STRUCTURAL ELUCIDATION OF A MOLLUSCICIDAL COMPOUND FROM THE LEAVES OF *ANNONA MURICATA* L. (ANNONACEAE)

A. E. G. Sant'Ana[a] , E. S. Bento[a,b], G. E. Hawkes[b], H. Toms[b], J. S. Luna[a], J. M. Carvalho[a].

[a]Depto de Química/CCEN - Universidade Federal de Alagoas – Maceió/AL - 57.072-970 Brasil: [b]Chem. Dept.– Queen Mary University of London – Mile End Road – London – E1 4NS - England

INTRODUCTION

The species *Annona muricata* L. (figure 1) (Annonaceae) is known in Brazil as graviola, cruaçá, araticum do grande or jaca do Pará. This species is cultivated because it produces an edible fruit of great commercial acceptance. The species *A. muricata* is used in folk medicine due to its various properties: antidiarreic, sedative, antidiabetes, insecticidal and parasiticidal. Some biological studies had already been carried out with diferent parts of this plant, showing antiparasitic, antidepressive, anti-herpes, cytotoxic and antileishmania activities and chemical studies lead to the isolation of various compounds, most of them being acetogenins.

Figure 1: *Annona muricata* L.

METODOLOGY

The leaves (3.5 kg) were dried, ground and extracted with ethanol (10L). This produced 390g of crude extract. This extract was later submitted to larvicidal and moluscicidal assay and toxicity testing against *Artemia salina*. The separation of the components of the extract was carried out with the use of silica gel columns, some impregnated with KOH, with the use of active charcoal and sephadex columns (Figure 2). This yielded 69.4 mg of the molluscicidal component, and its purity was demonstrated through the observation of only one spot in TLC.

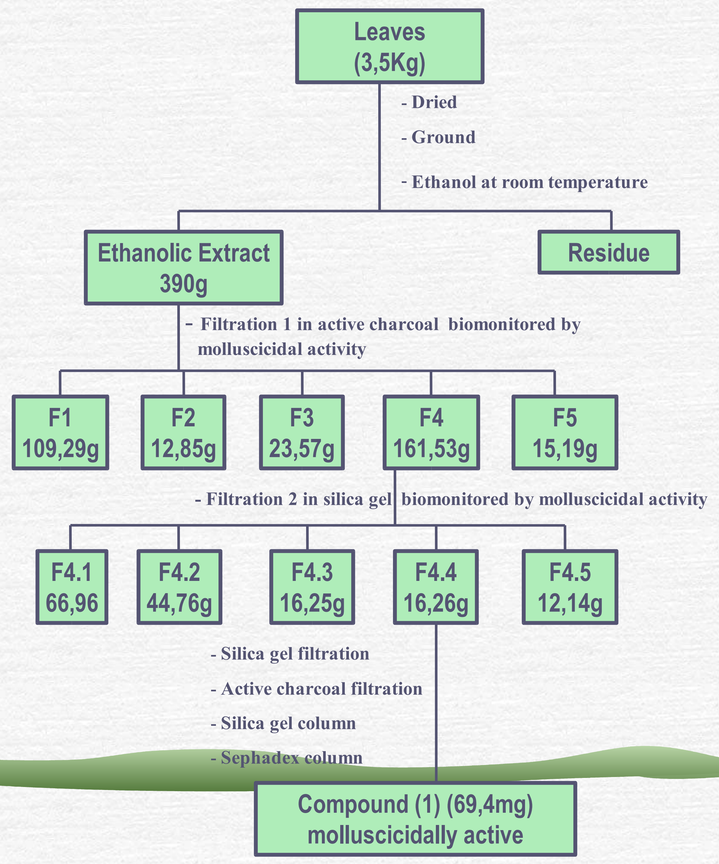

Figure 2: Scheme for the isolation of the compound 1

EXPERIMENTAL CONDITIONS

All 1D and 2D NMR spectra were obtained on Bruker spectrometers (AMX 400 and AVANCE 400 and 600), using $CDCl_3$ as solvent, at 300K. Exponential multiplication were applied to all 1D NMR time domain data, with LB=0.3 and 1.0, respectively, for the proton and ^{13}C spectra. NOESY and COSY spectra were acquired in phase sensitive mode. CH correlation spectra, one-bond and long-range spectra, were obtained in indirect detection mode.

RESULTS AND DISCUSSION

The data from mass spectometry gave a molecular weight of 580. A base peak at 43 ($C_3H_7^+$) and other signals at 562 ($M^+ - H_2O$), 309, 281, 273 and 241 were observed in the mass spectrum. Based on the molecular peak the molecular formule $C_{35}H_{64}O_6$ was proposed. The NMR analysis was performed using normal ^1H, ^{13}C, DEPT, COSY, HMQC, HMBC and NOESY spectra. All 1D and 2D spectra were obtained on Bruker spectrometers (AMX400 and AVANCE 400 and 600).The ^{13}C and DEPT spectra showed 35 carbons reonances, of which 2 are quaternary (one carbonyl), 24 methylenes, 7 methines and 2 methyls (Table 1). The identification of a γ-lactone-α,β-unsaturated ring, with a hydroxyl group in the 4 position was evidenced by carbon signals at 174.89, 152.11, 131.46, 78.25, 70.17, 33.75 and 19.38 ppm and proton signals at 7.19, 5.06, 3.82, 2.35-2.55 and 1.44 ppm (Table 1). A THF ring with an OH group flanking and a *threo-trans-erithro* stereochemistry is proposed, with carbon signals at 82.99, 74.37 and 29.05 ppm, and proton signals at 3.80, 3.40, 1.98 and 1.62 ppm (Table 1).

Position	δ (ppm)	
	^1H	^{13}C
1	---	174.89
2	---	131.46
3a, 3b	2.35 – 2.55 m	33.75
4	3.82 m	70.17
5	1.47m	37.57
6 - 13	1.25 – 1.60 brs	25.78, 25.88, 28.89-30.00, 32.20
14	1.40 m	33.66
15	3.40 brs	74.37
16	3.80 m	82.99
17a	1.67 m	29.05
17b	1.98 m	---
18a	1.67 m	29.05
18b	1.98 m	---
19	3.80 m	82.99
20	3.40 m	74.37
21	1.40 m	33.66
22 - 30	1.25 – 1.60 brs	25.78, 25.88, 28.89-30.00, 32.20
31	1.28 m	22.95
32	0.88 t (7.0)	14.37
33	7.19 q (1.3)	152.11
34	5.06 qd (7.0, 1.3)	78.25
35	1.44 d (7.0)	19.38

Table 1: NMR data from ^1H and ^{13}C spectra

From the above data analysis structure 1 was proposed, corresponding to Murisolin A, which was previously isolated from the seeds of *Asimina triloba* and from the seeds of *Annona muricata*. This compound shows moluscidal activity against the snail Biomphalaria grabata, the vector of human schistosomiasis, showing 100% death at a concentration of 50 ppm