Separating and characterizing products generated by Nocardia cyriacigeorgica

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Introduction

Nocardia cyriacigeorgica, commonly found in the soil, is a bacterium belonging to actinomycetales which produces a pigment as well as other compounds and may inhibit the Ubiquitin/proteasome system. The proteasome destroys abnormal proteins in peptides, as long as ubiquitin marked them. If this system is faulty, in association with other factors, the patient may develop Parkinson's disease. The task I was given during my placement at the CNRS (National Center of Scientific Research) within the Research Unit in microbial ecology, was to isolate the pigment and the damaging compounds of culture nutrient broth to study the pathology of this bacterium.

Experimental method

The different analyses which were carried out on Nocardia samples showed that the compounds had a high polarity and lie in the aqueous phase. A lot of HPLC columns were tested to find the appropriate column which could separate or isolate any Nocardia's compounds. To be attractive, these compounds mustn't be present in the non inoculated medium.
The column which had the best retention was the Eclipse Xtreme Dense Bonding (XDB) of Agilent, grafted C18, which is a not end capped HPLC column. Its dimensions are 250mm*4.6mm with a particle size of 5µm. To obtain a good separation between the different peaks, a gradient of solvent was used as shown in table 1. All solvents were acidified by one percent of formic acid because acid refines peaks and increases retention. Moreover the flow was regulated at 0.8 mL/min.
First, compounds of the nutrient broth were separated on Sephadex's column (G25) and all fractions were injected in High Pressure Liquid Chromatography (HPLC) using previous parameters. Sephadex's column sorted compounds by molecular size and mass and was an important step to purify the sample. The fraction containing attractive compounds was injected again several times in HPLC to collect peaks' characteristics by their retention times and to concentrate the solution to obtain a more important mass of pure compounds. Then the new pure sample was analysed with a mass spectrometer and in further analyses biological tests should be realised.

gradient of solvant		
time (min)	%water	%metanol
0	100	0
4	100	0
25	70	30
35	0	100
40	0	100
41	100	0
47	100	0

Table 1: Proportions of solvents used in HPLC column

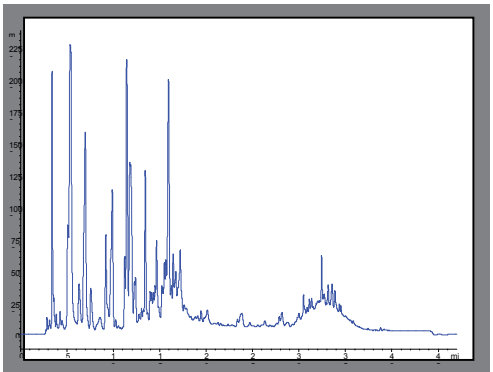

Figure 1 : Chromatogram of a Nocadia's fraction nutrient broth

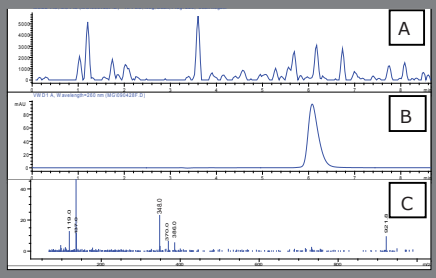

Figure 2 : LCMS of a Nocardia product

Results and discussion

Figure 1 presents the chromatogram of the global attractive fraction after Sephadex separation at 260 nm.
On figure 2, the chromatogram A represents the positive total ionization current using a mass spectrometer which contained an electrospray source and a quadripole analyser. The chromatogram B shows the absorption of the pure compound at 260 nm. Figure 2C shows the mass spectrum of selected peak B.
Finally, a mass of 347u was obtained for this compound.
If this pure new sample is biologically active, scientists will soon be searching for its structure thanks to its molecular mass.

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