《CHEMISTRY & BIODIVERSITY》

NewPyran-2-ones from Alkalophilic Actinomycete, Nocardiopsis alkaliphila sp. Nov. YIM-80379

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1620183003

2016.11.26

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Section A-Abstract and Knowledge introductions

1）Abstract

2）Learning More

1、Abstract

Two new pyran-2-ones, nocardiopyrones（吡喃酮） **A** and **B** (**1** and **2**, resp.), along with four known compounds, pyridinols（吡啶酚） 3–5, and 1-acetyl（乙酰）-β-carboline（咔啉） were isolated from the alkalophilic actinomycete（嗜碱性放线菌） Nocardiopsis alkaliphila（嗜碱性拟诺卡氏菌属） sp. **nov. YIM-80379.**

Relevant experimental methods：
spectroscopic analysis,
CD spectra,
the quantum-chemical ECD calculation（量子化学电子捕获计算），
HR-ESI-MS，
HPLC……

Knowledge introductions

1）What is Pyran?

Pyran is completely containing one atom of oxygen unsaturated six-member heterocyclic compounds. Not replace pyran has not been found in nature, its value is not big, but pyran derivatives（衍生物）, especially pyrone, widely exists in many natural natural substances.

For example: Coumarin（香豆素）、 Flavones（黄酮）、 Isoflavones（异黄酮）、 anthocyanidin（花青素）

2） Characteristics of the Nocardiopsis

Base yarn breaking into aureus corpuscle.

Section of the wire breaking into different size.

Aerobic（需氧）.

Gram positive.

No acid.

Etc.

1、Abstract

Compounds 1 and 2 showed weak antibacterial activities against Pseudomonas aeruginosa（绿脓假单胞菌）, Enterobacter aerogenes（产气肠杆菌）, and Escherichia coli（大肠杆菌） with MIC values of 20–48 μM. Compound 2 showed weak antimicrobial activities against Candida albicans（白色念珠菌） and Staphylococcus aureus（金黄色酿脓葡萄球菌） with MIC values of 24 and 48 μM, respectively.

MIC: Minimum inhibitory concentration（最小抑菌浓度）

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Section B-Introduction

1）Generate and environment

2）Simple examples

3）Chemical structures of compounds

1、 Generate and environment

Alkalophilic microorganisms as a kind of extreme microbes can grow optimally最适宜 at pH values above 8.5 ,and maintain pH homeostasis稳态 by Na + (Li +)(K +)/H + antiporters（逆向运输蛋白） to adapt the high-pH environment . Previously, they identified a new alkalophilic actinomycete strain that they named as Nocardiopsis alkaliphila sp. nov.YIM-80379 from a desert soil sample collected in Egypt .

- Some genes encoding（编码） the enzymes（酶） of secondary metabolism can be controlled by the microbial regulatory system in the microorganisms

2）For instance

1、The *ipn* A gene encoding isopenicillin（异青霉素） N synthetase（合成酶） in the fungus *Aspergillus nidulans* （*构巢曲霉*）is under the control of the pH-regulatory system, and external外部 alkaline pH can make the strain produce higher levels of penicillins （青霉素）. These suggested that the secondary metabolites of microorganisms might be regulated by external pH. In some cases, the pH effects were stronger than C and N effects.

2、 Since 1980, some new antibiotics抗生素 have been identified from alkalophilic microorganisms, such as 1907-II, 1907-VIII. Interestingly, when alkalophilic strain 1907 was cultured at neutral中性 pH, the cells grew well, but none of 1907-II and 1907-VIII was detected in the broth.

IPN(Interpenetrating Polymer Network)
即共混聚合物，不同聚合物分子相互缠结形成一个整体，但聚合物 I 和 II 之间未发生化学键结合。

3）Chemical structures of compounds

Chemical studies on secondary metabolites at pH 10 resulted in the isolation and identification of two new pyran-2-ones, nocardiopyrones A and B (1 and 2, resp.) and four known compounds, pyridin-3-ol (3) ,6-methylpyridin-3-ol (4) ,5-methylpyridin-3-ol (5) ,and 1-acetyl-b-carboline (6) (Fig. 1).

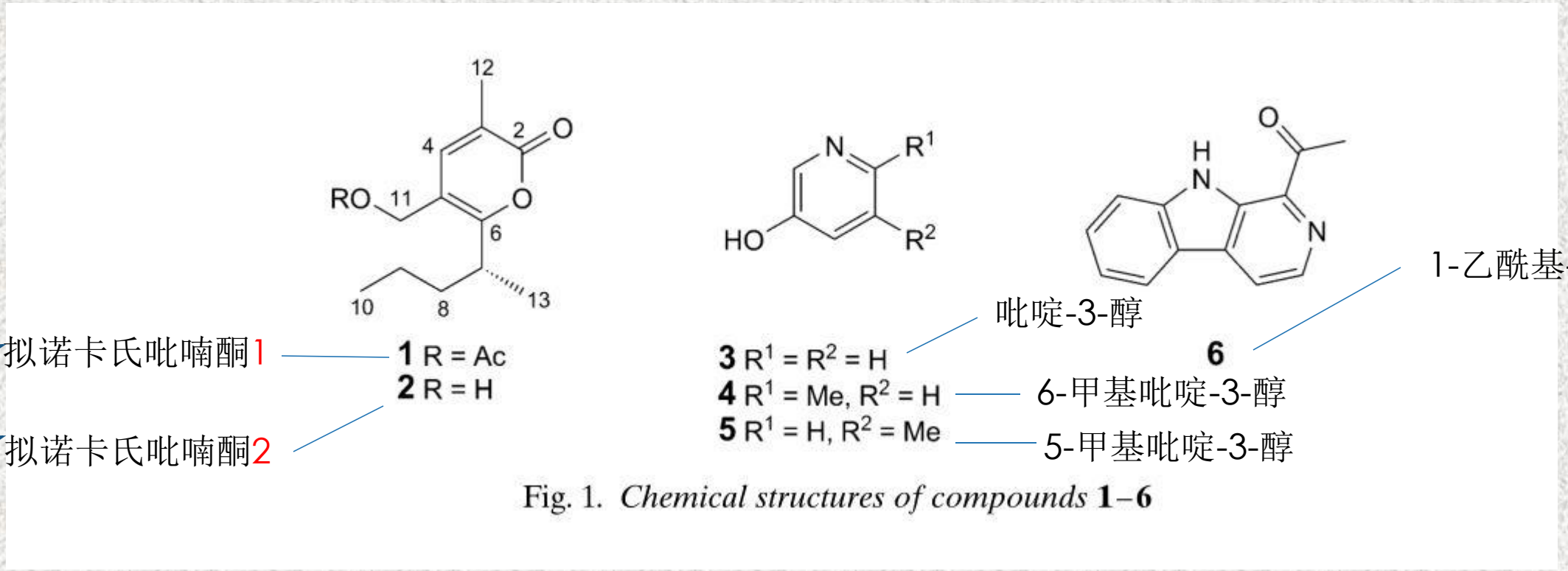

Fig. 1. *Chemical structures of compounds* **1–6**

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Section C-Experimental Part

1）Fermentation, Extraction(Crude extract)

2）Purification

1）Fermentation, Extraction(Crude extract)

containing liquid medium composed of glucose (10 g/l), yeast extract(10 g/l), beef extract(4g/l), and peptone (4 g/l) after adjusting its pH to 10.0

soil sample collected from the eastern desert of Egypt

shaky conditions at 180 rpm and 28°C for 14 d in 500ml Erlenmeyer flasks

filtered through cheese cloth to separate filtrate and mycelia

mycelia

filtrate

extracted with AcOEt

extracted with acetone

concentrated under reduced pressure to afford an aq. soln

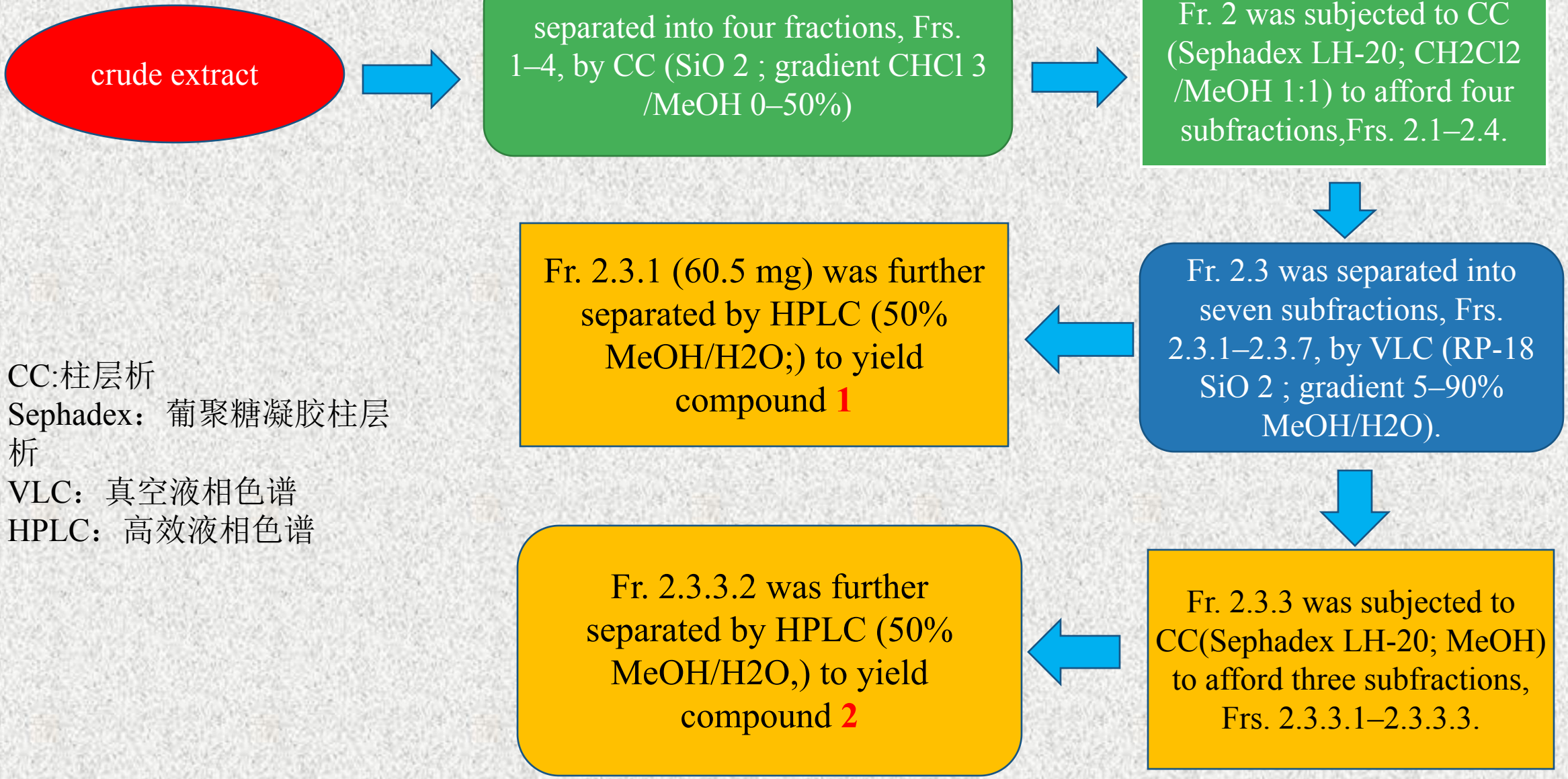
extracted three times with AcOEt to give another AcOEt soln

combined and concentrated under reduced pressure

crude extract

△ Aq.即水溶液

2）Purification



CC:柱层析
Sephadex：葡聚糖凝胶柱层析
VLC：真空液相色谱
HPLC：高效液相色谱

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Section D-Result and Discussion

1）Structure Elucidation. N. alkaliphila sp. nov. YIM-80379

2）Antimicrobial Activity

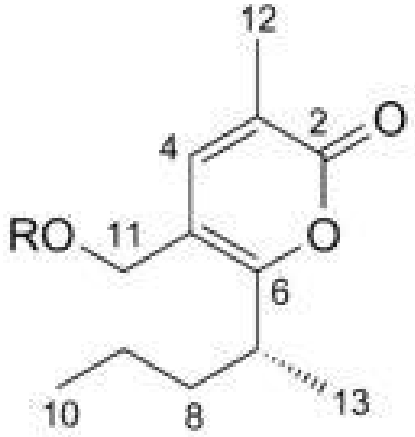
1）Structure Elucidation. N. alkaliphila sp. nov. YIM-80379

Nocardiopyrone **A (1)** was obtained as a yellow oil. Its molecular formula was determined as C14H20O4 by HR-ESI-MS， which accounts for five degrees of unsaturation.

Nocardiopyrone **B (2)** gave an HR-ESI-MS corresponding to the molecular formula C12H18O3 . The1 H- and 13 C-NMR data of 2were quite similar to those of 1 except for the replacement of the AcO signals by a OH signal.

Table 1. *^{1}H- and ^{13}C-NMR Data* (at 600 and 150 MHz, resp.; in (D_6)DMSO) *for Nocardiopyrones* A *and* B (**1** and **2**, resp.). Atom numbering as indicated in *Fig. 1.*

Position	**1**		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
2	–	163.0 (*s*)	–	163.0 (*s*)
3	–	121.9 (*s*)	–	121.6 (*s*)
4	7.36 (*q*, *J*=1.3)	143.6 (*d*)	7.34 (*s*)	143.5 (*d*)
5	–	111.0 (*s*)	–	115.7 (*s*)
6	–	165.8 (*s*)	–	163.4 (*s*)
7	2.97 (*ddq*, *J*=10.1, 8.9, 6.8)	34.2 (*d*)	2.93 (*ddq*, *J*=10.1, 8.8, 6.6)	33.7 (*d*)
8	1.43 (*ddt*, *J*=13.3, 10.0, 5.6), 1.55 (*ddt*, *J*=13.2, 9.0, 5.5)	36.8 (*t*)	1.41 (*ddt*, *J*=13.3, 10.0, 5.8), 1.54 (*ddt*, *J*=13.2, 9.0, 5.6)	36.9 (*t*)
9	1.14, 1.22 (2*m*)	20.6 (*t*)	1.14, 1.22 (2*m*)	20.6 (*t*)
10	0.84 (*t*, *J*=7.4)	14.4 (*q*)	0.83 (*t*, *J*=7.3)	14.4 (*q*)
11	4.84, 4.82 (2*d*, *J*=12.4)	61.1 (*t*)	4.23, 4.18 (2*dd*, *J*=12.4, 5.0)	58.0 (*t*)
12	1.95 (*d*, *J*=1.2)	16.4 (*q*)	1.95 (*s*)	16.5 (*q*)
13	1.13 (*d*, *J*=6.8)	19.3 (*q*)	1.11 (*d*, *J*=6.6)	19.2 (*q*)
11	–	–	5.10 (*t*, *J*=5.0)	–
Ac	2.02 (*s*)	21.2 (*q*), 170.8 (*s*)	–	–



1 R = Ac
2 R = H

1）Structure Elucidation. N. alkaliphila sp. nov. YIM-80379

How about the recorded CD and calculated ECD spectra of **1** and **2**?

The recorded CD curve of **1** showed Cotton effects at 231 and 300 nm,in accordance with the calculated ECD curve of (R)-**1** and opposite to the calculated ECD curve of (S)-**1** (Fig. 3), indicating (R)-configuration of **1**.

The CD Cotton effects of **2** at 193 、 231 and 301nm were similar to those of **1**, in accordance with the calculated ECD curve of (R)-**2** (Fig. 3), indicating that **2** has the same absolute configuration as **1**.

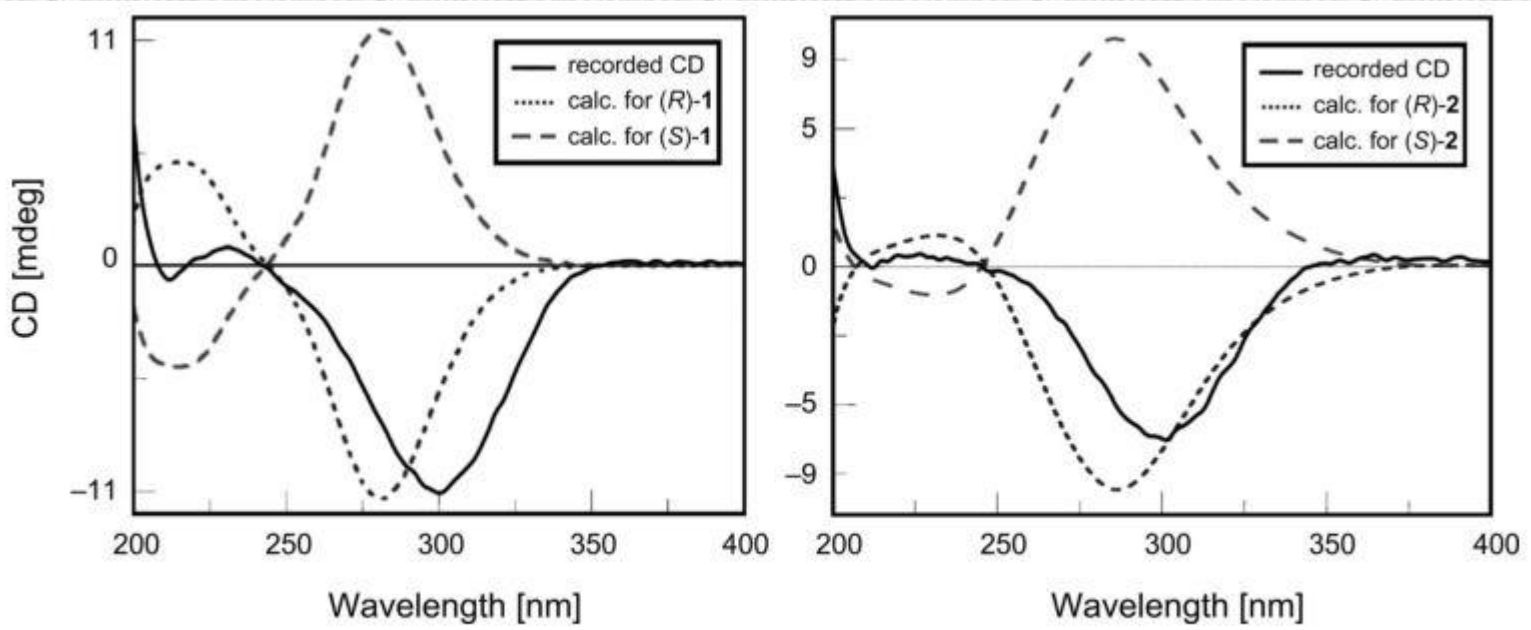


Fig. 3. *The recorded CD and calculated ECD spectra of* **1** *and* **2**

CD:圆二色散吸收光谱法

ECD spectra： 电子捕获光谱

2）Antimicrobial Activity

The antimicrobial activities of the new isolates **1** and **2** against *Pseudomonas aeruginosa*（绿脓杆菌）, *Enterobacter aerogenes*（产气肠杆菌）, *Escherichia coli*（大肠杆菌）, *Staphylococcus aureus*（金黄色葡萄球菌）, and *Candida albicans*（白色念珠菌）were evaluated.

Table 2. *Antimicrobial Activities* (*MIC* [μM]) *of Nocardiopyrones A and B* (**1** and **2**, resp.)

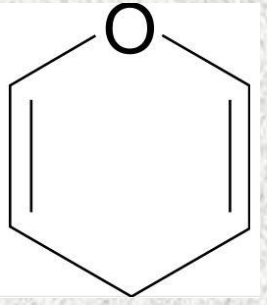
Compound	*P. aeruginosa*	*E. aerogenes*	*E. coli*	*S. aureus*	*C. albicans*
1	20	40	40	>100	>100
2	48	48	48	48	24
Ciprofloxacin	1.9	3.7	0.93	30	–
Ketoconazole	–	–	–	–	0.18

The results indicated that the removal of the Ac group increased the antifungal activity on C. albicans.

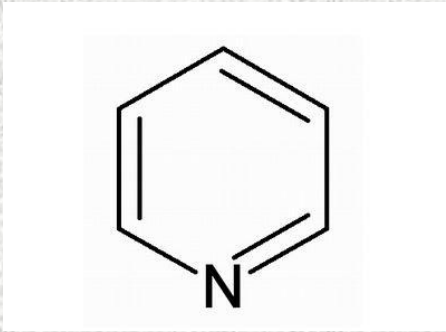
Ciprofloxacin：环丙沙星、环丙氟哌酸

Ketoconazole：酮康唑

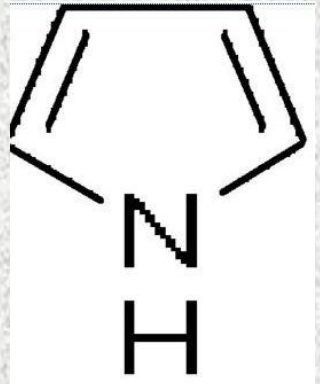
吡喃、吡啶、吡咯与咔啉



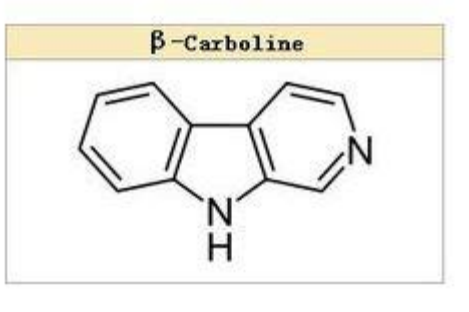
吡喃的电子结构与苯系类似，环中氧原子具有极强的碱性，成盐后，即被稳定下来。许多重要的天然物如色素、糖、抗生素、生物碱，均含有吡喃或吡喃盐的环系。



吡啶，有机化合物，是含有一个氮杂原子的六元杂环化合物。可以看做苯分子中的一个（CH）被N取代的化合物，故又称氮苯，无色或微黄色液体，有恶臭。吡啶可用作变性剂、助染剂，以及合成一系列产品（包括药品、消毒剂、染料等）的原料



吡咯，是含有一个氮杂原子的五元杂环化合物，其分子式为C_4H_5N。其衍生物广泛用作有机合成、医药、农药、香料。用作色谱分析标准物质，也用于有机合成及制药工业。



咔啉，又称二氮杂芴。吡啶环与吲哚的吡咯环稠合的杂环化合物。用作有机合成试剂

谢谢观赏

请多多批评指正

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2016.11.26