Identification of a Bioactive Compound from *Myrcianthes cysplatensis*

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ABSTRACT

Myrcianthes cisplatensis (Cambess.) O. Berg (Myrtaceae) grows freely in Uruguay especially in the banks of rivers and streams. It is locally known as guayabo colorado and it´s fruits are edible and used for the preparation of marmalades.

In this work we present the results of the bioguided isolation and structural elucidation of the main active compound as well as it´s antibacterial activity. Through repeated chromatography a pure compound could be obtained. The compound was studied by different spectroscopic techniques and could be unambiguously identified as α-methyl-1-(2´, 4´, 6,-trimethoxyphenyl)-1-propanone.

When assaying for antistaphylococcal activity, it showed MICs of 62.5 μg/mL for the sensible strain (ATCC 6538p) and 250 μg/mL for the multirresistant ones (ATCC 43300 and ATCC 700699). This shows that the bioguided fractionation is appropriate even when not very active compounds are isolated

Key words: *Myrcianthes cysplatensis, Staphylococcus aureus*.

INTRODUCTION

In spite of the great advances in chemotherapeutics, infectious diseases are still one of the leading causes of death in the world. The World Health Organization[1] states that infectious and parasitic diseases account for nearly 11 million among the 57 million total deaths in 2003.

Although there seems to be a great array of antibacterial and antifungal drugs in clinical use, the appearance of resistant organisms makes them sometimes ineffective or lead to recurrence as stated by the World Health Organization.[2] Amongst some of the most problematic clinically relevant pathogens at present, methicillin-resistant *Staphylococcus aureus* (MRSA) ranks as one of the most difficult bacteria to treat.[3]

The use of higher plants and preparations made from them to treat infections is an age-old practice in a large part of the world population, especially in developing countries, where there is dependence on traditional medicine for a variety of diseases.[4] This wealth of experience and information about medicinal plants as well as the current problems associated with the use of antibiotics has renewed the interest in plants with antimicrobial properties.[5-11]

In previous work we undertook the biological and chemical prospection of the gallery forest of the northern Uruguay River basin.[12] Plants were selected after an exhaustive review of the available literature according at its ethnopharmacological use and submitted to antimicrobial assays and phytochemical characterization.[13] Among them, *Myrcianthes cisplatensis* extracts showed striking activity with a broad spectrum of activity that deserves further investigation. Many species belonging to the Myrtaceae family(that comprises, *Eucalyptus*, *Psidium* and *Syzygium* genus) have been studied for their antimicrobial properties.[14-16]

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MATERIAL AND METHODS

Plant material

M. cisplatensis leaves were collected in the banks of Rio Uruguay, Paysandu and identified by Lic. F. Haretche, Museo y Jardin Botanico "Atilio Lombardo", Montevideo. Voucher specimens (N° 26349) were kept in the MVJB Herbarium, Jardín Botánico, Montevideo.

Analytical methods

GC analysis was performed in a Shimadzu GC 14 apparatus with an SE-52 column using a temperature program from 100° to 280° with a 5°/min gradient. A Brucker micrOTOF-Q-TOF with ESI source in positive mode was used for MS spectra and a Shimadzu QP 5050 with a SE 52 column was used for the GC-MS analysis.

TLC was performed on silicagel or RP C18 plates (Macherey Nagel, Dürin, Germany) using $CHCl_3$/MeOH (80:20) or isopropanol/H_2O (50:50) as solvent respectively and H_2SO_4/heating or anisaldehyde as detection reagents.

^1H NMR and ^{13}C NMR spectra were obtained at 400 MHz and 100 MHz reapectively, on a Brucker Avance DPX 400 spectrometer, using $CDCl_3$ as solvent and TMS (δ_H 0.00) and acetone (δ_C 31.00) as references. 2D (different H,H-COSY, HMBC, HSQC) experiments were carried out with programs available in the Brucker software.

Bioautography

Bioautographies were made on developed and dried TLC plates according to the agar overlay method of Rahalison et al.[17] using *Staphylococcus aureus* (ATCC 6538p).

Extraction and isolation

Air dried and coarse milled *M. cisplatensis* leaves were twice extracted with dichloromethane for one week in the dark. The combined extracts were evaporated under vacuum and used for the following procedures.

The extract was dissolved in a minimum volume of methanol and submitted to column chromatography on Polyamide (Macherey-Nagel, 815600) with MeOH and acetone as eluents. The second MeOH fraction was submitted to vacuum column chromatography (VLC) on flash Silicagel (Macherey-Nagel, 815380) with CH_2Cl_2/MeOH (100:0 to 90:10) as eluent and the active fraction (95:5) was further purified a C_{18} cartridge to give a single compound (by TLC and GC)

Conglomerone (1)

$C_{13}H_{18}O_4$, dark yellow oil. UV (CH_2Cl_2) λ_{max}.= 279 nm. EI-MS *m/z*: 238 $[M]^+$, 195 $[C_{10}H_{11}O_4]^+$. HR-ESI-MS *m/z*: 239.2879 $([M+H]^+$, 261.2694 $[M+Na]^+$. ^1H NMR ($CDCl_3$): 1.13 (6H, d, 8Hz) α-Me and 3-Me, 3.03 (1H, m) H-2, 3.78 (6H, s) 2´ and 6´OMe, 3.84 (3H, s) 4´OMe, 6.12 (2H, s) H-3´and H-5´. 13 C NMR ($CDCl_3$): 18.0 C-1 and α-Me, 41.7 C-2, 55.4 4´-OMe, 55.8 2´and 6´-OMe, 90.0 C-3´and C-5´, 113.0 C-1´, 158.0 C2´ and C-6´, 161.0 C4´, 208.0 C-1.

Antibacterial analysis

Minimum inhibitory concentration (MIC) was determined by the microdilution technique according to Clinical and Laboratory Standards Institute (CLSI, 2006) using sensitive (ATCC 6538p) and resistant (ATCC 43300 and ATCC 700699) *Staphylococcus aureus* strains.

RESULTS AND DISCUSSION

Repeated column chromatography of the dichloromethane extract of *M. cisplatensis* leaves gave a compound (**1**) that showed only a spot in TLC and one peak in GC.

The ESI mass spectrum of **1** showed ions at *m/z* 239.2879 and 261.2694 ($[M+H]^+$ and $[M+Na]^+$, respectively) indicating a molecular formula $C_{13}H_{18}O_4$ (needs 238,2801). In the GC-MS spectra a prominent ion at *m/z* 195 is shown along with the 238 ion. The UV spectrum showed a maximum absorption at λ 279 nm indicating the presence of an aromatic group.

The ^1H NMR spectra showed few signals, with a doublet (6H) at 1.13 ppm, a septuplet at 3.03 ppm and singlets at 3.78, 3.84 and 6.12 ppm. In the ^{13}C NMR spectra 9 signals could be identified corresponding to 5 methyl, 3 methine and 5 quaternary carbons according to DEPT. Using a combination of 2D (H,H COSY, HSQC, HMBC) experiments all the signals can be assigned.

Especially useful were the correlations between the protons at δ_H 1.13 (d, 6H) with the signal at 3.03 and carbons at δ_C 18.0 (*via* HSQC) and 41.7 (*via* HMBC) defining a isopropyl group that in turn is correlated to the carbonyl carbon at 208.2 ppm as can be seen in Figure 1. This carbon did not have any other correlation suggesting that is directly linked to the phenyl moiety. This suggestion is further supported by the presence of the peak at *m/z* 195 in the GC-MS characteristic of a trimethoxyphenyl-carbonyl ion.

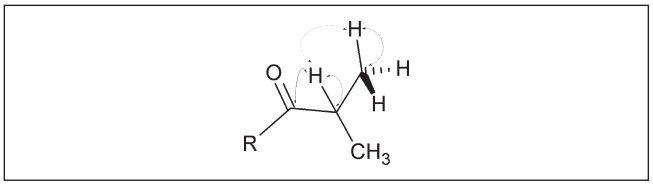

Figure 1: Main correlations in the isopropyl moiety.
Key to the figure:
COSY HSQC HMBC

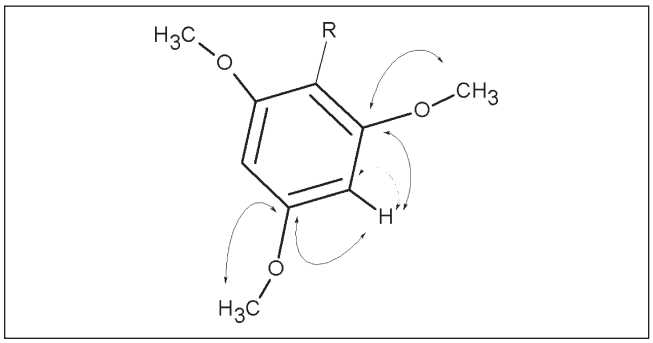

Figure 2: Main correlations in the aromatic moiety.
Key to the figure:
COSY HSQC HMBC

In the same way the correlations between the aromatic protons at δ_H 6.12 ppm with carbons at 158.0 and 161.0 ppm and the absence of correlation with carbon at 113.0 ppm determined the 2´, 4´, 6´ pattern of substitution in the aromatic group (Figure 2). Thus the compound could be unambiguously identified as α-Methyl-1-(2´, 4´, 6´-trimethoxyphenyl)-1-propanone.

When assaying for antistaphylococcal activity, Compound 1 showed . a MIC of 62.5 μg/mL for the sensible strain (6538p) and 250 μg/mL for the multirresistant ones (43300 and 700699). This shows that the bioguided fractionation is appropriate even when not very active compounds are isolated.

CONCLUSIONS

The bioguided fractionation of *M. cisplatensis* dichloromethane extract gives a pure compound which using different spectroscopic techniques could be identified as a propiophenone derivative: α-Methyl-1-(2´,4´,6´-trimethoxyphenyl)-1-propanone. From a biosynthetic point of view the compound could be rationalized as a product of the polyketide pathway with an isobutirylCoA starter and the usual malonylCoA prolonger units through Claisen reaction.[19]

The compound has been previously isolated by Lahey from *Eucalyptus conglomerata* who named it conglomeone.[20] Conglomerone was also proposed by Ricciardi as a phyletic marker for chemosystematics studies in the Myrtaceaee family.[21] However this is the first complete spectroscopic study of the compound as well as the first antibacterial activity reported.

Both the extract and the pure compound showed antibacterial activity against methicillin-sensitive and resistant *Staphylococcus aureus* strains

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