JOURNAL OF NATURAL PRODUCTS

Note

pubs.acs.org/jnp

# On the Antibiotic and Antifungal Activity of Pestalone, Pestalachloride A, and Structurally Related Compounds

Daniel Augner,[†] Oleg Krut,*[,‡] Nikolay Slavov,[†] Dario C. Gerbino,[†] Hans-Georg Sahl,[§] Jürgen Benting,[⊥] Carl F. Nising,[⊥] Stefan Hillebrand,[⊥] Martin Krönke,[‡] and Hans-Günther Schmalz*[,†]

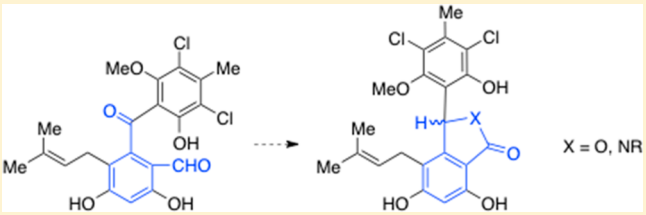
[†]Department of Chemistry, University of Cologne, Greinstrasse 4, 50939 Köln, Germany

[‡]Institute for Medical Microbiology, Immunology and Hygiene, University of Cologne, Goldenfelsstrasse 19-21, 50935 Köln, Germany

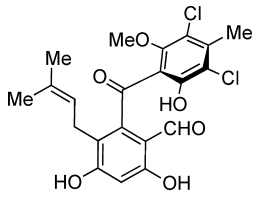
[§]Institute for Medical Microbiology, Immunology and Parasitology, University of Bonn, Meckenheimer Allee 168, 53155 Bonn, Germany

[⊥]Bayer CropScience, Research−Chemistry Disease Control, Alfred-Nobel-Strasse 50, 40789 Monheim, Germany

**ABSTRACT:** Pestalone (**1**) is a prominent marine natural product first isolated by M. Cueto et al. in 2001 from a co-fermentation of a marine fungus with a marine bacterium. For more than 10 years, **1** had been considered as a promising new antibiotic compound, the reported MIC against methicillin-resistant *Staphylococcus aureus* (MRSA) being 37 ng/mL. After overcoming the limited availability of **1** by total synthesis (N. Slavov et al., 2010) we performed new biological tests, which did not confirm the expected degree of antibiotic activity. The observed activity of pestalone against different MRSA strains was 3−10 μg/mL, as determined independently in two laboratories. A number of synthetic derivatives of **1** including pestalachloride A and other isoindolinones (formed from **1** by reaction with amines) did not exhibit higher activities as compared to **1** against MRSA and a series of plant pathogens.



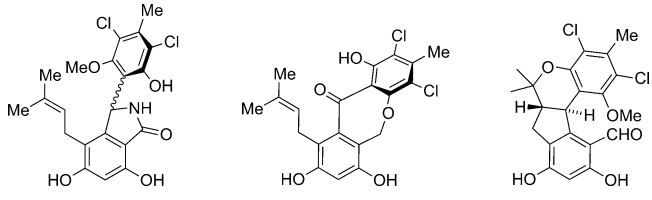

The story of pestalone (**1**) started in 2001 in the context of a research program by Fenical and co-workers, who tried to trigger the expression of new antibiotics against drug-resistant pathogens from marine microorganisms in response to bacterial challenge.[1] After co-culturing a fungus of the genus *Pestalotia* and a unicellular marine bacterium (strain CNJ-328) they isolated **1** as a new antibiotic agent. Structural elucidation revealed **1** to be a highly functionalized, chlorinated benzophenone derivative (Figure 1).[2]




**1**

**Figure 1.** The marine natural product pestalone (**1**).

Pestalone (**1**) was reported to exhibit strong antibiotic activity against methicillin-resistant *Staphylococcus aureus* (MRSA, minimum inhibitory concentration (MIC) = 37 ng/mL) and vancomycin-resistant *Enterococcus faecium* (VRE, MIC 78 = ng/mL). The authors even underlined that "the potency of this agent toward drug-resistant pathogens suggests that pestalone should be evaluated in more advanced, whole animal models of infectious disease".[1] Due to its pronounced activity, **1** was highlighted in several publications as a promising new lead structure in the struggle against multiresistant microorganisms.[3] Nevertheless, because of its limited availability, no further biological investigations of **1** have been reported so far. In 2008, Che and co-workers reported the discovery of the pestalachlorides A−C (Figure 2) from the plant endophytic fungus *Pestalotiopsis adusta*.[4] These compounds are structurally closely related to **1**, from which they may be biosynthetically derived. Recently, we demonstrated that **1** can be easily converted into pestalachloride A (*rac*-**2a**) by treatment with ammonia.[5] The fact that this compound occurs in nature also as a racemic




*rac*-**2a** **3** **4**

**Figure 2.** The natural products pestalachloride A (*rac*-**2a**), pestalachloride B (**3**), and pestalachloride C (**4**).

**Received:** April 15, 2013
**Published:** August 1, 2013

mixture indicates its nonenzymatic "biosynthesis" from pestalone (**1**).

Pestalachlorides A–C were reported to exhibit antimicrobial activity against certain plant pathogenic fungi (Table 1). The highest antifungal activity was observed for pestalachloride A (*rac*-**2a**) against *Fusarium culmorum* (MIC = 3.2 μg/mL). In contrast, pestalachloride B (**3**) was most effective against *Gibberella zeae* (MIC = 5.0 μg/mL) and *Verticillium aibo-atrum* (MIC = 5.0 μg/mL), while no biological activity was observed for pestalachloride C (**4**) in these in vitro test systems.[4]

**Table 1. Activity (Minimum Inhibitory Concentration) of Pestalachloride A against Plant Pathogenic Fungi: *Fusarium culmorum, Gibberella zeae,* and *Verticillium aibo-atrum,* As Reported by Che[4]**

| | MIC (μg/mL) | | |
|---|---|---|---|
| compound | *F. culmorum* | *G. zeae* | *V. aibo-atrum* |
| *rac*-**2a** | 3.2 | 50.1 | 50.1 |
| **3** | 20.1 | 5.0 | 5.0 |
| **4** | >100 | >100 | >100 |

The limited availability of **1** from natural sources challenged efforts toward its chemical synthesis. A synthesis of deformylpestalone and some related analogues was achieved in 2003,[6] and a first total synthesis was disclosed a little later.[7] However, no further biological experiments were reported since then.

Recently, we developed an efficient total synthesis and were able to produce substantial amounts of pestalone (**1**), the identity of which was secured by X-ray crystal structure analysis.[5] In the course of this work, we also demonstrated that **1** reacts with ammonia to give *rac*-**2a** or with primary amines to afford isoindolinones *rac*-**2b** and *rac*-**2c**, respectively. In addition we found that the conversion of **1** into pestalalactone *rac*-**5** proceeds smoothly either under photochemical conditions or in the presence of a nucleophilic catalyst such as cyanide (Scheme 1).[8]

The availability of material enabled us to further explore the antibacterial potency of pestalone (**1**), the related isoindolinones (*rac*-**2a–c**), pestalalactone (*rac*-**5**), and analogues (Figure 3). For this purpose, we utilized a standardized broth microdilution method to determine the activity against three different clinically relevant *Staphylococcus aureus* strains, i.e., USA300 (epidemic community acquired MRSA), Mu50 (hospital acquired MRSA, vancomycin-intermediate *S. aureus*, VISA), *S. aureus* MW2 (community acquired MRSA), and one reference strain, ATCC 29213. The results are shown in Table 2.

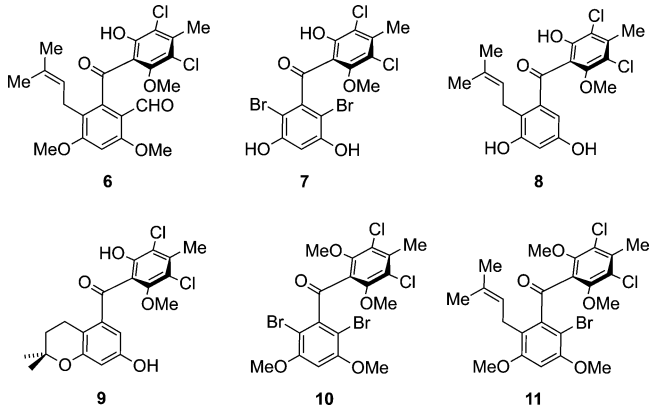



**Figure 3.** Further synthetic analogues of pestalone (**1**) employed in this study.

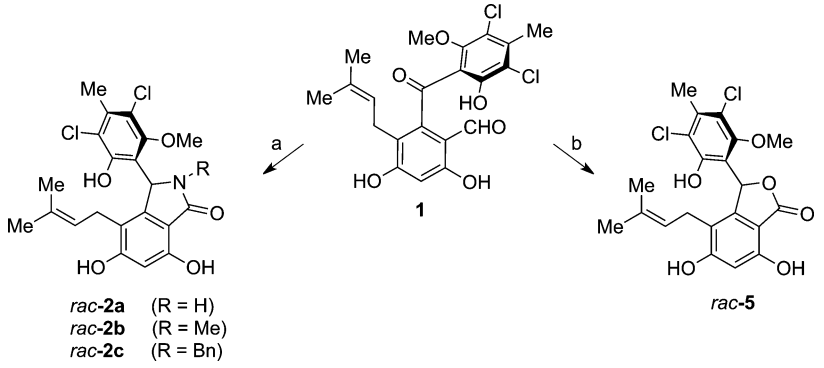
**Table 2. Activity of Pestalone (1) and Analogues against Different *S. aureus* Strains**

| | MIC [μg/mL] against different *S. aureus* strains[a] | | | |
|---|---|---|---|---|
| compound | USA300 | Mu50 | MW2 | ATCC29213 |
| **1** | 10 | 10 | | 16 |
| *rac*-**2a** | | | 10 | 10 |
| *rac*-**2b** | | | >50 | >50 |
| *rac*-**2c** | | | >50 | >50 |
| *rac*-**5** | 25 | | | |
| **6** | 10–15 | 15–20 | | |
| **7** | 30–35 | 30–45 | | |
| **8** | 5–10 | 5–10 | | |
| **9** | 7–10 | 7–10 | | |
| vancomycin | 0.25 - 0.5 | 4 | 1 | 1 |

[a]$10^5$ cfu/mL; controls with vancomycin and **1**.

Our experiments revealed a significant biological effect of pestalone (**1**), pestalachloride A (*rac*-**2a**), and some analogues against methicillin-resistant *Staphylococcus aureus*, however, only at comparably high concentrations (MIC = 10 μg/mL for **1**). Pestalone (**1**) was found to be about 270 times less active then reported.[1] Pestalachloride A (*rac*-**2a**) also exhibited a certain activity (comparable to **1**), in contrast to the N-alkylated derivatives (*rac*-**2b**/**c**), which were not active at any

**Scheme 1. Conversion of Pestalone (1) into Pestalachloride A and Derivatives (*rac*-2a–c) or Pestalalactone (*rac*-5)[a]**




[a]Conditions: (a) $RNH_2$, 1,4-dioxane/$H_2O$, AcOH; (b) cat. NaCN, DMSO (or *hν*, DMSO).


dx.doi.org/10.1021/np400301d | *J. Nat. Prod.* 2013, 76, 1519–1522

reasonable concentration. Pestalalactone (*rac*-**5**) displayed lower antibacterial potency (MIC = 25 μg/mL). Deformylpestalone (**8**) showed a similar activity to **1**, against both USA300 and Mu50 (MIC = 5−10 μg/mL).

The activity of pestalone (**1**) was confirmed in an independent series of broth microdilution experiments employing other bacterial strains (Table 3). *Staphylococcus aureus* strain SG511 was affected by **1** at a MIC of 3.1 μg/mL, while the growth of a randomly chosen clinical isolate, MRSA LT-1334, was inhibited at a MIC of 6.25 μg/mL. As a reference, a strain of *Bacillus subtilis* 168 was also tested (MIC = 1.6 μg/mL). It was demonstrated again that **1** acts as an antibacterial agent, however, not at the very low concentrations originally reported.[1]

**Table 3. Activity of Pestalone (1) against Different Pathogenic Bacteria**

| | MIC [μg/mL] | | |
|---|---|---|---|
| compound | *B. subtilis* 168 | *S. aureus* SG511 | *MRSA* LT-1334 |
| **1** | 1.6 | 3.1 | 6.25 |

We also examined the antifungal potency of these compounds against various plant pathogens.[9] The results summarized in Table 4 indicate that the compounds behaved very differently. Pestalone (**1**) displayed significant antimicrobial activity against several of the microorganisms tested (7 out of 15 plant pathogens), while pestalachloride A (*rac*-**2a**) interfered only with *Pyrenophora teres* ($ED_{50}$ = 33.1 μg/mL) and *Fusarium culmorum* ($ED_{50}$ = 31.9 μg/mL) with moderate activities. ($ED_{50}$ = effective dose that causes 50% growth inhibition.) Pestalalactone (*rac*-**5**) selectively affected *Phytophthora cryptogea* (MIC = 18.9 μg/mL), *Trametes versicolor* ($ED_{50}$ = 13.1 μg/mL), and *Pyrenophora teres* ($ED_{50}$ = 4.3 μg/mL). The dibromo derivative (**7**) was selective against the most sensitive organism, i.e., *Fusarium culmorum* ($ED_{50}$ = 0.014 μg/mL). While compounds **6** and **10** are active, only compound **11** showed no antimicrobial activities (at ≤50 μg/mL).

In conclusion, we have demonstrated that the antimicrobial activity of synthetic pestalone (**1**) is significantly less than that reported for the natural product.[1] In addition, we demonstrated that pestalalactone (*rac*-**5**), pestalachloride A (*rac*-**2a**), and related isoindolinones, which are readily formed from **1** under mild conditions, also do not exhibit a particular level of activity. Compounds **1**, *rac*-**2a**, and the deformyl derivatives **8** and **9** were found to be active against methicillin-resistant *Staphylococcus aureus* strains at concentrations of 3−10 μg/mL (MIC). The natural products **1** and *rac*-**2a** as well as a series of synthetic derivatives were also tested against a spectrum of plant pathogenic fungi. While **1** displayed the broadest activity, none of the compounds proved to be sufficiently active to qualify as a potential crop-protecting agent.

At this point we have no explanation for the discrepancy between our results and the reported data concerning the antibiotic activity of pestalone (**1**). Since no reference strain was used in the susceptibility testing of natural pestalone, it is possible that the employed isolate was particularly sensitive by chance. However, our data, which are based on repeated testing in different laboratories using both reference stains and clinical isolates, should reflect the susceptibility of *S. aureus* to pestalone rather precisely. Finally, having identified **1** as a reactive agent (e.g., forming isoindolinones with primary amines), we cannot exclude the possibility that a highly active derivate of **1** had formed and caused the adventitious antimicrobial effect.

## EXPERIMENTAL SECTION

**General Experimental Procedures.** MIC determinations (Table 2) were carried out in microtiter plates according to CLSI standards with 2-fold serial dilutions of the compounds (Clinical and Laboratory Standards Institute; NCCLS, 2006: Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard M7-A6). Strains were grown in Mueller−Hinton broth (Oxoid). Bacteria were added at an inoculum of $10^5$ cfu/mL in a final volume of 0.2 mL. After incubation for 24 h at 37 °C the MIC was read as the lowest compound concentration causing inhibition of visible growth. *S. aureus* reference strains were obtained from American Type Culture Collection (ATCC 29213) or from the Network on Antimicrobial Resistance in *Staphylococcus aureus* (USA300, Mu50, MW2). The bacterial stocks were stored at −70 °C and recovered on Mueller-Hinton agar a few days before experiments.

MIC determinations (Table 3) were carried out in 96-well microtiter plates according to CLSI standards with 2-fold serial

**Table 4. Activity ($ED_{50}$ Values) of Pestalone (1), Pestalachloride A (*rac*-2a), and Other Derivatives (see Figure 3) against Different Plant Pathogens**

| | $ED_{50}$ [μg/mL] | | | | | | |
|---|---|---|---|---|---|---|---|
| organism | **1** | *rac*-**2a** | *rac*-**5** | **6** | **7** | **10** | **11** |
| *Phytophthora infestans* | >50 | >50 | n.d. | n.d. | >50 | n.d. | >50 |
| *Phytophthora cryptogea* | 33.9 | >50 | 18.9 | >50 | >50 | >50 | >50 |
| *Pythium aphanidermatum* | >50 | >50 | >50 | 44.4 | n.d. | >50 | >50 |
| *Alternaria mali* | 22.4 | >50 | >50 | >50 | >50 | >50 | >50 |
| *Botrytis cinerea* | >50 | >50 | >50 | >50 | >50 | >50 | >50 |
| *Septoria tritici* | 26.7 | >50 | >50 | >50 | >50 | >50 | >50 |
| *Pyrenophora teres* | 2.2 | 33.1 | 4.3 | 5.4 | >50 | 42 | >50 |
| *Leptosphaeria nodorum* | 22.4 | >50 | >50 | 22.5 | >50 | >50 | >50 |
| *Fusarium culmorum* | 0.12 | 31.9 | >50 | 1.2 | 0.014 | 2.2 | >50 |
| *Gibberella zeae* | >50 | >50 | >50 | >50 | >50 | >50 | >50 |
| *Pyricularia oryzae* | 28.6 | >50 | >50 | 5 | >50 | 12.6 | >50 |
| *Ustilago avenae* | >50 | >50 | >50 | >50 | >50 | >50 | >50 |
| *Aspergillus niger* | >50 | >50 | >50 | >50 | >50 | >50 | >50 |
| *Trametes versicolor* | >50 | >50 | 13.1 | >50 | >50 | >50 | >50 |
| *Pseudomonas fluorescens* | >50 | >50 | >50 | >50 | >50 | >50 | >50 |


dx.doi.org/10.1021/np400301d | *J. Nat. Prod.* 2013, 76, 1519−1522

dilutions of the compounds. Strains were grown and tested in half-concentrated Mueller–Hinton broth (Oxoid). Bacteria were added at an inoculum of $10^5$ cfu/mL in a final volume of 0.2 mL. After incubation for 24 h at 37 °C the MIC was read as the lowest compound concentration causing inhibition of visible growth.

**In Vitro Test for the Calculation of the $ED_{50}$ Value with Microorganisms (Table 4).** Wells of 96-well microtiter plates were filled with 10 μL of a solution of the test compound in methanol together with the emulsifier alkylaryl polyglycol ether. Thereafter, the solvent was evaporated in a hood. At the next step, into each well 200 μL of liquid potato dextrose medium was added that had been amended with an appropriate concentration of spores or mycelium suspension of the test fungus. With the aid of a photometer the extinction in all wells was measured at the wavelength of 620 nm. The microtiter plates were then transferred for 3–5 days onto a shaker at 20 °C and 85% relative humidity. At the end of the incubation time the growth of the test organisms was measured again photometrically at the wavelength of 620 nm. The difference between the two extinction values (taken before and after incubation) was proportional to the growth of the test organism. Based on the Δ extinction data from the different test concentrations and that of the untreated test organism (control) a dose–response curve was calculated. The concentration that is necessary to give 50% growth inhibition is defined and reported as $ED_{50}$ value in ppm (= μg/mL).

## AUTHOR INFORMATION

### Corresponding Author

*(O. Krut) Phone: +49-221-478-32103. E-mail: oleg.krut@uni-koeln.de. (H.-G. Schmalz) Phone: +49-221-470-3063. E-mail: schmalz@uni-koeln.de.

### Notes

The authors declare no competing financial interest.

## REFERENCES

(1) Cueto, M.; Jensen, P. R.; Kauffman, C.; Fenical, W.; Lobkovsky, E.; Clardy, J. *J. Nat. Prod.* **2001**, *64*, 1444–1446.

(2) A closely related structure, formally derived from **1** by cleavage (*O*-demethylation) of the methoxy group, was reported: Wachi, Y.; Yamashita, T.; Komatsu, K.; Yoshida, S. JP Patent, JKXXAF JP 07061950 A2 19950307, 1995.

(3) (a) Donia, M.; Hamann, M. T. *Lancet Infect. Dis.* **2003**, *3*, 338–348. (b) Scherlach, K.; Hertweck, C. *Org. Biomol. Chem.* **2009**, *7*, 1753–1760. (c) Pettit, R. K. *Appl. Microbiol. Biotechnol.* **2009**, *83*, 19. (d) Villa, F. A.; Gerwick, L. *Immunopharm. Immunotox.* **2010**, *32*, 228–237. (e) Hughes, C. C.; Fenical, W. *Chem.—Eur. J.* **2010**, *16*, 12512–12525. (f) Rahman, H.; Austin, B.; Mitchell, W. J.; Morris, P. C.; Jamieson, D. J.; Adams, D. R.; Spragg, A. M.; Schweizer, M. *Mar. Drugs* **2010**, *8*, 498–518.

(4) Li, E.; Jiang, L.; Guo, L.; Zhang, H.; Che, Y. *Bioorg. Med. Chem.* **2008**, *16*, 7894–7899.

(5) Slavov, N.; Cvengros, J.; Neudörfl, J.-M.; Schmalz, H.-G. *Angew. Chem., Int. Ed.* **2010**, *49*, 7588–7591.

(6) Kaiser, F.; Schmalz, H.-G. *Tetrahedron* **2003**, *59*, 7345–7355.

(7) Iijima, D.; Tanaka, D.; Hamada, M.; Ogamino, T.; Ishikama, Y.; Nishiyama, S. *Tetrahedron Lett.* **2004**, *45*, 5469–5471.

(8) (a) Augner, D.; Gerbino, D. C.; Slavov, N.; Neudörfl, J.-M.; Schmalz, H.-G. *Org. Lett.* **2011**, *13*, 5374–5377. (b) Gerbino, D. C.; Augner, D.; Slavov, N.; Schmalz, H.-G. *Org. Lett.* **2012**, *14*, 2338–2341.

(9) For a review on the relevance of natural products in crop protection, see: Nising, C. F.; Hillebrand, S.; Rodefeld, L. *Chem. Commun.* **2011**, *47*, 4062–4073.