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Insecticidal Activity of Paraherquamides, Including Paraherquamide H and Paraherquamide I, Two New Alkaloids Isolated from *Penicillium cluniae*

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ABSTRACT

Paraherquamide H (**1**) and paraherquamide I (**2**), two new compounds of the paraherquamide family (PHQ), together with the already known paraherquamide A (**3**), paraherquamide B (**4**), paraherquamide E (**5**), VM55596 (N-oxide paraherquamide) (**6**), paraherquamide VM55597 (**7**), and five known dikepiperazines (**8**-**12**) (**Figures 1** and **2**) (*1*-*6*) have been isolated from the culture broth of *Penicillium cluniae* Quintanilla. The structure of **1** and **2,** based on NMR and MS analysis, was established. It is worth noticing that, in both cases, an unusual oxidative substitution in C-16 was found, which had only previously been detected in PHQ **7** (*4*).

Isolated compounds were tested for insecticidal activity against the hemipteran *Oncopeltus fasciatus* Dallas. Mortality data have allowed preliminary structure activity relationships to be proposed. The most potent product was **5** with a LD_{50} of 0.089 μg/nymph.

KEYWORDS: *Penicillium cluniae*, paraherquamides, paraherquamide H and paraherquamide I, marcfortines, brevianamides, diketopiperazines, insecticide activity, *Oncopeltus fasciatus.*

INTRODUCTION

Following our interest in the search for biologically active metabolites from fungal origin, we have investigated the culture broth of *Penicillium cluniae*, due to its potent insecticidal activity.

The study of this active extract has led to the isolation of several active compounds belonging to the paraherquamide family (PHQ). These compounds have been previously isolated as fungal metabolites (**3**-**7**) (**Figure 1**) (*1*-*4*), but they have never been reported as *P. cluniae* metabolites. Among isolated compounds, two new paraherquamides, named H (**1**) and I (**2**), have been described.

The first known PHQ, paraherquamide A (**3**), was isolated in 1980 from *Penicillium paraherquei* (*1*). Since then several PHQ belonging to other *Penicillium* species (*2*-*4*, *7*) have been found. On the other hand, several compounds possessing a similar skeleton have also been isolated from fungal sources: brevianamides (*8*,*9*), marcfortines (*10*,*11*), sclerotiamide (*12*), aspergillimides (*13*), asperparalines (*14*,*15*), avrainvillamide (*16*), etc. All of them, including PHQ, have shown a potent antiparasitic activity, especially as antihelmintic compounds (*17*-*21*).

The unusual structures of these oxindole alkaloids and their interesting biological activities have attracted the attention of synthetic chemists. Now several routes are proposed for the synthesis of PHQ and other related compounds (*18*-*28*).

Furthermore, five known diketopiperazines (DKP) have been isolated in this *P. cluniae* extract (**8**-**12**) (**Figure 2**) (*5*,*6*). According to several studies these DKP would be intermediates in the biosynthesis of PHQ and other related compounds (*12*,*27*).

Here we report on the isolation and identification of the two newly discovered PHQ. In addition, insecticidal activity of the PHQ has been evaluated in order to establish preliminary structure-activity relationships.

MATERIALS AND METHODS

General Experimental Procedures. IR spectra were obtained with a 710FT spectrophotometer (Nicolet, Madison, Wisconsin, USA). UV spectrum was obtained using a Shimadzu UV-2101PC spectrophotometer (Kyoto, Japan). ^{1}H, ^{13}C and COSY H-H NMR spectra were recorded on a Bruker AV 300 MHz instrument (Rheinstetten, Germany). The assignment of ^{13}C signals is supported by DEPT experiments. For HSQC and HMBC NMR experiments a Bruker 600 spectrometer (Rheinstetten, Germany) was used. HRESIMS was carried out on a Micromass Q-TOF micro (Milford, USA). TLC was run on silica gel F_{254} precoated plates (Merck) and spots were detected under UV light. Isolation and purification of **1-12** was carried by a Waters HPLC system, with a 600 pump and a 2996 Photodiode Array Detector (Milford, USA).

Biological Material. The fungus, *Penicillium cluniae* Quintanilla (CECT 2888), was provided by the "Colección Española de Cultivos Tipo (CECT)" of the Universidad de Valencia. The strain was seeded in Petri dishes containing PDA culture medium and incubated for 7 days at 28 ºC. Then a solution of Tween 80 (0.05%) in sterile distilled water was used to obtain a suspension containing ca. 10^6 conidia/ mL. This suspension was poured to an Erlenmeyer flask containing antibiotic test broth (1:9 volume ratio). The mixture was incubated for 15 days, in the dark, at 25 ºC.

Extraction and Preliminary Fractionation. After incubation the mycelium was removed from the culture broth by filtration. Then the broth (30 L) was partially evaporated in vacuum to 1 L and it was extracted with CH_2Cl_2/AcOEt 1:1 (3 x 1 L). The resulting organic extract was dried under reduced pressure to obtain a brown solid (7.03 g) which exhibited acute insecticidal activity and was partitioned by flash column chromatography on Silica gel (1:100, w/w) using stepwise gradient from CH_2Cl_2; CH_2Cl_2/EtOAc (9:1, 7:3, 5:5, and 3:7 (v/v)); EtOAc; EtOAc/MeOH (9:1, 8:2, 7:3, and 5:5 (v/v)); MeOH. The volume eluted in each step was 3.5 L, and eleven fractions, one from each step, were separated. These fractions were evaporated to dryness and tested

for insecticidal activity. Two fractions, F-9 (96.8 mg) and F-10 (152.6 mg) were active against *O. fasciatus* and were systematically studied in the search for insecticidal metabolites.

Isolation, Purification and Characterization of Compounds. The fraction F-9 was subjected to flash chromatography on Silica gel (1:100, w/w) using as mobile phase CH_2Cl_2/MeOH 94:6. This mixture was eluted and collected in aliquots of 3 ml, which were pooled in eleven fractions according to their similarity by TLC. Subfractions 1 (F9-1) and 2 (F9-2) showed insecticidal activity and were analyzed by HPLC.

Semipreparative HPLC of F9-1 (29.3 mg) was performed using the following conditions: Spherisorb ODS2 C18 column, 5 μm (25.0 x 0.7 cm); mobile phase MeOH/H_2O (70:30, v/v); flow, 1 mL/min; detection by Photodiode Array. Compounds **6** and **8-12** were isolated and then purified by analytical HPLC with an Explore Luna (Phenomenex) C18 column, 5 μm (25.0 x 0.46 cm); mobile phase MeOH/H_2O (70:30, v/v); flow, 0.5 mL/min; detection by Photodiode Array. Their chromatographic properties were the following: compound **8** (*cyclo*-*L*-Pro-*L*-Tyr): [Rt= 5.9 minutes; 4.9 mg], compound **9** (*cyclo*-*L*-Pro-*L*-Val): [Rt= 6.5 minutes, 5.5 mg], compound **10** (*cyclo*-*L*-Pro-*L*-Ile): [Rt= 7.2 minutes; 1.4 mg], compound **11** (*cyclo*-*L*-Pro-*L*-Leu): [Rt= 7.3 minutes; 1.6 mg], compound **12** (*cyclo*-*L*-Pro-*L*-Phe): [Rt= 7.6 minutes; 3.4 mg], and compound **6** (paraherquamide VM55596): [Rt= 11.1 minutes; 3.3 mg].

Semipreparative HPLC of F9-2 (8.4 mg) was carried out using the same conditions as those described for F9-1. Compound **2** (paraherquamide I) was isolated and then purified using the analytical HPLC column and chromatographic conditions described above. The properties of **2** were: [Rt= 14.5 minutes; 0.7 mg].

F-10 was directly subjected to semipreparative HPLC, also employing the above conditions, and compounds **1**, **3**, **4**, **5,** and **7** were isolated. These compounds were subsequently purified by the above-mentioned analytical HPLC conditions. The

chromatographical results were as follows: compound **1** (paraherquamide H): [Rt= 18.0 minutes; 0.5 mg], compound **3** (paraherquamide A): [Rt= 18.7 minutes; 2.7 mg], compound **4** (paraherquamide B): [Rt= 22.4 minutes; 1.8 mg], compound **5** (paraherquamide E): [Rt= 33 minutes; 2.1 mg], and compound **7** (paraherquamide VM55597): [Rt= 18.9 minutes; 1.1 mg].

Paraherquamide H (**1**) was obtained as a colorless amorphous substance with $[\alpha]_D$ – 30.0º (c 0.03, MeOH); UV (MeOH) λ_{max} (log ε) 222 (1.00) nm; IR (film) ν_{max} 1736, 1706, 1669, 1459, 1188, 1081, 968 cm^{-1}; ^1H (600 MHz, $CDCl_3$) and ^{13}C (75 MHz, $CDCl_3$) NMR data (see Table 1); HRESIMS *m/z* found 514.2292 (514.2318 calculated for $C_{28}H_{33}N_3O_5Na$). NOESY correlations: H-4 to H-5, H-12a, and C$\underline{H}_3$-22; H-10a to H-10b, and H-12a; H-12b to H-12a, H-20, and C$\underline{H}_3$-23; H-14 to H-19a; C$\underline{H}_3$-17 to H-15b and H-19b; H-20 to H-12b, H-19b, and C$\underline{H}_3$-23; C$\underline{H}_3$-22 to H-4 and C$\underline{H}_3$-23; H-25 to H-24 and C$\underline{H}_3$-28.

Paraherquamide I (**2**) was obtained as a colorless amorphous substance with $[\alpha]_D$ – 25.1º (c 0.04, MeOH); UV (MeOH) λ_{max} (log ε) 225 (1.01) nm; IR (film) ν_{max} 1740, 1716, 1674, 1470, 1178, 1081, 968 cm^{-1}; ^1H (600 MHz, $CDCl_3$) NMR data: 6.82 (H-4, d, $J_{4\text{-}5}$=8.2 Hz), 6.72 (H-5, d, $J_{4\text{-}5}$=8.2 Hz), 6.31 (H-24, d, $J_{24\text{-}25}$=7.2 Hz), 5.90 (H-15, s), 4.90 (H-25, d, $J_{24\text{-}25}$=7.2 Hz), 3.91 (H-12a, d, $J_{12a\text{-}12b}$=12.0 Hz), 3.60 (H-12b, d, $J_{12a\text{-}12b}$=12.0 Hz), 3.36 (H-20, dd, $J_{20\text{-}19}$=10.2 Hz), 3.07 (C-29, s), 2.85 (H-10a, d, $J_{10a\text{-}10b}$=16.2 Hz), 2.32 (H-19a, m), 2.26 (H-17, s), 2.05 (H-10b, d, $J_{10a\text{-}10b}$=16.2 Hz), 1.51 (H-19b, m), 1.46 (H-27, s), 1.44 (H-28, s), 1.10 (H-22, s), 0.95 (H-23, s). ^{13}C (75 MHz, $CDCl_3$) NMR data: 182.0 (C-2), 171.0 (C-16), 169.0 (C-18), 159.2 (C-14), 147.6 (C-6), 140.0 (C-24), 135.4 (C-7), 133.1 (C-8), 125.5 (C-15), 125.0 (C-9), 121.1 (C-4), 118.9 (C-5), 116.0 (C-25), 80.1 (C-26), 70.2 (C-13), 66.5 (C-11), 62.8 (C-3), 52.7 (C-20), 48.0 (C-12), 46.5 (C-21), 37.2 (C-10), 30.2 (C-27), 30.0 (C-28), 27.5 (C-19), 25.9 (C-29), 24.0 (C-23), 21.0 (C-22), 14.9 (C-17). ^1H-^1H COSY correlations: H-25 to H-24;

155 C$\underline{H}_3$-17 to H-15; H-20 to H-19a and H-19b; H-19a to H-19b; H-12a to H-12b; H-10a to H-10b; H-5 to H-4. ^{1}H-^{13}C HMBC correlations: C-2 to H-10a and H-10b; C-6 to H-4; C-7 to H-24 and H-5; C-8 to H-4; C-9 to H-5; C-14 to C$\underline{H}_3$-17; C-15 to C$\underline{H}_3$-17; C-18 to H-15 and C$\underline{H}_3$-29; C-24 to H-25; C-25 to H-24; C-26 to H-24; C-13 to C$\underline{H}_3$-17 and H-15; C-11 to H-10a, H-12b, H-20, and C$\underline{H}_3$-29; C-13 to H-10a, H-10b, C$\underline{H}_3$-22, and C$\underline{H}_3$-
160 23; C-20 to C$\underline{H}_3$-22 and C$\underline{H}_3$-23; C-12 to H-10b; C-21 to H-10a, H-20, C$\underline{H}_3$-22, and C$\underline{H}_3$-23; C-23 to C$\underline{H}_3$-22; C-22 to C$\underline{H}_3$-23. HRESIMS *m/z* found 512.2159 (512.2161 calculated for $C_{28}H_{31}N_3O_5Na$). NOESY correlations: H-4 to H-5, H-12a, and C$\underline{H}_3$-22; H-10a to H-10b and H-12a; H-12b to H-12a and C$\underline{H}_3$-23; C$\underline{H}_3$-17 to H-19b; H-20 to H-12b, H-19b, C$\underline{H}_3$-23, and NC$\underline{H}_3$-29; C$\underline{H}_3$-22 to H-24 and C$\underline{H}_3$-23; H-25 to H-24 and
165 C$\underline{H}_3$-28.

Insecticidal Activity. Insects *O. fasciatus* Dallas were maintained at 27 ± 1ºC, 50-60% relative humidity and a 16h/8h (light/ dark) photoperiod on a diet based on sunflower seeds. The entomotoxicity against *O. fasciatus* was evaluated by topical application at an appropriate dose in order to obtain the LD_{50} values (*29*). One
170 microliter of the appropriate dilution in acetone was applied, using a micropipette, on the ventral surface of the abdomen of ten newly moulted fourth-instar nymphs, which had previously been anesthetized with cloroform. After treatment, nymphs were confined in a 9 cm Petri dish with food and water provided *ad libitum*. Acute toxicity effects were considered according to the number of dead insects after 72 h of exposure
175 to the chemicals The surviving nymphs were transferred to a 500 cm^3 glass flask and held at standard conditions in order to follow their development. Controls were carried out in parallel and received the same amount of acetone as treated insects. All assays were conducted in triplicate (**Table 2**).

Statistical Analysis

180 Probit analysis (*30*) was used to determine the LD_{50} values (SPSS v.10).

RESULTS AND DISCUSSION

Along with the known paraherquamide A (**3**), paraherquamide B (**4**), paraherquamide E (**5**), paraherquamide VM55596 (**6**), and paraherquamide VM55597 (**7**), we have isolated from *Penicillium cluniae* culture broth two new PHQ, that we have named paraherquamide H (**1**) and paraherquamide I (**2**) (*1-4*). Five known diketopiperazines (DKP) were also isolated for the first time from *P. cluniae*: *cyclo*-(*L*-Pro-*L*-Tyr) (**8**), *cyclo*-(*L*-Pro-*L*-Val) (**9**), *cyclo*-(*L*-Pro-*L*-Ile) (**10**), *cyclo*-(*L*-Pro-*L*-Leu) (**11**), and *cyclo*-(*L*-Pro-*L*-Phe) (**12**) (**Figure 2**). The absolute stereochemistries of DKP were established according to their rotation power and bibliography data (*5*,*6*). The DKP isolation, together with these six PHQ, seems to confirm their already described role as biosynthetical intermediates of these and related PHQ (*12*,*27*).

The chemical caracterization of **1** was readily performed by comparison of its ^{1}H NMR and ^{13}C NMR spectroscopic data (**Table 1**) with those of already known PHQ. In addition, it was confirmed by two-dimensional experiments (COSY H-H, HSQC and HMBC) and MS data (*3*,*4*).

Paraherquamide H (**1**) had the molecular formula $C_{28}H_{33}N_3O_5$, according to HRESIMS $[(M + Na)^+]$. The analysis of NMR spectra led to recognition of a dioxygenated seven-membered ring possessing a 1,2-disubstituted olefin joined to a *spiro*-indole unit. This moiety can also be found in PHQ **3**-**7**, paraherquamide C, paraherquamide D and other ones (**Figure 1**) (*1-4*,*7*). Only some PHQ, as paraherquamide F and G, have a monooxygenated six-membered ring joined to the *spiro*-indole unit (*3*,*7*). Furthermore, the presence of a bridged tetracyclic portion (C-10 through C-23), consisting of a bicyclo[2,2,2] diazaoctane core joined to a proline unit, could be compatible with the NMR data for **1**. This last moiety being common for all known PHQ, differences among them can be established based on the proline unit substituents. Also, the methyl single resonance in the ^{1}H NMR spectrum at 2.90 ppm indicated the presence of the amide N-methyl functionality.

Resonances at 171.0, 174.8, and 182.2 ppm in the ^{13}C NMR spectrum were attributed to three carboxyl carbons, which seem to be amide carbonyl groups. The HMBC experiment led to locating the carbonyl signal at 174.8 ppm in C-16 of proline unit, because there was an heteronuclear correlation between this signal and protons at δ 2.43 (H-15a). This oxidative substitution at C-16 has only been demonstrated in PHQ **7**, which was also isolated from our *P. cluniae* extract and previously found in other P*enicillium* strains (*4*). In addition, COSY and HMBC experiments of **1** led to establishing the existence of a CH_2CHCH_3 subunit in this proline unit, with a methyl group located in C-14. Thus, the only structural difference between **1** and **7** was the presence in the latter of an hydroxy group at C-14.

The structure of **2** was established by comparison of its NMR and MS spectra with those of **1**. HRESIMS [$(M + Na)^+$] for compound **2** rendered $C_{28}H_{31}N_3O_5$ as its molecular formula, two units less than **1**. According to NMR spectra, the structures of **1** and **2** were very similar, with differences only noticeable in the proline unit. Compound **2** had a carbonyl group at C-16 and a methyl group at C-14, just like compound 1, but differed from it in that a double bond between C-14 and C-15 was observed. This was established by the absence in its ^{13}C NMR spectrum of signals at δ 34.2 and δ 39.6, corresponding to C-14 and C-15 in **1**, respectively. Instead, two new signals at δ 159.2 and δ 125.5 appeared in the spectrum of **2**, corresponding to a quaternary sp^2 carbon and a protonated sp^2 carbon, respectively, which were located in positions 14 and 15, according to HMBC spectrum data. Compound **2** seems to be the first documented example of the PHQ family possessing this double bond.

The relative stereochemistry of **1** and **2** was concluded to be the same as that shown by **3** and all the other described PHQ, at all centers. This was done on the basis of the close similarity of the ^1H NMR and ^{13}C NMR chemical shifts and NOESY correlations to the corresponding values for **3** and all the other known PHQ (**Table 1, Figure 3**, and **material and methods**). Thus, the NOESY correlations of **1** from H-4 to the H-5, H-

12a, and C$\underline{H}_3$-22 were critical in establishing the relative stereochemistry of the *spiro* center. Also, NOESY interactions from H-20 to H-12b, H-19b, and C$\underline{H}_3$-23 of **1**, placed the corresponding substituents on the opposite face of the cyclopentanoid ring. The NOESY data also allowed stereo-specific proton assignments of the other geminal proton pairs H-10 and H-15, H-14, and C$\underline{H}_3$-17. Furthermore, C-13 must have the described relative configuration to permit connection of the amide bridge to C-11. Similar NOESY correlations of **1** were obtained for **2**, indicating the same relative stereochemistry as **1** (**material and methods**). Assuming that the absolute configuration of C-20 in **1** and **2** was the same as that determined by a X-ray study on a derivative of **3** (*31*), it was possible to establish that the absolute configurations of **1** and **2** were 3*R*, 11*S*, 13*R*, 14*R*, 20*S* and 3*R*, 11*S*, 13*R*, 20*S*, respectively.

Toxicity against *O. fasciatus*. Taking into account the acute mortality calculated as the 72 hours LD_{50} (**Table 2**), the compounds could be ranged as follows in terms of their relative toxicity: paraherquamide E (**5**) > paraherquamide A (**3**) > VM55597 (**7**) > VM55596 (N-oxide PHQ) (**6**) > paraherquamide B (**4**). This information, as mentioned previously, allowed us to establish preliminary structure-activity relationships. Paraherquamide H (**1**) and paraherquamide I (**2**) were not assayed because we did not obtain a sufficient amount for testing. DKP, intermediates in the biosynthesis of PHQ, did not show insecticidal activity in our assay conditions (at 20 µg/nymph, the highest assayed dose, compounds were completely inactive).

Compound **5** was the most active compound followed by compound **3**. The only structural difference between them was the presence of a hydroxy group at C-14 in **3**, absent in **5.** This seems to indicate that the presence of this hydroxy group reduced the insecticidal activity of the molecule (**3** was approximately 3.5-fold lesser active than **5**).

On the other hand, metabolite **7** was the third more potent of the assayed compounds. It had a structure very similar to **3**; the only difference was the presence in the former of the carbonyl group at C-16. Taking into account that **3** was three times more potent than

7, it was possible to establish that the carbonyl group was hindering the insecticidal activity.

In addition, the fourth more potent PHQ was the compound **6**. Chemically, **6** and **3** differed only in the oxygen substitution in N(12) atom for **6**, because both compounds had the same substituents at C-14. Thus, it seems that the oxidative substitution at N impeded the insecticidal activity.

Finally, it was possible to notice that **4** is the least active of the five assayed compounds although it had a very similar structure to **5** the former only lacking the methyl group at C-14. Thus, the alkyl substitution at C-14 seems to be decisive for possessing a potent insecticidal activity.

Regarding all of these correlations, it is possible to conclude that oxidative substitutions in the PHQ proline unit hinder the insecticidal activity and that the alkyl substitution at C-14 favors it. The significance of this alkyl substitution had already been shown in other PHQ biological activities (*2*).

It is known that PHQ and marcfortines are effective against strains of parasites that are resistant to all known broad-spectrum antihelmintics (*17-2, 26*). The postulated mechanism of action for PHQ and related compounds is the blocking of invertebrate nicotinic acetylcholine receptors (nACh R) as competitive antagonists of acetylcholine and nicotine (*32-34*).

Insect nACh R are targets of growing importance for new insecticide compounds, but their antagonist compounds were always considerably less active as insecticides than their agonist compounds, such as imidaclopid. Furthermore, the insecticidal activity of antagonists such as dihydro-β-erythroidine was always associated to antifeedant effects in oral ingestion assays (*32*). However in this paper we have demonstrated that PHQ are potent insecticides against the hemipteran *O. fasciatus*, producing the typical symptoms of compounds that interfere with the insect's nervous system, such as slowness of movement and stiffness.

The utility of PHQ is compromised by the fact that PHQ are toxic for mice and dogs, because they are also antagonistic of mammal nACh R. However, comparative analysis between nematode and vertebrate nACh R reveal that paraherquamide A (**3**) is 1000-fold more potent at nematode nACh R than at human nACh R (*33*). These results seem to indicate intrinsic differences in PHQ-nACh R affinity between these *phyla*. Thus, although PHQ are toxic for mammals, their interesting activity against *O. fasciatus* argues for efforts focused on isolation of new PHQ and their chemical modifications to reduce the mammalian toxicity, keeping their selective insecticide and nematode toxicity.

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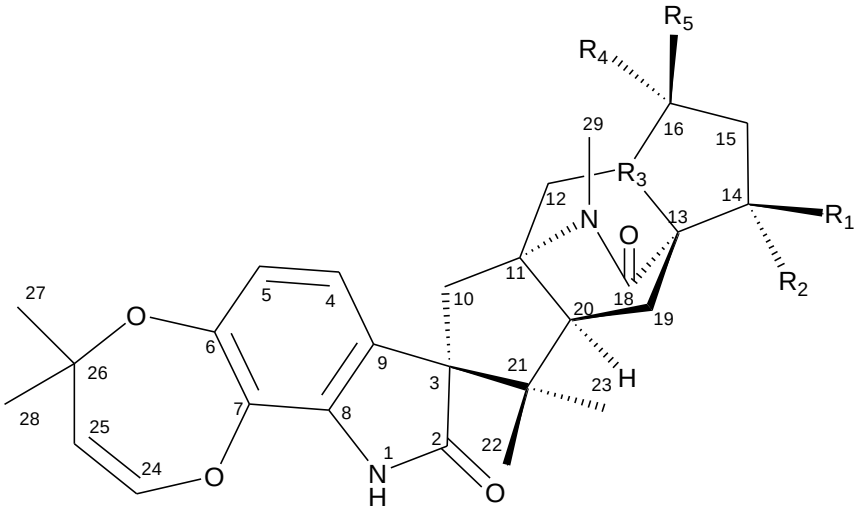
Figure 1. Structures of Paraherquamides isolated from *P. cluniae*.

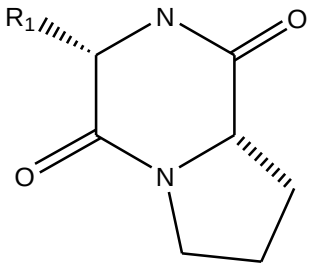


(**1**) Paraherquamide H; R_1=H, R_2= CH_3, R_3=N, R_4, R_5=O
(**2**) Paraherquamide I; R_2= CH_3, R_3=N, R_4, R_5=O, C_{14}:C_{15} dehydro
(**3**) Paraherquamide A; R_1=OH, R_2= CH_3, R_3=N, R_4=H, R_5=H
(**4**) Paraherquamide B; R_1=H, R_2=H, R_3=N, R_4=H, R_5=H
(**5**) Paraherquamide E; R_1=H, R_2= CH_3, R_3=N, R_4=H, R_5=H
(**6**) VM55596; R_1=OH, R_2= CH_3, R_3=N^+O^-, R_4=H, R_5=H
(**7**) VM55597; R_1=OH, R_2= CH_3, R_3=N, R_4, R_5=O

410

Figure 2. Structures of Diketopiperazines isolated from *P. cluniae*.

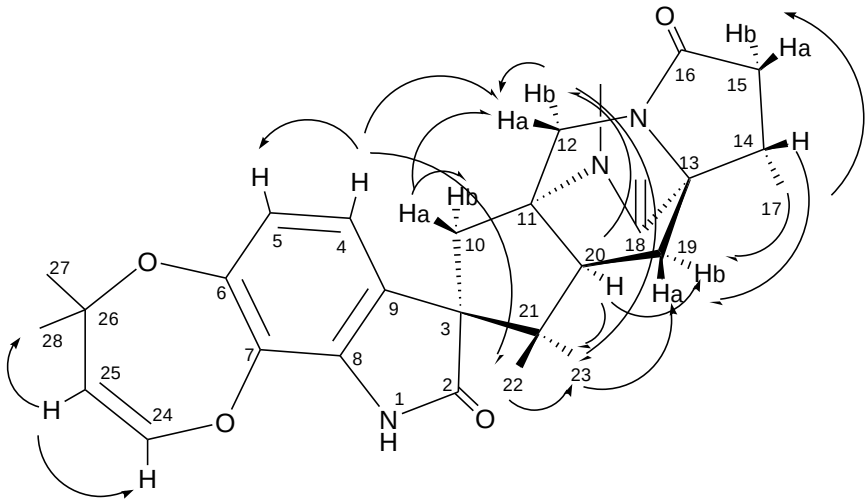
415



(**8**) *Cyclo*-*L*-Pro-*L*-Tyr; R_1=C_6H_4 *p*-OH
(**9**) *Cyclo*-*L*-Pro-*L*-Val; R_1=$CH(CH_3)_2$
(**10**) *Cyclo*-*L*-Pro-*L*-Ile; R_1=$CH_3CHCH_2CH_3$
(**11**) *Cyclo*-*L*-Pro-*L*-Leu; R_1=$CH_2CH(CH_3)_2$
(**12**) *Cyclo*-*L*-Pro-*L*-Phe; R_1=C_6H_5

Figure 3. NOE Correlations observed for compound **1**.

420


O
Hb
Ha
16
15
Hb
Ha
N
12
14
H
N
13
H
H
Hb
Ha
11
17
5
4
10
20
18
19
27
O
6
H
Hb
9
21
26
3
28
7
8
25
2
22
23
H
24
1
O
N
O
H
H

425

Table 1. ^{1}H and ^{13}C NMR Data of **1** ($CDCl_3$, 600 MHz and 75 MHz, respectively).

	δ_H (m,[a] *J* in Hz)	δ_C	HMBC with H	COSY
2	-	182.2	1.92, 2.69	-
3	-	63.2	2.69, 1.08, 0.88	-
4	6.80 (d, 8.1)	120.6	-	6.71
5	6.71 (d, 8.1)	117.4	-	6.80
6	-	147.2	6.71, 6.80	-
7	-	135.5	6.31, 6.71	-
8	-	131.5	6.80	-
9	-	125.0	1.92, 6.71	-
10a	2.69 (d, 15.7)	37.2	-	3.42, 1.92
10b	1.92 (d, 15.7)		-	2.69
11	-	64.0	2.69, 2.90, 3.16, 3.42	-
12a	3.53 (d, 11.6)	49.1	1.92	3.42
12b	3.42 (d, 11.6)		-	3.53
13	-	65.4	1.52, 2.43	-
14	2.20 (m)	34.2	-	1.52, 2.31, 2.43
15a	2.43 (dd, 16.15, 8.3)	39.6	1.52	2.31
15b	2.31 (dd, 16.15, 8.3)		-	2.43
16	-	174.8	2.43	-
17	1.52 (m)	24.8	2.2	2.20
18	-	171.0	2.90	-
19a	2.10 (m)	28.2	0.88, 3.16	3.16, 1.48
19b	1.48 (m)			3.16, 2.10
20	3.16 (td, 10.3, 1.3)	53.2	0.88, 1.08	-
21	-	46.4	0.88, 1.08, 2.69, 3.16	2.10, 1.48
22	0.88 (s)	23.8	1.08	1.08
23	1.08 (s)	20.4	0.88	0.88
24	6.31 (d, 7.7)	138.4	1.46, 4.90	4.90
25	4.90 (d, 7.7)	115.0	1.44, 1.46, 6.31	6.31
26	-	80.1	1.44, 1.46, 6.31, 4.90	-
27	1.46 (s)	30.6	1.44	1.44
28	1.44 (s)	30.0	1.46	1.46
29	2.90 (s)	26.8	-	-

[a]multiplicity.

Table 2.- Insecticidal Activity of *Penicilliun cluniae* Paraherquamides against *Oncopeltus fasciatus.*

Product	Slope ± ES	LD_{50} (95% CL)[a]	χ^2	df	P
Paraherquamide A (**3**)	2.43 ± 0.14	0.32 (0.26, 0.38)	24.58	8	0.002
Paraherquamide B (**4**)	1.36 ± 0.16	16.54 (13.14, 21.22)	15.73	8	0.046
Paraherquamide E (**5**)	33.65 ± 2.17	0.089 (0.086, 0.092)	27.41	4	0.000
VM 55596 (**6**)	1.62 ± 0.11	7.01 (4.68, 9.80)	22.47	6	0.001
VM 55597 (**7**)	7.81 ± 0.68	0.91 (0.85, 1.00)	21.68	6	0.001

435 [a] Values, in µg/nymph, were determined 72 h. after exposure to the chemical. Values into parenthesis correspond to LD_{50} confidence limits