Extraction and analysis of an anti-leishmaniasis natural substance from a Guyanese plant in NMR

Mayka YA NYIA

Introduction

Leishmaniasis is a skin disease, which is caused by an intracellular parasite, leishmania. It is generally transmitted by the bite of a female of the phlebotominae family.
Irlbachia alata (I.alata) is an ubiquitous herb found in Central and Tropical South America. The plant is commonly used by indigenous people of the Amazon for treating skin sores, dermatological fungal infections and vaginal thrush. This plant contains an interesting molecule "irlbacholine" (figure 1) with potent antifungal activity, but this molecule has never been tested on the parasite leishmania. The effective usage of I.alata may be explained by an activity linked to the presence of irlbacholine. The aim of this analysis was to detect irlbacholine's presence in an extract (enriched in irlbacholine).

Experimental section

Isolation of Irlbacholine from I.alata
An attempt of isolation of irlbacholine is made by following parts of a protocol of a paper [1]. 50g of crushed and dried flowers of I.alata were mechanically stirred for 63h at room temperature (rt), with IPA-water (1:1; 400mL) The solution was filtered and the filtrate was dried. The extract (9,13g) was dissolved in water (210mL) and partitioned against 1-butanol (3x70mL) and the combined butanol phases were concentrated. The butanol extract (3.14g) was washed with acetone (62mL) under vigorous mechanical stirring for 2h at rt, and the insoluble portion was filtered, dried and then partitioned between CH_2Cl_2-IPA-water (2:3:3; 80mL). The phases were allowed to separate overnight, and then the aqueous layer was dried.

Analysis of the extract enriched in irlbacholine by NMR.
A small quantity of the dried extract was dissolved in CD_3OD in a tube of 5 mm. Proton ^1H and Phosphor ^{31}P were recorded on a 400MHz spectrometer. NMR spectrums were treated with the software "ACD-NMR". Chemical movements are measures in ppm, compared with the TMS chemical movement. ^1H NMR theoretical spectrum of irlbacholine was performed by the software "Chemdraw", in order to make a comparison with the NMR spectrum of the extract and with the one of the paper.

Results

As shown in the figure 2, identifications signals of the theoretical ^1H NMR spectrum of irlbacholine (by chemdraw) are similar to the ^1H NMR spectrum of the paper, but a moderate scatter of chemical movements is observed.
The NMR spectrum of direct correlation ^{31}P-^1H, as shown in the figure 3, there are two spots which correspond to interactions between ^{31}P with two ^1H. These two ^1H are situated in 3.85 and 4.25ppm, they correspond respectively to the positions 4 and 3 of irlbacholine'structure. There is a strong probability that irlbacholine is present in this sample.

Conclusion

NRM is a technique which allows identifying molecular structure. Although these results do not allow having purely NMR spectra of irlbacholine, some signals can show the presence of the studied molecule. In an extract of a natural substance there are many molecules which can be observed in NMR and interfere with the irlbacholine'signals.

Figure 1: Structure of irlbacholine

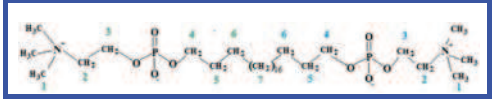
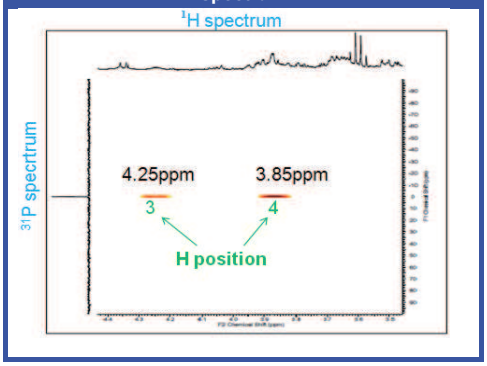


Figure 2: Chemical movement δ (ppm) of ^1H NMR spectrum of the extract enriched in irlbacholine

Chemical movement δ (ppm) of ^1H NMR spectrum		
Chemdraw	Paper	Identification of H
1,26	1,30	H7
1,43	1,38	H6
1,71	1,64	H5
3,30	3,22	H1
3,61	3,63	H2
4,07	3,87	H4
4,47	4,25	H3

Figure 3: Direct correlation ^{31}P-^1H NMR spectrum



[1] Bierer et al, «Isolation, Structure Elucidation, and Synthesis of Irlbacholine: A Novel Antifungal Plant Metabolite from Irlbacha alata and Anthocleista djalonensis», J.Org.Chem, 1995, 60, p 7022-702

CNRS-UMR EcoFoG
43 av L.Pasteur
97300 Cayenne