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Akanthomycin, a New Antibiotic Pyridone from the Entomopathogenic Fungus *Akanthomyces gracilis*

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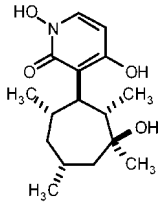
ABSTRACT



Akanthomycin

Organic extracts of the entomopathogenic fungus *Akanthomyces gracilis* ARS 2910 contained antibiotics active against *Staphylococcus aureus*. Bioassay-guided fractionation of the CH_2Cl_2 extract yielded the antibacterial compound akanthomycin as a mixture of atropisomers along with the closely related compounds 8-methylpyridoxatin and cordypyridone C. Akanthomycin was characterized using X-ray crystallography and NMR.

Fungi are prolific producers of structurally diverse, biologically active secondary metabolites.[1,2] Among the fungi, those that live in close association with other organisms are believed to be exceptional generators of biologically active compounds. High levels of environmental stress and intense and frequent interactions with other organisms promote the production of metabolically diverse compounds.[3] Entomopathogenic fungi, the pathogenic fungi found in association with insects, are an underexploited group of significant taxonomic diversity and represent one of the largest untapped pools of novel fungi.[4] The more than 750 described entomopathogens are distributed across all major fungal subdivisions and represent more than 100 genera.[5]

Previously reported biologically active metabolites from entomopathogenic fungi including tenellin from *Beauveria tenella*,[6] the red pigment oosporein from *B. bassiana*,[7] the destruxins from *Aschersonia* sp.,[8] and cyclosporin A from *Tolypocladium inflatum*[9] exemplify the complexity of compounds synthesized by entomopathogens.

In our search for new biologically active natural products, a fungus (ARS 2910[10]) isolated from the ant *Paltothyreus tarsatus* was active in an antimicrobial screen. ARS 2910, which was identified as *Akanthomyces gracilis*, was refer-

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(1) Henkel, T.; Brunne, R. M.; Muller, H.; Reichel, F. *Angew. Chem., Int. Ed.* **1999**, *38*, 643−647.
(2) Pearce, C. *Adv. Appl. Microbiol.* **1997**, *44*, 1−80.
(3) Dreyfuss, M. M.; Chapela, I. H. In *The Discovery of Natural Products with Therapeutic Potential*; Gullo, V. P., Ed.; Butterworth-Heinemann: Boston, MA, 1994; pp 49−80.
(4) Hawksworth, D. L. *Mycologist* **1997**, *11*, 18−22.
(5) Roberts, D. W.; Humber, R. A. In *Biology of Conidial Fungi*; Academic Press: New York, 1981; Vol. 2, pp 201−236.
(6) (a) MacLeod, D. M. *Can. J. Bot.* **1954**, *32*, 818. (b) McInnes, A. G.; Smith D. G.; Wat, C.; Vining, L. C.; Wright, J. C. C. *J. Chem. Soc., Chem. Commun.* **1974**, 281−282.
(7) El Basyouni, S. H.; Brewer, D.; Vining, L. C. *Can. J. Bot.* **1968**, *46*, 441−448.
(8) Krasnoff, S. B.; Gibson, D. M.; Belofsky, G. N.; Gloer, K. B.; Gloer, J. B. *J. Nat. Prod.* **1996**, *59*, 485−489.
(9) Ruegger, A.; Kuhn, M.; Lichti, H.; Loosli, H. R.; Huguenin, R.; Quiquerez, C.; von Wartburg, A. *Helv. Chim. Acta* **1976**, *59*, 1075−1092.
(10) The fungus *A. gracilis* (Agricultural Research Service (ARS) accession number 2910) was obtained from the ARS Collection of Entomopathogenic Fungi (ARSEF).

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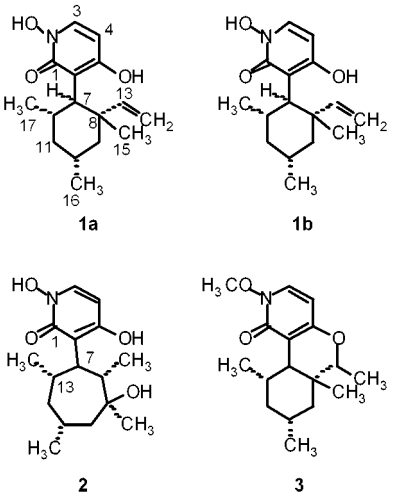
mented and the antimicrobial activity was found in the CH_2Cl_2 extracts of both the mycelium and the broth.[11] The crude CH_2Cl_2 extract of the mycelium was subjected to a modified Kupchan[12] scheme and the activity partitioned into both the CCl_4 and CH_2Cl_2 fractions. The CH_2Cl_2 fraction was chromatographed using RPHPLC (Supelcosil C-8 analytical column; 25 cm × 4.6 mm) with a method of 45:55 MeCN:H_2O isocratic for 5 min followed by a gradient to 100% MeCN over 15 min (flow rate 1.0 mL/min) to yield 6.2 mg of **1a** (t_R 11.0 min) and 5.6 mg of **1b** (t_R 12.5 min). The crude organic extract of the broth was partitioned using a modified Kupchan scheme. The antibiotic CH_2Cl_2 fraction was then subjected to countercurrent chromatography (1:1:1:1 hexanes:EtOAc:MeOH:H_2O supplemented with 0.1% HOAc; the lower phase was used as the mobile phase) followed by RPHPLC (Supelcosil C-8 analytical column; 25 cm × 4.6 mm; 45:55 MeCN:H_2O isocratic, flow rate 1.0 mL/min) to yield **2** as a mixture of rotamers (1.6 mg; t_R 5.1 min). The CCl_4 fractions from both the mycelium and the broth were combined and chromatographed (Supelcosil C-8 analytical column; 25 cm × 4.6 mm; 45:55 MeCN:H_2O for 5 min followed by a gradient to 100% MeCN over 15 min; flow rate 1.0 mL/min) to yield **1a**, **1b**, and **3** (1.8 mg; t_R 15.0 min). Analysis of the ^1H and ^{13}C NMR data suggested that the purified compounds were closely related.



NMR and X-ray crystallography experiments were used to elucidate the structures of **1a** and **1b** as atropisomers of 8-methylpyridoxatin with hindered rotation around the C-6−C-7 bond. In both **1a** and **1b** the *N*-hydroxy-2-pyridone ring is almost perpendicular with the cyclohexane ring system. However, the dihedral angle defined by C-1, -6, -7, and -8 is +95.5° in compound **1a** and −84.7° in compound **1b**. Rotamer **1b** of 8-methylpyridoxatin was first isolated from the fermentation extract of an unidentified fungus as a modulator of erythropoietin gene expression.[13] More recently, both **1a** and **1b**, confusingly renamed cordypyridone B and A, respectively, were isolated from the insect pathogenic fungus *Cordyceps nipponica* BCC 1389 and shown to display potent in vitro antimalarial activity.[14]

A molecular formula of $C_{16}H_{25}NO_4$ (HRMS-ESI (*m*/*z*) [M + H]$^+$ calcd for $C_{16}H_{26}NO_4$, 296.1861; found, 296.1860) was determined using high-resolution mass spectrometry for compound **2**.[15] Analysis of the ^{13}C and ^1H NMR spectra of compound **2** suggested that this compound also existed as a pair of atropisomers. Although the two atropisomers could not be chromatographically separated, one preferentially crystallized and was characterized by single-crystal X-ray diffraction techniques.[16] The X-ray crystal structure revealed that compound **2**, trivially named akanthomycin, is structurally related to 8-methylpyridoxatin. The major difference between 8-methylpyridoxatin and akanthomycin is the ring expansion of the cyclohexane moiety of 8-methylpyridoxatin to the cycloheptane unit present in akanthomycin. The skeleton of akanthomycin consists of an *N*-hydroxy-2-pyridone system linked through a single bond to a 3-hydroxy-2,3,5,7-tetramethylcycloheptyl unit. In the crystal structure, the two rings are almost perpendicular to each other as seen in the crystal structures of 8-methylpyridoxatin with the C-5 hydroxyl on the same side of the cycloheptyl ring as CH_3-14 as shown in Figure 1. The X-ray defined only the relative

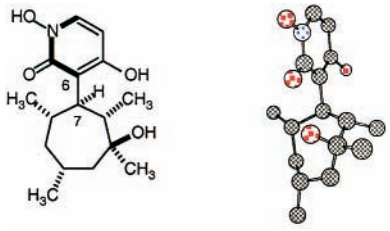


Figure 1. Chemical drawing and computer-generated perspective drawing of akanthomycin (**2**). Hydrogen atoms have been omitted from the perspective drawing for clarity.

stereochemistry, and akanthomycin is arbitrarily drawn to have the same absolute configuration as **1a** and **1b**. In the ^1H NMR of the mixture of rotamers of **2**, significant chemical shift differences of approximately 0.2 ppm are observed for H-7 (δ 2.78 and 3.01) and H-13 (δ 2.47 and 2.65). Similar differences in the chemical shifts for H-7 and H-12 were noted for the rotamers of 8-methylpyridoxatin. Therefore, akanthomycin (**2**) is proposed to exist as a mixture of two atropisomers with restricted rotation around the C-6−C-7 bond. Molecular mechanics calculations suggest that the C-6−C-7 torsional barrier in akanthomycin (**2**) is roughly 10 kcal/mol lower than the C-6−C-7 barrier in **1a**/**1b**.

(11) The entomopathogenic fungus *A. gracilis* was fermented in Sabouraud dextrose broth supplemented with 0.5% yeast extract for 21 days in shake culture. The broth was separated from the mycelium by filtration and each portion was extracted separately.

(12) The sample was dissolved in 90% MeOH in H_2O and extracted 3× with hexanes, then diluted to 80% MeOH in H_2O and extracted 3× with CCl_4, and finally diluted to 60% MeOH in H_2O and extracted 3× with CH_2Cl_2. Kupchan, S. M.; Britton, R. W.; Ziegler, M. F.; Sigel, C. W. *J. Org. Chem.* **1973**, *38*, 178−179.

(13) Cai, P.; Smith, D.; Cunningham, B.; Brown-Shimer, S.; Katz, B.; Pearce, C.; Venables, D.; Houch, D. *J. Nat. Prod.* **1999**, *62*, 397−399.

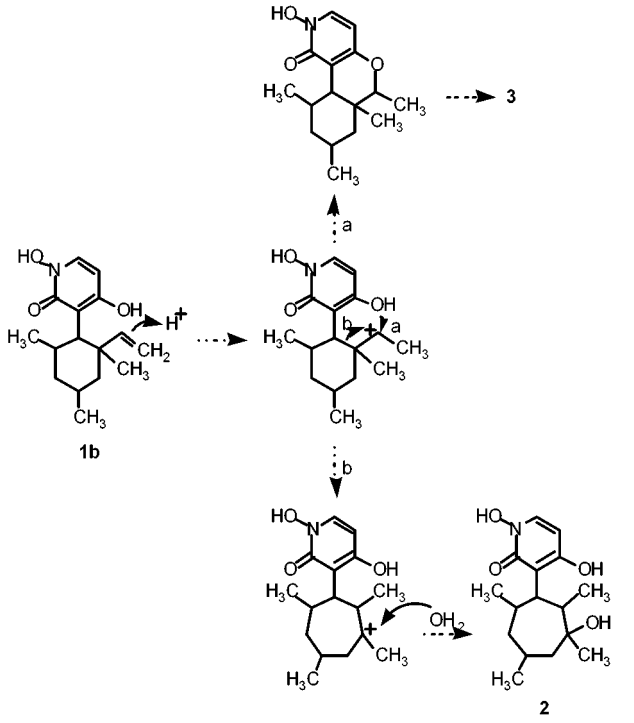
(14) Isaka, M.; Tanticharoen, M.; Kongsaeree, P.; Thebtaranonth, Y. *J. Org. Chem.* **2001**, *66*, 4803−4808.

Analysis of mass spectral, NMR, and X-ray crystallographic data of compound **3** indicated that it is identical to the known compound cordypyridone C.[14] In addition, compound **3** is the *N*-methoxy derivative of PF1140, a broad spectrum antifungal agent isolated from a species of *Eupenicillium*.[17]

Tenellin and bassianin,[6] compounds previously isolated from entomopathogenic fungi, are closely related structurally to the pyridones isolated from *A. gracilis*. Biosynthetic studies on these known compounds showed they are derived from the condensation of phenylalanine with a polyketide chain.[18] To form the pyridone moiety, the phenylalanine rearranges. Compounds **1a**, **1b**, and **3** could be formed in a similar manner with alanine in place of phenylalanine and a cyclization of the polyketide chain to form the cyclohexane system. Akanthomycin (**2**) could then form by oxidative ring expansion of **1b** to yield the cycloheptane ring system as shown in Scheme 1.

The rotamers of 8-methylpyridoxatin, akanthomycin, and cordypyridone C were assayed against *Staphylococcus aureus*. Direct application of each compound on an agar plate containing *S. aureus* indicated that the rotamers of 8-methylpyridoxatin (**1a** and **1b**) are equally potent antibiotics causing growth inhibition at between 25 and 250 ng per application. Akanthomycin (**2**) is slightly less potent with growth inhibition resulting from 250 ng per application. Cordypyridone C (**3**) is the least active compound assayed; 2.5 μg per application is needed to produce a kill zone. This difference in activity between the *N*-hydroxy-2-pyridones and the *N*-methoxy-2-pyridone (**3**) could result from either the formation of the tricyclic ring system or the methylation of the *N*-hydroxyl group. The closely related compound tolypocin, isolated from *Tolypocladium geodes*, is a known bactericidal agent that chelates iron.[19] Its ability to act as a siderophore has been proposed to play a role in the pathogenicity of *T. geodes*. This finding suggests that the observed decrease in antibacterial activity between 8-methylpyridoxatin and cordypyridone C may result from loss of the metal binding upon O-methylation.

Scheme 1. Proposed Biosynthesis of Akanthomycin (**2**)



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Supporting Information Available: ^{1}H and ^{13}C NMR spectra for akanthomycin. Archival X-ray data for **1a**, **1b**, and akanthomycin. This material is available free of charge via the Internet at http://pubs.acs.org.

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(15) **Akanthomycin**: ^{1}H NMR (CD_3OD, 500 MHz) δ 7.58 (1H, d, J = 8.0 Hz, H-3 or H-3′), 7.55 (1H, d, J = 8.0 Hz, H-3 or H-3′), 6.01 (1H, d, J = 8.0 Hz, H-4 or H-4′), 5.94 (1H, d, J = 8.0 Hz, H-4 or H-4′), 3.01 (1H, dd, J = 6.0, 11.0 Hz, H-7 or H-7′), 2.78 (1H, dd, J = 6.0, 11.0 Hz, H-7 or H-7′), 2.65 (1H, m, H-13 or H-13′), 2.47 (1H, m, H-13 or H-13′), 2.06 (4H, m, H-8, H-8′, H-11, H-11′), 1.72 (2H, m, H-12a, H-12′a), 1.62 (4H, m, H-12b, H-12′b, H-10a, H-10′a), 1.15 (3H, s, CH_3-15 or CH_3-15′), 1.10 (3H, s, CH_3-15 or CH_3-15′), 0.98 (2H, m, H-10b, H-10′b), 0.93 (3H, d, J = 6.5 Hz, CH_3-16 or CH_3-16′), 0.92 (3H, d, J = 6.5 Hz, CH_3-16 or CH_3-16′), 0.87 (6H, d, J = 7.5 Hz, CH_3-14, CH_3-14′), 0.63 (3H, d, J = 6.0 Hz, CH_3-17 or CH_3-17′), 0.60 (3H, d, J = 7.0 Hz, CH_3-17 or CH_3-17′); ^{13}C NMR[a] (CD_3OD, 400 MHz) δ 163.3/163.0[b] (C-1/C-1′), 162.4/161.4[b] (C-5/C-5′), 133.2/133.2 (C-3/C-3′), 118.5/118.4 (C-6/C-6′), 100.5/99.5 (C-4/C-4′), 77.3/76.8 (C-9/C-9′), 51.1/50.7[c] (C-10/C-10′), 50.7/50.5[c] (C-12/C-12′), 48.1/47.9 (C-8/C-8′), 45.8/45.6 (C-7/C-7′), 37.5/36.3 (C-13/C-13′), 29.6/29.5[d] (C-11/C-11′), 29.4/28.7[d] (C-15/C-15′), 25.5/25.5 (C-16/C-16′), 23.5/23.4 (C-17/C-17′), 17.6/17.5 (C-14/C-14′), Superscript a designates that signals could not be assigned to specific atropisomer. Superscripts b−d designate signals that may be interchanged. HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{16}H_{26}NO_4$, 296.1861; found, 296.1860.

(16) Akanthomycin (**2**) crystallized in space group $P2_12_12_1$ with a = 9.2802(6), b = 10.9945(8), and c = 32.125(2) Å. The final R-factor was 5.1% for the 2σ data.

(17) Hosoya, R.; Yugami, T.; Yoshida, S.; Yaguchi, T.; Komuro, K.; Sasaki, T. (Meiji Seika Co). Jpn. Kokai Tokkyo Koho JP 07,267,962 [95,267,962], 1995.

(18) Wright, J. L. C.; Vining, L. C.; McInnes, A. G.; Smith, D. G.; Walter, J. A. *Can. J. Biochem.* **1977**, *55*, 678−685.

(19) Jegorov, A.; Matha, V.; Husak, M.; Kratochvil, B.; Stuchlik, J.; Sedmera, P.; Havlicek, V. *J. Chem. Soc., Dalton Trans.* **1993**, 1287−1293.