Fitoterapia xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Fitoterapia

journal homepage: www.elsevier.com/locate/fitote

Induction of new metabolites from the endophytic fungus *Bionectria* sp. through bacterial co-culture

Ramsay S.T. Kamdem[a,b,*], Hao Wang[a], Pascal Wafo[b], Weaam Ebrahim[a,c], Ferhat Can Özkaya[a], Gamall Makhloufi[d], Christoph Janiak[d], Parichat Sureechatchaiyan[e], Matthias U. Kassack[e], Wenhan Lin[f], Zhen Liu[a,**], Peter Proksch[a,**]

[a] Institute of Pharmaceutical Biology and Biotechnology, Heinrich-Heine-University Düsseldorf, Universitätsstrasse 1, 40225 Düsseldorf, Germany
[b] Department of Organic Chemistry, Higher Teachers' Training College, University of Yaounde I, P. O. Box 47, Yaounde, Cameroon
[c] Department of Pharmacognosy, Faculty of Pharmacy, Mansoura University, Mansoura 35516, Egypt
[d] Institute of Inorganic and Structural Chemistry, Heinrich-Heine-University Düsseldorf, Universitätsstrasse 1, 40225 Düsseldorf, Germany
[e] Institute of Pharmaceutical and Medicinal Chemistry, Heinrich-Heine-University Düsseldorf, Universitätsstrasse 1, 40225 Düsseldorf, Germany
[f] State Key Laboratory of Natural and Biomimetic Drugs, Peking University, Beijing 100191, China

ARTICLE INFO

Keywords:
Bionectria sp.
Co-cultivation
Cytotoxicity

ABSTRACT

A new alkaloid, 1,2-dihydrophenopyrrozin (**1**), along with five known compounds (**2–6**) was isolated from an axenic culture of the endophytic fungus, *Bionectria* sp., obtained from seeds of the tropical plant *Raphia taedigera*. Co-cultivation of this fungus either with *Bacillus subtilis* or with *Streptomyces lividans* resulted in the production of two new *o*-aminobenzoic acid derivatives, bionectriamines A and B (**7** and **8**) as well as of two additional known compounds (**9** and **10**). None of the latter compounds (**7–10**) were detected in axenic cultures of the fungus or of the bacteria indicating activation of silent biogenetic gene clusters through co-cultivation with bacteria. The structures of the new compounds were unambiguously determined based on detailed NMR and MS spectroscopic analysis and by comparison with the literature. The crystal structure of agathic acid (**6**) is reported here for the first time. Penicolinate A (**4**) exhibited potent cytotoxic activity against the human ovarian cancer cell line A2780 with an IC_{50} value of 4.1 μM.

1. Introduction

Plant endophytic fungi are microorganisms that thrive in the inner tissues of plants usually without causing apparent harm to their hosts. Endophytes are known to produce a wide plethora of new bioactive secondary metabolites [1]. Tropical and temperate forests represent diverse terrestrial ecosystems, featuring taxonomically diverse endophytic fungi [2]. Since the isolation of the block buster anticancer agent paclitaxel from *Taxomyces andreanae*, formerly known only from *Taxus brevifolia*, endophytic fungi have attracted wide attention of natural product chemists. Fungi of the genus *Bionectria* have been reported to produce numerous new bioactive secondary metabolites such as the antibiotically active compounds verticillin G, bionectrins A-C, the tetramic acid derivatives virgineone and virgineone aglycone, or the cytotoxic pullularins E and F [3–6]. Bacteria and fungi co-exist in many ecosystems such as soil, water, or inside the living tissues of higher plants as endophytes [7,8]. An important interaction between fungi and bacteria is competition for limited nutrients, which is known as a major ecological factor that triggers natural product biosynthesis and accumulation in prokaryotes and eukaryotes alike [9,10]. Co-culture of different microbes rather than maintaining axenic cultures is increasingly practiced in microbial natural product research as the interaction of two or more different microbes may enhance the accumulation of constitutively present natural products [11–13], or may trigger the expression of silent biosynthetic pathways thereby yielding new compounds [14,15].

In the present study, investigation of an axenic culture of the endophytic fungus *Bionectria* sp. fermented on rice afforded one new alkaloid, 1,2-dihydrophenopyrrozin (**1**), along with five known compounds (**2–6**). Co-cultivation of this fungus either with *Bacillus subtilis* or with *Streptomyces lividans* resulted in the production of two new amides, bionectriamines A and B (**7** and **8**) in addition to two known compounds (**9** and **10**). The presence of compounds **7–10** in both co-culture extracts (*Bionectria* sp. with *B. subtilis* or with *S. lividans*)

* Corresponding author at: Institute of Pharmaceutical Biology and Biotechnology, Heinrich-Heine-University Düsseldorf, Universitätsstrasse 1, 40225 Düsseldorf, Germany.
** Corresponding authors.
E-mail addresses: Ramsay.Kamdem@uni-duesseldorf.de (R.S.T. Kamdem), zhenfeizi0@sina.com (Z. Liu), proksch@uni-duesseldorf.de (P. Proksch).

http://dx.doi.org/10.1016/j.fitote.2017.10.021
Received 25 August 2017; Received in revised form 23 October 2017; Accepted 25 October 2017

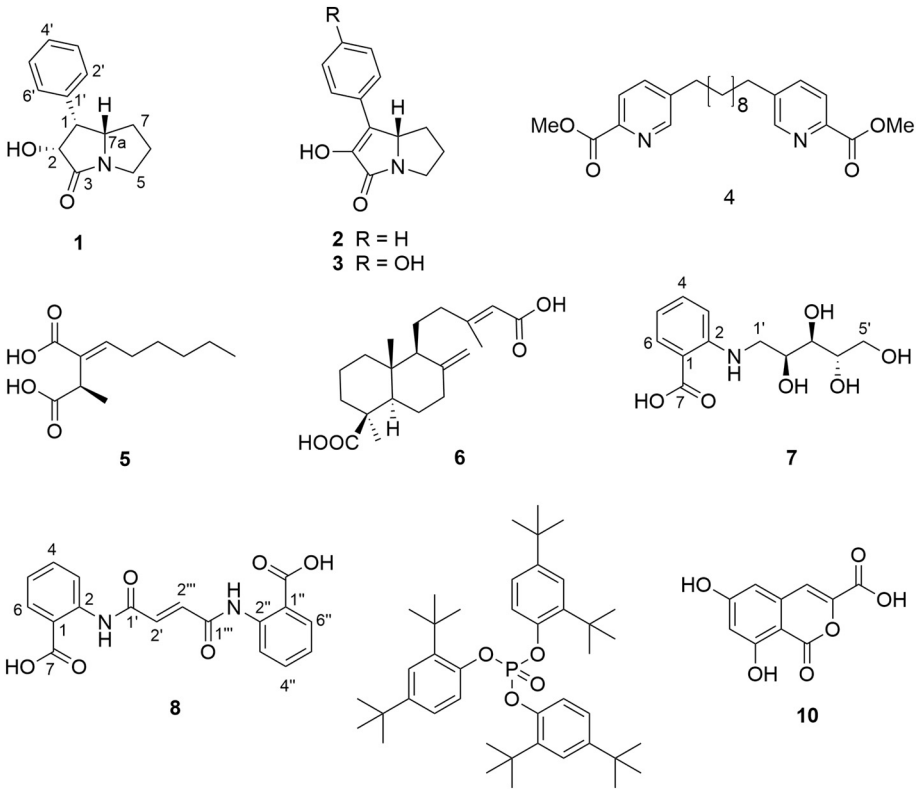

Fig. 1. Structures of isolated compounds.

suggested that they are of fungal origin. Compounds **7–10** were not detected in axenic cultures of the fungus or of the bacteria indicating an activation of silent biogenetic fungal gene clusters through fungal-bacterial co-cultivation. Herein, we report the structure elucidation of the new compounds (**1**, **7** and **8**), the X-ray diffraction study of agathic acid (**6**) and the biological activities of the isolated compounds (Fig. 1).

2. Result and discussion

Compound **1** was isolated as colorless powder. The HRESIMS data of **1** revealed the pseudomolecular ion peak at *m/z* 218.1176 $[M + H]^+$, corresponding to the molecular formula $C_{13}H_{15}NO_2$ with seven degrees of unsaturation. The ^1H NMR spectrum of **1** exhibited five aromatic methines at δ_H 7.28 (H-3′ and 5′, t, J = 7.2 Hz, 2H), 7.25 (H-4′, t, J = 7.2 Hz, 1H) and 7.03 (H-2′ and 6′, d, J = 7.2 Hz, 2H) while the ^{13}C NMR spectrum of **1** showed four peaks of aromatic carbons at δ_C 137.0 (C-1′), 130.8 (C-2′ and 6′), 129.3 (C-3′ and 5′) and 128.2 (C-4′), suggesting the presence of a mono-substituted benzene ring in **1**. This was confirmed by the COSY correlation between H-2′(6′)/H-3′(5′) and H-3′(5′)/H-4′ as well as the HMBC correlations from H-2′(6′) to C-4′, from H-3′(5′) to C-1′, and from H-4′ to C-2′(6′). Apart from these signals, three methylene groups at δ_C 42.5 (C-5), 26.7 (C-7) and 26.5 (C-6), three aliphatic methines at δ_C 75.5 (C-2), 62.3 (C-7a) and 53.2 (C-1), and a carbonyl carbon at δ_C 175.8 (C-3) were observed in the ^{13}C NMR and DEPT spectra of **1**. The COSY correlations between H-2 (δ_H 4.95)/H-1 (δ_H 3.92), H-1/H-7a (δ_H 4.13), H-7a/H_{ab}-7 (δ_H 1.63 and 0.98), H_{ab}-7/H_{ab}-6 (δ_H 1.93 and 1.77), and H_{ab}-6/H_{ab}-5 (δ_H 3.44 and 3.13) established the partial structure C(2)-C(1)-C(7a)-C(7)-C(6)-C(5). The attachment of the mono-substituted benzene ring at C-1 was confirmed by the HMBC correlations from H-2 and H-7a to C-1′, from H-1 to C-1′ and C-2′(6′), and from H-2′(6′) to C-1. Presence of a hydroxy group at C-2 was suggested based on the chemical shifts of C-2 and H-2. The HMBC correlations from H-1 and H-2 to C-3, combined with the molecular formula and the chemical shifts of C-5 and C-7a, indicated the linkages from C-2 to C-5 and C-7a via an amide bond. Thus, the planar structure of **1** was elucidated as shown (Fig. 2). The NOE correlations between H-1/H-2 and H-1/H-7a suggested these protons to be on the same side of the lactam ring. Based on the close biogenetic relationship between 1,2-dihydrophenopyrrozin (**1**) and the two co-isolated analogues (*S*)-phenopyrrozin (**2**) [16,17] and (*S*)-*p*-hydroxyphenopyrrozin (**3**) [17,18], compound **1** is suggested to possess the same absolute configuration at C-7a as the known compounds. Thus, the absolute configuration of **1** is proposed to be (1*R*, 2*R*, 7a*S*).

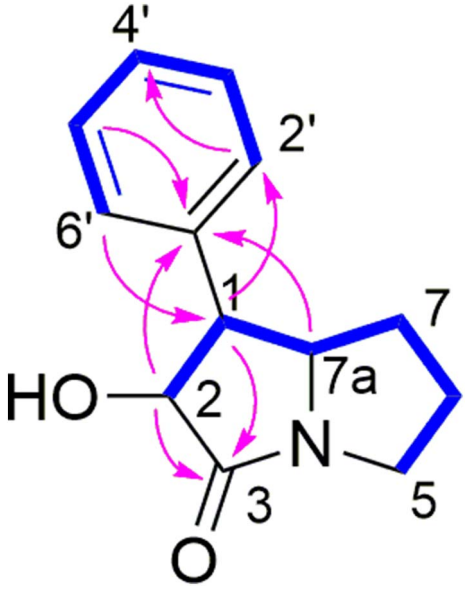

Fig. 2. COSY and key HMBC correlations of **1**.

In addition to the new natural product 1,2-dihydrophenopyrrozin (**1**), five known compounds including (*S*)-phenopyrrozin (**2**) [16,17], (*S*)-*p*-hydroxyphenopyrrozin (**3**) [17,18], penicolinate A (**4**) [18], (*R*)-piliformic acid (**5**) [19,20] and agathic acid (**6**) [21,22] were also isolated from the axenic culture of the endophytic fungus *Pestalotiopsis* sp.