separated aqueous layer was acidified with HCl (5%) and then extracted with diethyl ether. The combined organic extracts were washed with brine, dried and concentrated to dryness. Chromatography of the residue on silica gel (gradient elution with mixtures of EtOAc and hexane, between 0 and 20% of the former) provided 6-octenoic acid (**7**) (8.4 g, 49%) as a brown oil. − IR (film): $\tilde{\nu}_{max}$ = 3600 cm^{-1} (COO−H), 1705 (C=O), 1590, 1405, 1280, 1230, 1100, 960, 810, 750, 690, 660. − ^1H NMR (300 MHz, $CDCl_3$, 25°C, TMS): δ_H = 10.2 (br. s, 1 H, OH), 5.4 (m, 2 H, 6-H + 7-H), 2.3 (t, $^3J_{H,H}$ = 8 Hz, 2 H, 2-H), 2.0 (m, 2 H, 5-H), 1.6 (m, 5 H, 3-H + 8-H), 1.4 (m, 2 H, 4-H). − ^{13}C NMR (75 MHz, $CDCl_3$, 25°C, TMS): δ_C = 180.4 (C-1), 130.7 (C-6), 125.2 (C-7), 33.9, 32.1(C-2 + C-5), 28.8, 24.1 (C-3 + C-4), 17.9 (C-8). − MS (70 eV, EI); *m/z* (%): 142 (8) [M^+], 124 (35), 113 (4), 109 (4), 106 (2), 100 (13), 96 (50), 95 (15), 87 (17), 83 (47), 82 (100), 81 (30), 79 (13), 73 (34), 68 (39), 67 (91), 60 (39), 56 (25).[17b]

***N*-(2-Methyl-3-oxodec-8-enoyl)pyrrolidine (4):** 6-Octenoic acid (1.24 g, 7.5 mmol) and thionyl chloride (1.3 mL, 17.6 mmol) were stirred overnight at room temp. Excess of chloride was removed by vacuum distillation. The resulting acid chloride was added dropwise to a cooled solution (0°C) of 2,2-dimethyl- 1,3-dioxane-4,6-dione (1.32 g, 9.0 mmol) and pyridine (1.5 mL, 17.9 mmol) in dichloromethane (8.0 mL) by double-ended technique under nitrogen. The solution was stirred at 0°C for 1 h 45 min; thereafter, it was allowed to warm up to room temp. over an additional period of 2 h 35 min. The dichloromethane solution was washed with dilute HCl, water and brine, dried and concentrated to dryness, to give the acylated Meldrum′s acid, which was used for the aminolysis without further purification. A solution of the acylated Meldrum′s acid and pyrrolidine (1.2 mL, 14.9 mmol) in benzene (40.0 mL) was refluxed for 13 h 30 min. The solvent was evaporated under reduced pressure and the residue was purified by column chromatography on silica gel (gradient elution with mixtures of EtOAc and hexane, between 20 and 40% of the former) to obtain a viscous oil which was used directly in the next reaction. To a stirred slurry of prewashed NaH (60% dispersion in oil; 28 mg, 0.7 mmol) in DMF (2.50 mL) at 0°C was added dropwise a solution of the previously obtained oil (148 mg,) in DMF (2.50 mL) by double-ended needle. After the hydrogen evolution had ceased, the mixture was warmed to room temp., stirred for 1 h 40 min and then re-cooled to 0°C. Then iodomethane (0.05 mL, 0.8 mmol) was added. After being stirred at room temp. for 5 h 50 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. Chromatography of the residue on silica gel (gradient elution with mixtures of EtOAc and hexane, between 15 and 30% of the former) provided the desired monomethylated β-oxo amide **4** (86 mg, 55%) as a yellow oil and the dimethylated compound **8** (13 mg, 8%) as a secondary product

***N*-(2-Methyl-3-oxodec-8-enoyl)pyrrolidine (4):** HRMS (EI); *m/z* (%): 251.1890 ($C_{15}H_{25}NO_2$ requires 251.1885). − IR (film): $\tilde{\nu}_{max}$ = 2910 cm^{-1}, 2860, 1710 (C=O), 1630 (C=O), 1450, 1430, 960. − ^1H NMR (300 MHz, $CDCl_3$, 25°C, TMS): δ_H = 5.4 (m, 2 H, 8′-H + 9′-H), 3.4 (m, 5 H, 2-H + 5-H + 2′-H), 2.5 (m, 2 H, 4′-H), 2.0−1.8 (m, 6 H, 3-H + 4-H + 7′-H), 1.6 (m, 3 H, CH=CHCH_3), 1.5 (m, 2 H, 5′-H), 1.3 (d, $^3J_{H,H}$ = 7 Hz, 3 H, CHCH_3), 1.4−1.2 (m, 2 H, 6′-H). − ^{13}C NMR (75 MHz, $CDCl_3$. 25°C, TMS): δ_C = 207.4 (C-3′), 168.5 (C-1′), 130.9 (C-8′), 125.0 (C-9′), 53.2 (C-2′), 46.8 (C-2), 46.1 (C-5), 39.2 (C-4′), 32.3, 28.9, 26.1, 24.2, 23.0 (C-3, C-4, C-5′−C-7′), 17.9, 13.3 (2 × CH_3). − MS (70 eV, EI); *m/z* (%): 251 (18) [M^+], 236 (17), 222 (11), 210 (4), 207 (7), 196 (8), 182 (99), 169 (34), 154 (12), 152 (13), 140 (6), 127 (93), 126 (100), 113 (41), 98 (99), 81(18), 70 (92), 55 (97).

***N*-(2,2-Dimethyl-3-oxodec-8-enoyl)pyrrolidine (8):** HRMS (EI); *m/z* (%): 265.2043 ($C_{16}H_{27}NO_2$ requires 265.2041). − IR (film): $\tilde{\nu}_{max}$ = 2920 cm^{-1}, 2860, 1700 (C=O), 1620 (C=O), 1395, 1355, 960. − ^1H NMR (300 MHz, $CDCl_3$, 25°C, TMS): δ_H = 5.4 (m, 2 H, 8′-H + 9′-H), 3.5 (t, $^3J_{H,H}$ = 7 Hz, 2 H, 2-H), 3.1 (t, $^3J_{H,H}$ = 7 Hz, 2 H, 5-H), 2.4 (t, $^3J_{H,H}$ = 7 Hz, 2 H, 4′-H), 1.9 (m, 2 H, 7′-H), 1.8 (m, 4 H, 3-H + 4-H), 1.6 (m, 3 H, CH=CHCH_3), 1.5 (m, 2 H, 5′-H), 1.3 [s, 6 H, C(CH_3)$_2$], 1.4−1.2 (m, 2 H, 6′-H). − ^{13}C NMR (75 MHz, $CDCl_3$, 25°C, TMS): δ_C = 210.6 (C-3′), 170.9 (C-1′), 130.8 (C-8′), 125.2 (C-9′), 56.3 (C-2′), 47.4 (C-2), 46.6 (C-5), 37.7 (C-4′),

32.3, 29.0, 26.6, 23.5, 23.3 (C-3, C-4, C-5′−C-7′), 22.3, 17.9 (3 × CH_3). − MS (70 eV, EI); *m/z* (%): 265 (24) [M^+], 250 (7), 221 (1), 196 (20), 183 (6), 166 (4), 141 (100), 140 (62), 126 (30), 113 (56), 98 (100), 81 (15), 70 (46), 55 (74).

2-Methoxy-*N*-(2-methyl-3-oxodec-8-enoyl)pyrrolidine (9): A solution of β-oxo amide **7** (144 mg, 0.6 mmol) in methanol (20.0 mL) containing tetrabutylammonium *p*-toluenesulfonate (431 mg, 1.4 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 had 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 layers were dried with 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 EtOAc 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, using a hexane/EtOAc mixture (8:2) as eluent, to afford two diastereomers of the methoxylated β-oxo amide **9** (17 + 14 mg, 20%) as an oil. Some unreacted β-oxo amide **7** (90 mg, 0.4 mmol) was recovered**.**

9a: HRMS (CI); *m/z* (%): 282.2066 (M + H^+; $C_{16}H_{28}NO_3$ requires 282.2069). − IR (film): $\tilde{\nu}_{max}$ = 2920, 2880, 1710 (C=O), 1630 (C=O), 1390, 1230, 1150, 950 cm^{-1}. − ^{1}H NMR (300 MHz, $CDCl_3$, 25°C, TMS): δ_H = 5.5 and 5.0 (d + d, $^3J_{H,H}$ = 5 Hz, 1 H, 2-H), 5.4 (m, 2 H, 8′-H + 9′-H), 3.7−3.3 (m, 3 H, 5-H + 2′-H), 3.4 and 3.3 (s + s, 3 H, OMe), 2.5 (m, 2 H, 4′-H), 2.2−2.1 (m, 2 H, 7′-H), 2.0−1.8 (m, 4 H, 3-H + 4-H), 1.6 (m, 3 H, CH=CHCH_3), 1.5 (m, 2 H, 5′-H), 1.4, 1.3 (d + d, $^3J_{H,H}$ = 7 Hz, 3 H, CHCH_3), 1.4−1.2 (m, 2 H, 6′-H). − ^{13}C NMR (75 MHz, $CDCl_3$, 25°C, TMS): δ_C = 208.2, 206.9 (C-3′), 170.6, 169.9 (C-1′), 130.9 (C-8′), 125.1 (C-9′), 88.5, 87.2 (C-2), 56.7, 54.1 (OMe), 53.2, 53.1 (C-2′), 46.2, 46.0 (C-5), 39.3, 38.9 (C-4′), 32.3, 31.4, 30.6, 28.9, 23.0, 22.9, 20.8 (C-3, C-4, C-5′−C-7′), 17.9, 14.0, 13.2 (2 × CH_3). − MS (70 eV, EI); *m/z* (%): 282 (38) [M^+ + 1], 266 (4), 250 (100) and 249 (30).

9b: HRMS (CI); *m/z* (%): 282.2077 (M + H^+; $C_{16}H_{28}NO_3$ requires 282.2069). − IR (film): $\tilde{\nu}_{max}$ = 2910 cm^{-1}, 2880, 1700 (C=O), 1640 (C=O), 1400, 1360, 1055. − ^{1}H NMR (300 MHz, $CDCl_3$, 25°C, TMS): δ_H = 5.4, 4.9 (d + d, $^3J_{H,H}$ = 5 Hz, 1 H, 2-H), 5.4 (m, 2 H, 8′-H + 9′-H), 3.8−3.3 (m, 3 H, 5-H + 2′-H), 3.4, 3.2 (s + s, 3 H, OMe), 2.5 (m, 2 H, 4′-H), 2.2−2.1 (m, 2 H, 7′-H), 2.0−1.8 (m, 4 H, 3-H + 4-H), 1.6 (m, 3 H, CH=CHCH_3), 1.5 (m, 2 H, 5′-H), 1.4, 1.3 (d + d, $^3J_{H,H}$ = 7 Hz, 3 H, CHCH_3), 1.4−1.2 (m, 2 H, 6′-H). − ^{13}C NMR (75 MHz, $CDCl_3$, 25°C, TMS): δ_C = 207.2, 206.5 (C-3′), 170.9, 170.7 (C-1′), 131.1, 130.9 (C-8′), 125.1, 124.9 (C-9′), 88.8, 87.5 (C-2), 56.7 (OMe), 53.7, 52.4 (C-2′), 46.3, 46.0 (C-5), 39.7, 39.2 (C-4′), 32.3, 32.2, 30.5, 28.9, 23.0, 22.9, 21.0 (C-3, C-4, C-5′−C-7′), 17.9, 14.0, 13.4 (2 × CH_3). − MS (70 eV, EI); *m/z* (%): 282 (10) [M^+ + 1], 266 (22), 250 (100), 249 (37).

***N*-(2-Methyl-3-oxodec-8-enoyl)2-pyrroline (2) and 2-(Hept-5-enyl)-3-methyl-4-oxo-6,7,8,8a-tetrahydro-4*H*-pyrrolo[2,1-*b*]-1,3-oxazine (3):** A mixture of the two diastereomeric methoxy amides **11** (19 mg, 0.07 mmol) and silica gel (6 mg, 0.10 mmol) was heated at 150−160°C in a flask under reduced pressure and nitrogen. After 5 h, water was added to the residue and the slurry was extracted with CH_2Cl_2. The combined organic layers were dried with anhydrous sodium sulfate. Then, the drying agent was removed by filtration, the solvent was evaporated to dryness and the residue was purified by column chromatography on silica gel (gradient elution with mixtures of EtOAc and hexane between 20 and 30% of the former) to provide, in order of elution, the enamine **2** (2 mg, 12%) and the bicyclic enone **3** (10 mg, 59%). Both compounds were oils, which showed spectral data identical to those reported above for the natural products.

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