HETEROCYCLES, Vol. 60, No. 7, 2003, pp. 1639 - 1644
Received, 26th March, 2003, Accepted, 16th May, 2003, Published online, 19th May, 2003

A NEW 16*N*-CARBOXYETHYL DERIVATIVE OF 3,12-DIHYDRO-ROQUEFORTIN

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Abstract – New 16*N*-carboxyethyl derivative (**2)** of 3,12-dihydroroquefortin (**1**) was isolated from the fermentation broth of *Penicillium aureovirens* VKM FW-766. Structure of **2** was settled on the basis of MS spectrometry, 1D and 2D NMR spectroscopy.

INTRODUCTION

Roquefortine, 3,12-dihydroroquefortine (**1**), meleagrin and glandicolins A and B are diketopiperazine alkaloids which are formed from tryptophane and histidine as precursors. They are produced by several hyphomycetes belonging to the genus *Penicillium.*[1~3] Representatives of thís type of diketopiperazines display interesting biological activities such as antibiotic and nootropic effects.[4,5]

In the course of our continuing screening for producers of new alkaloids we investigated recently fungal isolates from various permafrost regions of the Russian Federation. Thereby the strain *Penicillium aureovirens* VKM FW-766 was disclosed as producer of roquefortine, and 3,12-dihydroroquefortine (**1**) as the main components of a mixture of co-produced alkaloids.[6] Thus in addition to roquefortin and **1** several components were visible showing distinguishable chromatographic properties. In our searching

for new alkaloids we isolated such minor components and analyzed their structures. Here we report a new *N*-acylated derivative (**2)** of 3,12-dihydroroquefortine (**1**) from *Penicillium aureovirens* VKM FW-766 and its structure elucidation by MS spectrometry and NMR spectroscopy.

RESULTS AND DISCUSSION

Elucidation of structure of **2** (Figure 1) was carried out by optical spectroscopy (UV-VIS and IR spectrum, polarimetry), MS spectrometry (HREI-MS, ESI-MS) and 1D/2D NMR spectroscopy. In the UV-VIS spectrum of **2** absorbances were visible with λ_{max} 208, 239 and 301 nm as reported, too, for 3,12-dihydroroquefortine (**1**). However compound (**2)** differed in chromatographic properties from roquefortine (**2**: R_f 0.42 (solvent 1, Table 1) and 0.23 (solvent 2; Table 1); **1**: (R_f 0.17, solvent 2, Table1)). The FTIR spectrum of **2** showed ν_{max} 1663 cm^{-1} attributable to a diketopiperazine structure. But in addition ν_{max} 1763 cm^{-1} suggested the occurrence of an additional carbonyl or carboxyl group.

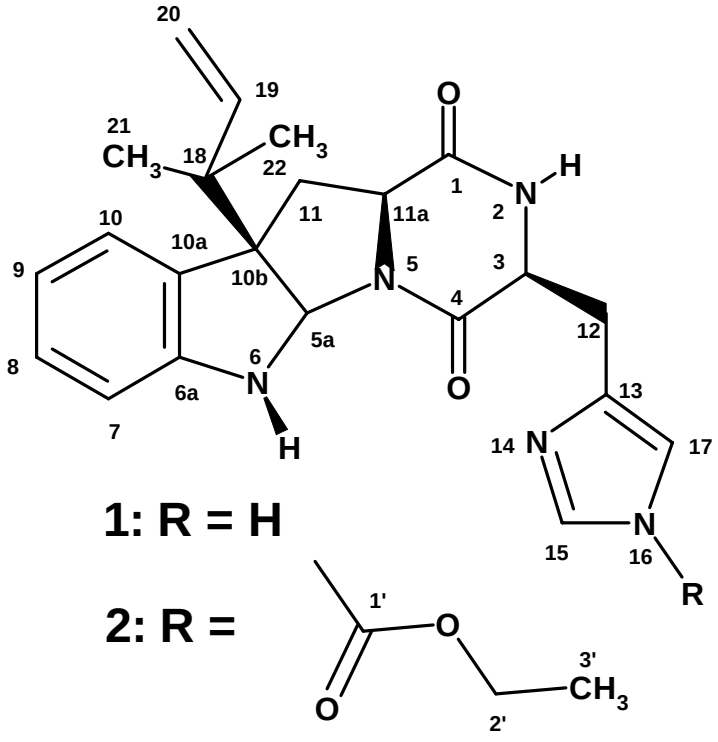

Figure 1: Structure of 3,12-dihydroroquefortin (**1**) and new 16*N*-carboxyethyldihydroroquefortin (**2**)

Table 2:

Assignment of ^{1}H and ^{13}C NMR spectra of 16*N*-carboxyethyldihydroroquefortin (**2**) (in $CDCl_3$, chemical shifts (δ) in ppm).

position	δ_C	δ_H
1	165.6 (s)	-
2	-	4.27 br s
3	55.5 (d)	4.10 br dd
4	169.0 (s)	-
5a	77.8 (d)	5.52 s
6	-	4.30 br s
6a	149.9 (s)	-
7	109.2 (d)	6.57 d, 8.1
8	128.9 (d)	7.08 dd, 7.7, 8.1
9	118.9 (d)	6.74 dd, 7.7; 7.4
10	125.1 (d)	7.13 d, 7.4
10a	129.1 (s)	-
10b	61.5 (s)	-
11	36.0 (t)	H_A 2.46 dd; 12.6, 9.5; H_B 2.54 dd, 12.6, 6.5
11a	53.3 (d)	3.98 dd, 9.5, 6.5
12	28.1 (t)	H_A 2.8 dd, 15.2, 9.5 H_B 3.40 dd, 15.2, 3.1
13	139.3 (s)	-
15	137.1 (d)	8.03 br s
17	114.6 (d)	7.25 br s
18	40.5 (s)	-
19	143.5 (s)	-
20	114.5 (t)	H_A 5.05 dd, 16.5, 1.0; H_B 5.10 dd, 9.5, 1.0
21	22.4 (q)	1.01 s
22	22.9 (q)	1.00 s
1‘	148.3 (s)	-
2‘	64.7 (t)	4.45 q, 7.2
21	14.2 (q)	1.42 t, 7.2

ESI-MS of **2** furnished m/z 464.3 ($[M+H]^+$) and 926.8 ($[2M+H]^+$). HREI-MS afforded m/z 463.2237 (M^+, 30 %; Calcd 463.2254 for $C_{25}H_{29}N_5O_4$) suggesting the presence of nine double bonds or rings. In addition to M^+ diagnostic fragments were visible such as m/z 394.1527 ($M-C_5H_9$; Calcd $C_{20}H_{20}N_5O_4$; 100 %) and m/z 322.0 ($M^+-C_5H_9$;- $C_3H_5O_3$).

Conclusive evidence for the structure of **2** as shown in Figure 1 was furnished by 1D and 2D NMR spectroscopy (^1H, ^{13}C, DEPT, HMQC, HMBC, NOESY). In the ^{13}C and DEPT NMR spectra 25 carbon atoms (Table 2) and their bonding type were visible (11 double bond carbons, 3 carbonyls (165.6 ppm, 169.0 ppm, 148.3 ppm), 7 CH and/or CH_3 groups, 2 methylenes and two additional quaternary carbons (C-11b: 61.5 ppm; C-18: 40.5 ppm)). The unusual upfield shift of C-1' was readily ascribed to the presence of an urethano structure. One of the double bond carbons (C-20; 145.5 ppm) appeared as triplet. Moreover, downfield shifted carbon signals at 55.5 ppm, 55.4 ppm, 64.7 ppm and 77.8 ppm suggested the occurrence of several heteroatom-bonded carbons. The latter signal was assigned to a cyclic aminal structure.

The ^1H and $^1H,^1H$-COSY spectra suggested partial structures for the benzene ring, the ethene substitutent at C-18, methylene groups attached to the diketopiperazine ring and the ethyl ester group (Table 1). C,H long-range spectra (HMBC) furnished diagnostic couplings as shown in Figure 2 which enabled full assignment of the dihydroroquefortine moiety of **2**. But no connectivities were visible confirming the presence of a carboxyethyl substituent at 16-N. However evidence was furnished by the NOESY correlations found between H-2' and H-3', at the one side, and H-15 and H-17, respectively, at the other side (Figure 2). Negative optical rotation of **2** ($[\alpha]_D$ -66.8 ^{o} in MeOH, see Table 1) was determined as was shown for **1** ($[\alpha]_D$ - 370 ^{o}, pyridine), too, suggesting that **2** possesses the same stereochemistry as **1**. Thus **2** differs only by the side chain at 16-N of **1**.

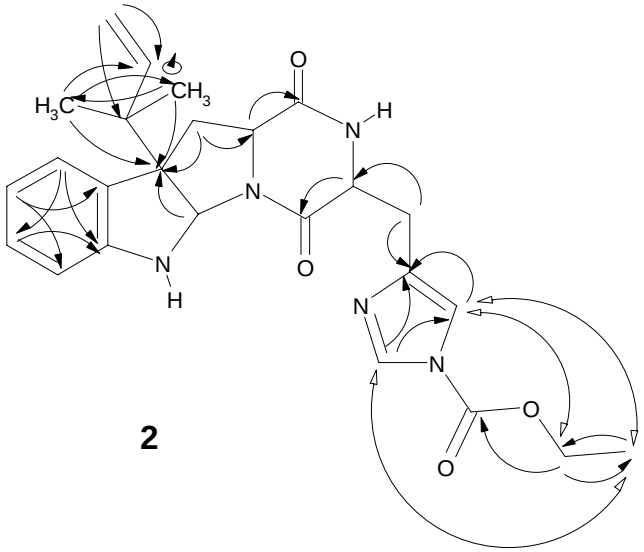

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Figure 2: Instructive C,H long-range couplings (HMBC, arrows at one side) and NOESY correlations (arrows at both sides) in the NMR spectra of **2**

Compound (**1)** (> 200 µg/mL) displayed moderate inhibitory activity against *Sporobolomyces salmonicolor* 549 during the common agar well diffusion[6] assay (50 µL per agar well) but was inactive against bacteria and yeasts.

EXPERIMENTAL

The strain *Penicillium aureovirens* VKM FW-766 obtained from the permafrost region of Northern Russia was deposited in the All Russian Culture Collection of Microorganisms (VKM, Pushchino, Moscow region, Russia). Cultivation was carried out at 24 °C on rotary shakers (220 r.p.m.) in Erlenmeyer flasks (750 mL) containing 150 mL medium. The medium was composed as follows (g/L) mannitol 50, succinic acid 5.4, $MgSO_4 \cdot 7\ H_2O$ 0.3, KH_2PO_4 1.0 (pH adjusted to 5.2 by addition of 25 % ammonia solution). Inoculation occurred with spore suspensions. After 11 - 13 days of cultivation the metabolites were extracted. First the culture broth was adjusted to pH 4 - 5 by 2 % tartaric acid. Thereafter the broth was extracted threetimes by chloroform as to remove lipids and acidic metabolites. These first extracts were discarded. Subsequently, the pH was brought to 8 - 9 by conc. NH_4OH, and again extraction occurred three-times by $CHCl_3$. The combined organic layer was dried over Na_2SO_4, and evaporated in vacuum. The residue of the evaporated extract was subjected to column chromatography on silica gel 60 (Merck, 0.063 - 0.1 mm). Elution was done first by $CHCl_3$/MeOH/25 % aqueous ammonia (90:10:0.1; v/v) and subsequently by the same solvent mixture but in ratio 80:20:0.2 (v/v). Thereby 25 mg of **2** was obtained as waxy solid. (see Table 1).

Table 1: Physicochemical properties of 16*N*-carboxyethyl-3,12-dihydroroquefortin (**2**)

Appearance:	wax
Molecular weight:	463
HREI-MS:	463.2237 (Calcd 463.2254)
Chemical formula:	$C_{25}H_{29}N_5O_4$
UV-VIS ($\lambda_{max.}$; nm, MeOH):	210, 239, 300
IR ($\nu_{max.}$, cm^{-1}, film):	923, 1019, 1080, 1214, 1244, 1408, 1663, 1763, 2850, 2950, 3300
$[\alpha]_D^{22}$ (l = 0.5 cm 10.1 mg/mL MeOH):	- 66.8°
R_f on TLC (silica gel aluminium sheets Merck)	
Solvent 1: $CHCl_3$-MeOH-conc. NH_4OH: (80:20:0.2):	0.85
Solvent 2: $CHCl_3$-MeOH-conc. NH_4OH (90:10:0.1):	0.75

ACKNOWLEDGEMENT

Support of this work by Deutsche Forschungsgemeinschaft DFG (Bonn, Germany, 436 RUS 113/677/1-1) is gratefully acknowledged.

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