

Inhibitory activity of a compound isolated of *Selaginella lepidophylla* on rat's uterus

ROSARIO VARGAS S., SALUD PÉREZ G., MIGUEL ZAVALA S., CUAUHTÉMOC PÉREZ G.
Universidad Autónoma Metropolitana-Xochimilco; A.P. 23-181, Mexico D.F., Mexique.

INTRODUCTION

Selaginella lepidophylla known as "doradilla" grows in arid regions of the southwester U.S.A., and into Mexico this plant is so named for its ability to recover from the dried brown "balled". Leaves are small and microphyllous, they may be arranged in a dense helix^{1,2}.

S. lepidophylla has been used in folk medicine as diuretic, as remedy for gallstone an for the treatment of cystitis³.

We studied the effect of different extracts of *S. lepidophylla* on Wistar rat's uterus. We found a stimulating activity on chloroform extract of this plant⁴. It was isolated from *S. rupestris* an amenoflavone wich increases the contraction of the cardiac muscle of the guinea pig⁵.

MATERIAL AND METHODS

Biological material. The plants were bought in "Sonora market" in Mexico city. They were dried in the shade and powdered.

The plant was authenticated by Biol. Aurora Chimal of the Department *el Hombre y su Ambiente*, Universidad Autónoma Metropolitana-Xochimilco, and a voucher specimen was deposited in the Herbarium of UAM-X.

Methanolic extract. In a 10 l round bottom flask fitted with a reflux condenser, 1 kg of dried and powdered leaves with 5 000 ml of water were heated and refluxed for 2 h, cooled to room temperature and filtered, the solvent was removed from the extract by evaporation under vacuum in a rotatory evaporator.

The residue was chromatographed on silica gel eluted with chloroform and increasing its polarity with methanol.

Crystallization from methanol yielded white crystals m.p. 214-216 °C, its purity was determined by thin-layer chromatography and IR spectra was mesured in KBr on Perkin-Elmer 1 600 FT, UV/Vis Varian Cary 219; MS analysis was carried out with a 5 985 Instrument (Hewlett Packard) at 70 eV with direct inlet, ¹HNMR and ¹³CNMR spectra was taken at 360 MHz using TMS, as internal standard.

Pharmacologic method. Wistar female rats (220-250 g) were housed in isolated cages under artificial light (dark: noon 10.00 pm) and supplied with Purina and water *ad libitum*.

Physiological stage (œstrus) was determined by vaginal smears⁶.

The animals were sacrificed by cervical dislocation and the uterus extracted surgically.

Uterus rings fixed in glass chambers (volume 10 ml) in a Krebs-Hansseleit solution⁷. The pH was adjusted to 7.4 and a mixture of 5 % CO₂ and 95 % O₂ was bubbled continuously into the chamber at 37 °C. Uterine contractions were sensed by a force displacement transducer connected to a polygraph Narco Biosystems. The uterine contractions were stabilized over 20 mn. Five minutes before the sample application were used as control register.

To the prepared uterus were applied two different doses of the compound (2 doses) in a range from 5 and 10 mg/ml.

Table 1.
¹³C Chemical Shifts in Cyclic Ketal

C - 1	60.873
C - 2	70.192
C - 3	71.667
C - 4	72.523
C - 5	72.947
C - 6	97.321

RESULTS AND DISCUSSION

The compound was crystallized from methanol as white crystals m.p. 214-216 °C.

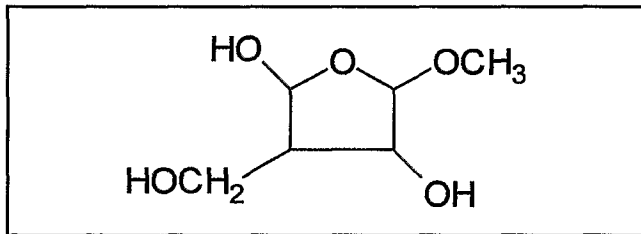
Found: C: 47.83 %; H: 7.06 %; O: 45.11 %, for C₆H₁₀O₄.

Anal. Calc. for: C: 49.32 %; H: 6.85 %; O: 43.84 %.

I.R. V^{KBr} cm⁻¹ 3 442, 3 011, that are characteristic of the hydroxyl group C-OH at 1 084 cm⁻¹, and shows the bands of ethers absorption at 1 146; 1 426 cm⁻¹; in 3 050 cm⁻¹ a signal characteristic of double bond and substituted double bond at 834 cm⁻¹. There is not absorption in UV/VIS spectrum, this excluding the possibility of conjugated double bond.

Fig. 1

Structure suggested for the compound isolated from *Selaginella lepidophylla*



^1H NMR (360 MHz, DMSO) spectrum showed signals for Methoxyl group at 3.45 (s), 4.65 (d), 4.8 (d), 4.9 (d).

The ^{13}C NMR spectrum (table 1) contains signals of five carbon atoms bearing oxygen function.

EIMS (probe) 70 eV m/z (rel. int.) 145 [$m-1$] $^+$ ($\text{C}_6\text{H}_9\text{O}_4^+$), 127 (20), 84 (10), 73 (98). The structure suggested for this compound is showed in figure 1.

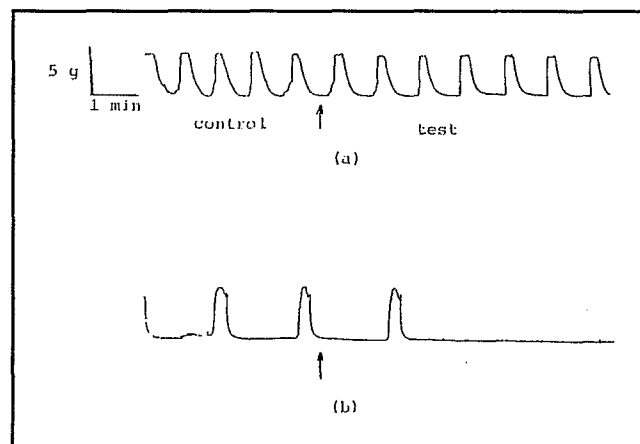
In figure 2 is shown the inhibition of contractile response of rats uterus with dose of 10 mg/ml of the cristals. In the presence of 5 mg/ml it was observed 50 % inhibition and with 10 mg/ml was 60 %.

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Fig. 2

Effect of compound isolated from *Selaginella lepidophylla* on rat's uterus



(a) (mg; (b) 10 mg.

The arrow indicates the application of the compound.