Rapid Structural Elucidation of Microbial Cyclopeptides with AntiBase

Felix Huth[1], Iris Grün-Wollny[2] und Hartmut Laatsch[1] *

[1]Institut für Organische Chemie der Universität Göttingen, Tammannstr. 2, 37077 Göttingen, Germany
[2]Labor für Bodenmikrobiologie, Burggarten 9, D-35102 Lohra-Kirchvers, Germany

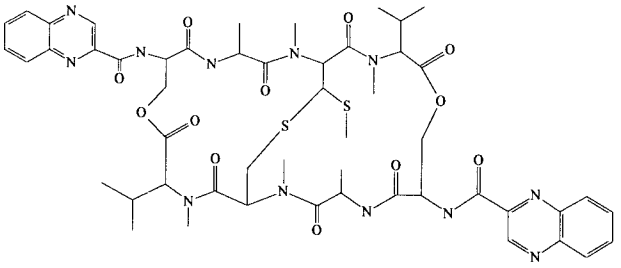
Analytical natural products research is a job with high risk: More than 20,000 natural products from microorganisms are known, and every year more than 700 new compounds from microorganisms are added. Therefore the chance to isolate a new metabolite is decreasing, while the probability to re-isolate known compounds increases.

To minimize this risk, we developed AntiBase, a ChemBase® or IsisBase® supported database, of microbial natural products. Substructures or spectroscopic data searched in this pool lead reliable to the correct structure. It is possible to search for molecular mass, molecular formulae, coupling-pattern in ^{1}H-NMR, ^{13}C-values, chromophors or biological activities. Structure elements not present in the molecule can be excluded in further searches. In most cases the database helps to find at least the type of a new structure.

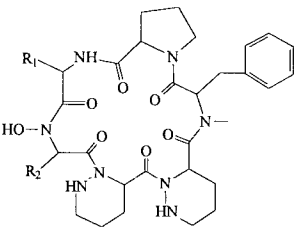
In our course of screening for secondary metabolites we found the strain GW 8/1753 to be active against gram-positive and gram-negative bacteria. The strain produced an ethyl acetate extractable mixture of antibiotics, which was seperated by a sequence of chromatographic steps leading to four pure compounds.

For substance **1**, the ^{1}H-NMR data were sufficient for identification: The search for 1 N-methyl group, 1 C_{quart} methyl and at least 1 phenyl ring reduced the number of positive hits from 21,000 to 79. The exclusion of fragments not present in the molecule (ketone carbonyls, methoxy groups) further reduced the number of compounds to 33. All of these were peptides, 16 were analogues of echinomycine [1], but only the latter fitted all the data. The second compound gave phenyl alanine and proline in an 1:1 ratio after hydrolysis in the amino acid analysis. A search in AntiBase for phenyl alanine, proline and for each one CH-CH_3, CH_2-CH_3, N-CH_3 fragment led to 63 hits. The molecular mass fitted only with L-156373 [2], an oxytocin and vasopressin antagonist.

Two further peptides proved to be structural analogues of L-156373 as comparison of the ^{1}H-NMR data showed. Extensive NMR measurements (H,H-COSY, HMQC, HMBC) identified the two compounds to have the structure **3** and **4**.

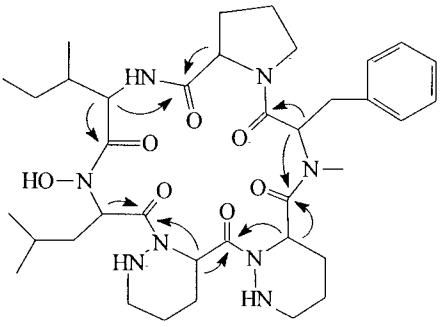


Echinomycin (**1**)



	L-156373 (**2**)	**3**	**4**
R1	Bn	2-Bu	Bn
R2	2-Bu	i-Bu	i-Pr

Selected long range correlation signals (HMBC) of **3**



3

Experimental

The strain was fermentated in a malt/yeast/glucose medium for 72 h at 28 °C. The ethyl acetate extract was separated by silica gel 60 column chromatography to five fractions. The active fraction was re-chromatographed on Sephadex LH-20. Further separation by preparative TLC followed by preparative RP-HPLC led to the four peptides in pure state.

[1] A. Dell et al., *J. Am. Chem. Soc.* **97**, 1975, 2497-2502

[2] M. Goetz et al., *Eur. Pat. Appl. EP 327*,**744**, (Cl C12P21704), 8/16/1989