

CHEMICAL CONSTITUENTS FROM THE LEAVES OF *MUCOA DUCKEI* (MARKGRAF) ZARUCCHI (APOCYNACEAE) A MEDICINAL PLANT FROM THE AMAZON REGION

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ABSTRACT

This paper describes the isolation and characterization of some metabolites present in the leaves of *Mucoa duckei* (Apocynaceae), popularly known as Sorvarana and used by the Amazonian population in the treatment of fevers. The ether and methanol extracts were fractionated by chromatographic techniques providing the triterpenes α -amyrin, β -amyrin, lupeol, betulinic acid, ursolic acid, oleanic acid and the acetyl derivate of the cyclitol methoxy-*myo*-inositol. All substances had their structures elucidated by MS and NMR analysis.

Keywords: *Mucoa duckei*, Triterpenes, Cyclitols.

INTRODUCTION

Various plants products have been used worldwide since time immemorial for medicinal purposes¹. Many applications of the medicinal plants are confirmed around the world² and its not different in the Amazon region.

The Apocynaceae family comprises from 3700 to 5100 species distributed in 250 to 550 genera^{3,4}, being found predominantly in the tropics. In Brazil the Apocynaceae family has over than 400 species distributed in 41 genera, being 32 found only in the Amazon region⁵. Several species of this family are known as sources of bioactive substances⁶ highlighting alkaloids, terpenoids and iridoids⁷. The use of plants from the Apocynaceae family is extensive in the popular medicine of the Amazon region⁷. The genus *Mucoa* belonging to the Ambelanieae tribe has only two species of relatively rare occurrence, being found only in central and western Amazon⁸. This genus is the result of a reclassification of two taxonomic species from the genus *Ambelania*⁸. The strong similarity between the species *Mucoa duckei* and *Ambelania occidentalis* confuses the local population so they are used with the same purpose, both have their latex popularly used as insect repellent and its leaves used to treat strong fevers, and their fruits are used as food people in poor regions of Peru⁹. The species *Mucoa duckei*, popularly known as Sorvarana is a middle sized tree that occurs in upland areas near rivers and lakes⁸. Until this moment no previous chemical studies with *M. duckei* have been conducted. In order to contribute to the chemotaxonomic study of the family Apocynaceae we conducted the chemical study of the leaves from *Mucoa duckei* to compare with previous data for *Ambelania occidentalis* that are known by producing indole alkaloids¹⁰.

MATERIALS AND METHODS

Used equipments

The NMR spectra were obtained in a DRX-300 (Bruker, Billerica, USA) spectrometer operating at 300 MHz for ¹H and 75 MHz for ¹³C. TMS was used as internal standard. EI-MS Spectra were obtained in a Q-mass 910 equipment (Perkin-Elmer, Waltham, USA) at 70 eV. TLC was performed using silica gel 60 PF₂₅₄ (Merck, Darmstadt, Germany) and spots were visualized under UV light and sprayed with vanillin-H₂SO₄ reagent followed by heating at 100 °C. The fractionation was performed in CC using normal phase silica 230-400 mesh (Merck, Darmstadt, Germany). Melting points were performed in Kofler block.

Collection and Identification of plant materials

Leaf samples of *Mucoa duckei* were collected in an upland forest in the municipality of Aximim, Amazon state. The identification of the

voucher specimen was performed by James L. Zarucchi from the Botanical Garden of Missouri in The United States, the voucher specimens are deposited in the Herbarium of the New York Botanical Garden, USA under the number 2973.

Extraction and Isolation

The dried leaves of *Mucoa duckei* were crushed and extracted at room temperature with petroleum ether and methanol three times with 1 L of each solvent. The ether extract (34.0 g) was chromatographed by CC using normal phase silica gel eluted with hexane, dichloromethane, ethyl acetate and methanol affording 25 fractions. The fractions 2-15 were combined and fractionated in normal phase silica gel with hexane, ethyl acetate and methanol yielding a white amorphous solid with a melting point of 145-150 °C, constituting the mixture of **1,2** and **3** (62.7 mg). The methanolic extract (23.0 g) had 20.0 g chromatographed in normal phase silica gel eluted with dichloromethane and methanol providing 27 fractions. The fractions 6-14 were recrystallized with ethyl acetate and afforded **4** (73.3 mg, melting point 280-282 °C), the same was conducted to the fractions 21-23 that provided the mixture of **5** and **6** (84.2 mg, melting point 220-225 °C). The remaining 3.0 g of the methanolic extract were acetylated with pyridine and acetic anhydride then fractionated in normal phase silica gel with hexane, dichloromethane and methanol affording 218 fractions where the fraction 203 appeared like a white amorphous solid with a melting point of 128-132 °C yielding **7** (85.3 mg).

RESULTS AND DISCUSSION

The comparison of the ¹H, ¹³C NMR and EIMS data for the mixture of **1,2** and **3** with the literature^{11,12,13,14}, lead to the identification of the triterpenes α , β amyrins and lupeol. In the ¹H NMR spectrum from the compound **4**, were observed six singlets characteristic of methyl groups (δ 1.93, δ 1.23, δ 1.21, δ 1.08, δ 1.02 and δ 0.83). The signals at δ 5.69 (1H, s) and δ 5.21 (1H, d, $J = 2$ Hz) indicated the presence of terminal olefinic hydrogens. The ¹³C spectrum showed 30 signals, of which two from olefinic carbons (δ 150.6 and 109.2), one carbinolic (δ 78.9) and a carboxylic (δ 180.5). The EIMS spectrum showed the molecular ion at m/z 456 and the base peak at m/z 189, characteristic of lupane type triterpenes, which comes from a rupture of the bond between C-8 and C-14 by hydrogen and Mac Lafferty rearrangements. The comparison with literature data lead to the identification of **4** as the betulinic acid^{15,16} this triterpene has a potent anti-aids activity¹⁷. The compounds **5** and **6** were identified as the oleanic and ursolic acids by comparison of the spectra with literature data^{15,18,19}, these two substances have biological activities like antiinflammatory, antihyperlipidemic and antitumor²⁰. The compound **7** in its ¹H NMR spectrum showed six carbinolic type

signals at δ 3.42 (1H, dd, $J = 3$ and 10 Hz), δ 4.96 (1H, dd, $J = 3$ and 10 Hz), δ 5.11 (1H, t, $J = 10$ Hz), δ 5.37 (1H, t, $J = 10$ Hz), δ 5.47 (1H, t, $J = 10$ Hz) and δ 5.76 (1H, t, $J = 3$ Hz), a signal at δ 3.35 (1H, s) characteristic from a methoxyl group and three singlets typical of methyl groups from acetyl substituents (δ 2.18, δ 2.04 and δ 1.98). The EIMS spectrum from 7 showed molecular ion at m/z 404 and the peak at m/z 345 typical by product ions resulting of acetate losses²¹, the comparison of the ^1H and ^{13}C NMR and EIMS data from the compound 7 with the literature^{22,23} together with the consideration that the original substance it's not acetylated lead to the identification of 7 as the cyclitol 1-*O*-methyl-*myo*-inositol, this substance has chemopreventive activities²⁴. The determination of

the *myo* orientation from the hydroxyl substituents were made by comparing the constant couplings with previous data²⁵. The family Apocynaceae has been studied extensively over the years because of their secondary metabolites, mainly alkaloids, which metabolites were not found in the present study. The triterpenoids and the glycoside methoxy *myo*-inositol (Figure 1) are all new in the genus *Mucoa*. Cyclitols, including *myo*-inositol and 1-*O*-methyl-*myo*-inositol, also named bornesitol, have already been identified in the Apocynaceae species *Hancornia speciosa* and *Ochrosia nakaiana*^{24,26}. Their occurrence in several Apocynaceae leaves was investigated and the significance from the viewpoint of chemotaxonomy was discussed²⁷.

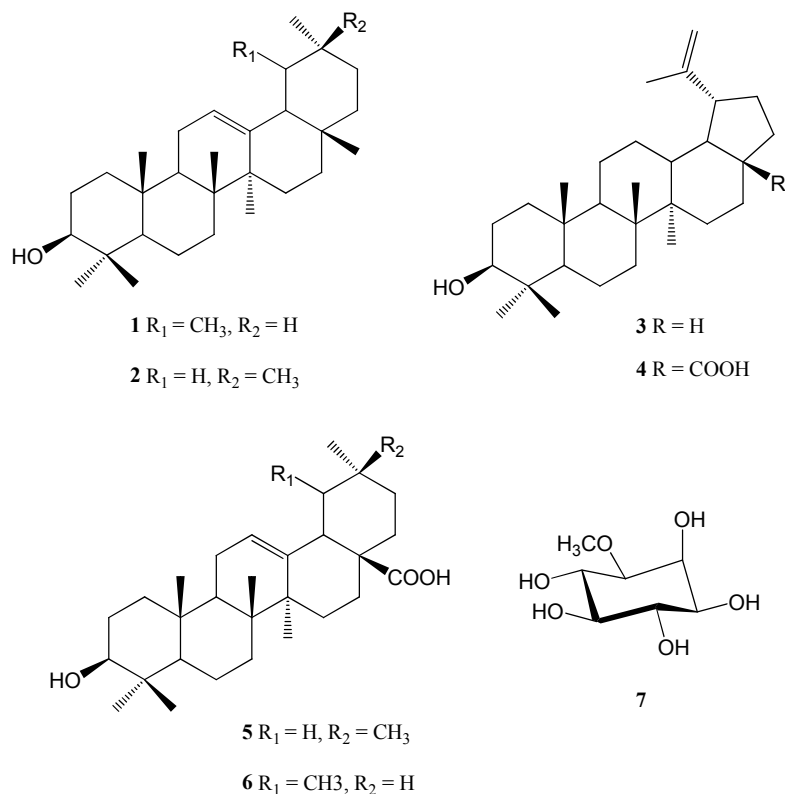


Fig. 1: Structures of the compounds 1-7

CONCLUSIONS

The phytochemical study from the leaves of *Mucoa duckei* lead to the isolation of six triterpenes, some of them with biological activities previously comproved, together with these substances we also isolated the cyclitol 1-*O*-methyl-*myo*-inositol which together with the absence of alkaloids in the extracts of *Mucoa duckei* indicates that the split of the genus *Ambelania* (Zarucchi, 1987) can also be supported by the chemotaxonomy.

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