SECONDARY METABOLITES FROM *SENNA VERSICOLOR*

Narda Blanco, Yonny Flores, Giovanna R. Almanza

Laboratorio de Productos Naturales, Instituto de Investigaciones Químicas, Carrera de Ciencias Químicas, Universidad Mayor de San Andrés, Campus Universitario de Cota Cota c. 27, La Paz, Bolivia

Key Words: Senna versicolor, vegetal origin secondary metabolites, antibacterial, NMR, structural elucidation

ABSTRACT

Three compounds were isolated from the leaf of
Senna versicolor (Vogel) Irwin & Barneby: Stigmasterol, Apigenin and d-Pinitol. The structures of all compounds were determined by spectroscopic techniques, mainly by NMR experiments. In adition, the DCM and EtOH extracts were tested against bacteria./ *Tres compuestos fueron aislados de las hojas de Senna versicolor (Vogel) Irwin & Barneby: Estigmasterol, Apigenina y d-Pinitol. Las estructuras de todos los compuestos fueron determinadas por técnicas espectroscópicas, principalmente por experimentos de RMN. Además los extractos de DCM y EtOH fueron evaluados contra bacterias.*

Corresponding author: giovyalmanza@yahoo.com.ar

INTRODUCTION

The genus *Senna* includes 260 species throughout the tropical American zone and some species in cool regions [1]. *Senna versicolor* is shrub or little tree that can grow until 3 meters tall, with florescence from August to October. In La Paz-Bolivia was found in the shore of Lake Titicaca [2] where is commonly known as "Takarkea", "Alcaparilla" or "Mutu Mutu" [1]¨. The leaf is used in folk medicine against diarrhea and herpes. According to literature, several *Senna* species have been studied phytochemically and pharmacologically, yielding antraquinones and flavonoids [3],[4],[5]. In this paper, we report the isolation and structural elucidation of three compounds from the leaf *S. versicolor*: Stigmasterol (1), Apigenin (2) and d-Pinitol (3).
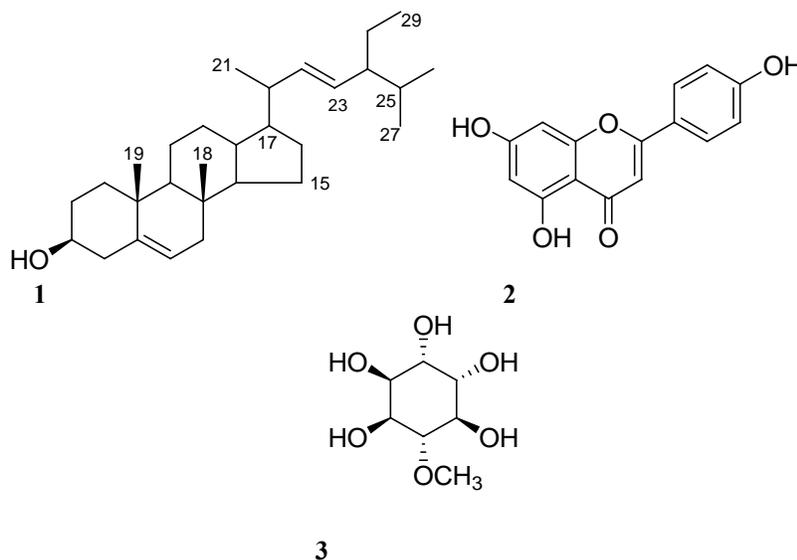
Figure 1. Isolated compounds from *Senna versicolor*.

RESULTS AND DISCUSSION

Senna versicolor was collected in June of 2003 in share of the Lake Titicaca (La Paz-Bolivia) and was identified by experts from National Herbarium from Bolivia. The leaves were ground and extracted successively with

petroleum ether, DCM, AcOEt and EtOH. The DCM and EtOAc were joined and analyzed following diverse chromatographic techniques isolating two compounds: Stigmasterol (1) and Apigenin (2). On the other hand, d-Pinitol (3) was isolated by direct crystallization from EtOH extract giving a yield around 40% respect of EtOH extract. So, d-Pinitol is the major compound found in this plant. The structures of the all compounds were established by spectroscopic means. Compound (1) is a well known compound finding in several plants. Then, its identification was done by comparison with an authentic sample of our laboratory. In addition, its ^{1}H and ^{13}C NMR spectroscopic data were compared with those reported in literature[6].

Compound (2) was isolated as a yellow powder. The ^{13}C NMR spectrum exhibited 15 signals: one corresponding to a carbonyl group and 14 to olefinic or aromatic sp^2 carbons, suggesting a flavonoid structure.[7] The ^{1}H NMR spectrum together with the ^{1}H-^{1}H COSY experiment allowed the identification of a *para-* disubstituted aromatic ring [δ 7.58 (2H, *d*, 8.8 Hz), 6.73 (2H, *d*, 8.8 Hz)]; one tetra-substituted aromatic ring [δ 6.29 (1H, *d*, 2.1 Hz), 6.06 (1H, *d*, 2.1 Hz)], and one olefinic proton [δ 6.31 (1H, *s*)] assigned to H-3 (Table 1). Full assignments of the proton and carbon NMR signals were made using the heteronuclear HMQC and HMBC experiments confirming the structure as apigenin, some pertinent HMBC are showed in the Figure 2. Finally and its NMR data were compared with thoses reported in literature[8] confirming the proposal structure.
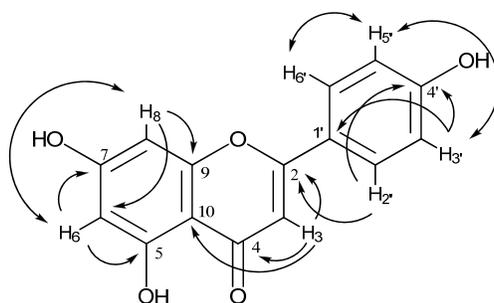
Figure 2. Some pertinent COSY (↔) and HMBC (→) correlations.

Table 1: ^{1}H NMR, ^{1}H-COSY, HMQC and HMBC data for compounds **2**

Proton	δ$_H$	^{1}H-COSY	HMQC	HMBC
H-3	6.31 *s*		102.66	C-4, C-2 ,C-10
H-6	6.06 *d*	H-8	98.87	C-5,C-7,C-8,C-10
H-8	6.29 *d*	H-6	93.90	C-6,C-7,C-9
H-2'	7.58 *d*	H-3', H-6'	127.89	C-2, C-4', C-6'
H-3'	6.73 *d*	H-2', H-5'	115.58	C-1', C-4', C-5'
H-5'	6.73 *d*	H-6', H-3'	115.58	C-1', C-4', C-3'
H-6'	7.58 *d*	H-5', H-2'	127.89	C-2, C-4', C-2'

Solvent: CDCl₃　　　　　Frequency: 75 MHz

In a similar way, the compound **3** was elucidated mainly by the analysis of ^{1}H and ^{13}C NMR data. But, in this case we needed two ^{1}H NMR spectra, one in DMSO and the other in D₂O. Initially we obtained the ^{1}H spectrum in DMSO (Figure 3) where we can observe one proton at δ$_H$ 3.00 *dd* assigned to H-1 followed by five protons between 3.30 and 3.62 ppm, among them we can distinguish a high signal corresponding to a methoxi group. Finally, we found five more protons between 4.3 and 4.8 ppm which are coupled with the previously named protons, probably corresponding to hydroxyl protons in a sugar structure. But, the coupling among the hydroxyl protons and their geminal protons difficult the analysis of the proton multiplicity and, in consequence, the assignment of the relative stereochemistry.
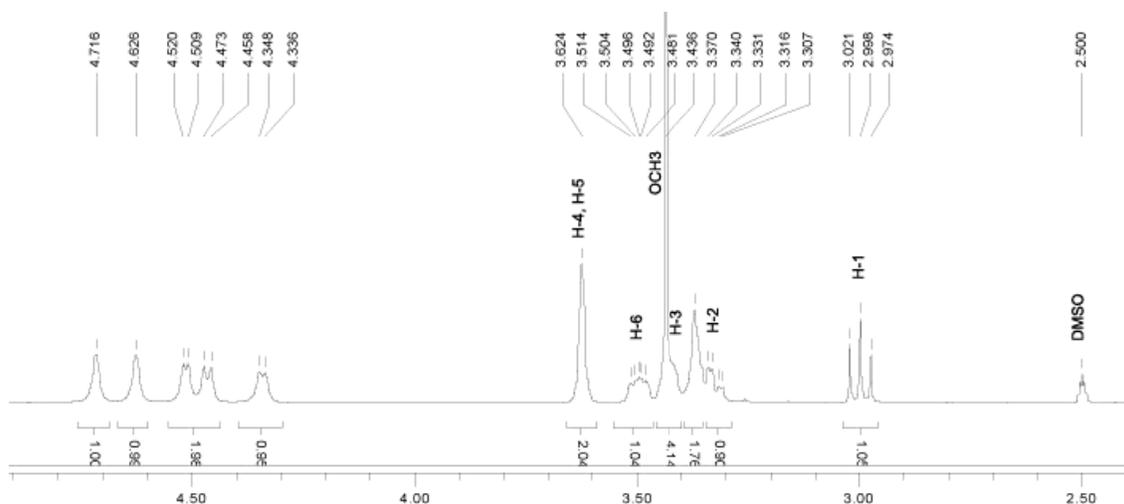
Figure 3. ^{1}H NMR spectrum of compound 3 using DMSO as solvent
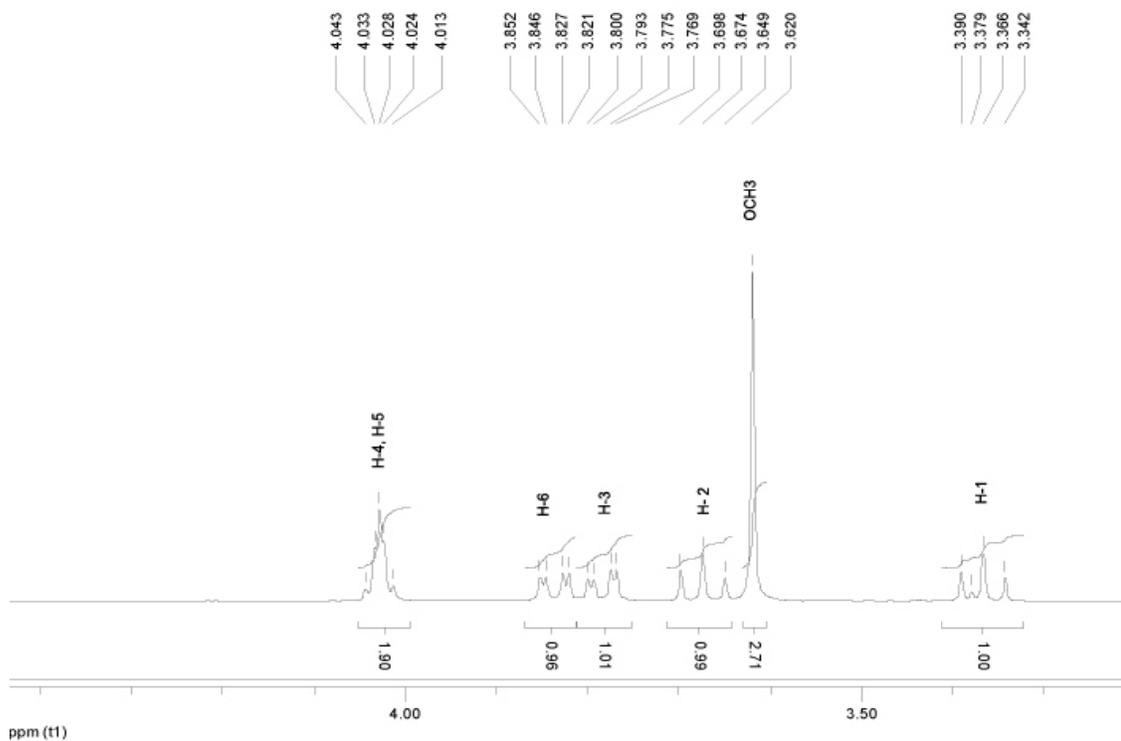
Figure 4. ^{1}H NMR spectrum of compound 3 using D$_2$O as solvent

For the reasons exposed above, we decided to obtain other spectrum using D$_2$O as solvent (Figure 4). In this case we can distinguish very well the multiplicity of the protons *gem* to hydroxyls. So, we have the proton H-1 at δ 3.37 *dd* (*J*= 9.6 & 9.6 Hz) with two big couplings indicating a *trans*-diaxial position respect of the protons H-2 and H-6. In a similar way, the proton H-2 at δ 3.67 *dd* (*J*= 9.6 & 10.0 Hz) also showed two *trans*-diaxial couplings with H-1 and H-3, respectively. The proton H-3 have a different performance at δ 3.78 *dd* (*J*= 2.8 & 10.0 Hz) showing two different couplings, one trans-diaxial with H-2 and the other axial-equatorial with H-4. The proton

H-4 at δ 4.03 *dd* (*J*= 2.8 & 2.4 Hz) showed an equatorial-axial coupling with H-3 and one *trans*-diequatorial coupling with H-5. The proton H-5 at δ 4.03 *dd* (*J*= 2.8 & 2.4 Hz) showed also two little couplings indicating a relation *trans*-diequatorial with H-4 and equatorial-axial with H-6. Finally, the signal of proton H-6 at δ 3.84 *dd* (*J*= 2.8 & 10.0 Hz) confirmed its axial position (see Figure 5). So, we arrived to the structure of d-pinitol confirming all its data by RMN 2D as we can see in the Table 2
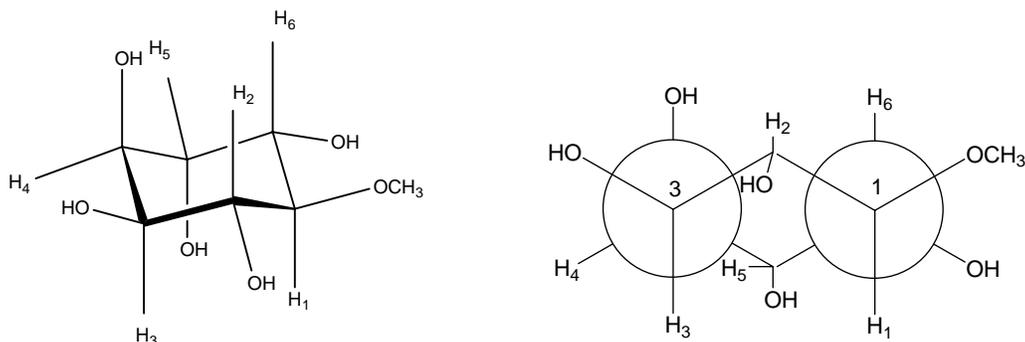
Figure 5. Chair structure and Newman projection of compound 3.

Table 2: ^{1}H NMR, ^{1}H-COSY, HMQC and HMBC data for compound **3**

Proto	δ$_H$ (D$_2$O)	δ$_H$ (DMSO)	^{1}H-COSY	HMQC (DMSO)	HMBC (DMSO)
H-1	4.03 *m*	3.62 *m*	OH-1	71.9	C-3, C-5
H-2	3.84 *dd (2.8,10)*	3.50 *m*	H-1, H-3, OH-2	70.0	C-3
H-3	3.37 *dd (9.6, 9.6)*	3.00 *t*	H-2, H-4	83.8	C-1, C-2, C-4, OCH$_3$
H-4	3.67 *dd (9.6, 10.)*	3.32 *dd*	H-3, H-5, OH-4	72.48	C-5,C-3
H-5	4.78 *dd (2.8,10)*	3.41 *m*	H-4, H-6, OH-5	70.9	C-3
H-6	4.03 *m*	3.62 *m*	OH-6	72.5	C-2.C-4
OCH$_3$	3.62 *s*	3.44 *s*		59.63	C-3
OH-1		4.63 brs	H-1		C-6
OH-2		4.46 d	H-2		C-1, C-2, C-3
OH-4		4.51	H-4		C-3, C-4, C-5
OH-5		4.34	H-5		C-5
OH-6		4.72 brs	h-6		C-1, C-5

The antibacterial activities of the raw extracts were evaluated against four bacteria: *Staphylococcus aureus* (Table **3**). The results show that any extract showed antibacterial activity, for this any compound was submitted trough this evaluation.

Table 3. Bacteriological analysis

Extracto Hoja	Sal.	E. c	S. aure	S.
Éter de petróleo	-	-	-	
CH_2Cl_2	-	-	-	
AcOEt	-	-	-	
EtOH	-	-	-	
Extracto Tallos				
Éter de petróleo	-	-	-	
CH_2Cl_2	-	-	-	
AcOEt	-	-	-	
EtOH	-	-	-	
Extracto Semil				
Éter de petróleo	-	-	-	
CH_2Cl_2	-	-	-	
AcOEt	-	-	-	
EtOH	-	-	-	

EXPERIMENTAL SECTION

General Experimental Procedures.

The melting points (uncorrected) were recorded on a FISATOM 430 D Melting Point Apparatus. ^{1}H and ^{13}C NMR spectra were recorded with a BRUKER DRX-400 using as solvent $CDCl_3$; D_2O and DMSO and the chemical shifts are reported in δ units (ppm) and coupling constants (*J*) in Hz. Sephadex LH-20 was used for gel filtration; Silica gel (E.M. Merck,70-230 mesh) and silica gel G-60 (E.M. Merck) were used for CC and VLC, respectively, while aluminum plates impregnated with silica gel 60 F_{254} (E.M. Merck) were used for analytical (0.25 mm) TLC analyses. Spots on chromatograms were detected under UV light (254 and 365 nm) and by spraying the plates with 10% H_2SO_4, followed by heating.

Plant material The areal part of Senna versicolor Meyen ex Vogel, H.S, Irwin & Barneby. was collected in june 2003 at Lake Titicaca (North of the ANDEAN PART OF La Paz, Bolivia) and identified by Lic. Emilia Garcia (Universidad Mayor de San Andres, La Paz). A voucher specimen is kept at the Bolivian National Herbarium in La Paz.

Extraction and Isolation. The dry and ground plant material (1667 g) was first extracted with 3 L of petroleum ether for 72 h at room temperature, the solution was filtered off and evaporated to yield the petroleum ether extract. The solid residue was macerated for 72 hours with 3 L ethanol, which after filtering and evaporation of the solvent gave the ethanolic extract. Finally, the ethanolic extract was extracted with 150 mL mixture (CH_2Cl_2:MeOH 9:1) this process was done for 4 times. The petroleum ether extract (6,7 g) was subjected to VLC on silica gel, eluting with increasing amounts of CH_2Cl_2 in petroleum ether, followed by increased amounts of MeOH in CH_2Cl_2 finalizing with MeOH, to give nine main fractions. The fractions 3, 4 and 5 where submitted to other chromatographies followed by recrystallization with MeOH giving the compounds **1** (22.3 mg), **2** (17.9 mg) and **3** (37.2 mg). The CH_2Cl_2 extract was also submitted to an chromatographic analysis by VLC on silica gel followed by gel filtration on Sephadex LH-20 giving two compounds in pure form, compound **4** (20 mg) and compound **5** (45 mg).

Compound **1** (Stigmasterol) White crystals of mp. 165-167ºC, ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.52 *m* (H-3); 5.35 *d* (H-6); 0.67 *s* (H-18); 1.00 *s* (H-19); 0.92 *d* (H-21); 5.15 *m* (H-22); 5.01 *m* (H-23); 1.47 *m* (H-25); 0.86 *d* (H-26); 0.79 *d* (H-27) and 0.84 *d* (H-29). ^{13}C NMR see Table 1.

Compound **2** (Ethyl galleate) White crystals of mp. 213-215ºC, ^{1}H and ^{13}C NMR see Table 2.

Compound **3** (Rhuschalcone VI) Amorphous yellow solid, mp 184-186°C, ^{1}H and [13] C NMR see Table 3. HREIMS *m/z* 511.1342, calc. for $C_{30}H_{22}O_9$+H, 511.1387.

Microorganisms

The antibacterial activity was tested against *Staphylococcus aureus* (ATCC-25923/6538).

Biological tests

Methodologies employed for *in vitro* assays against protozoa are given in full details in a previous papers [**¡Error! Marcador no definido.**].The bacterial assays was done by dilution and the protocol is described in Gimenez et al., 1996 [17].

Minimal Inhibitory Concentration (MIC)

The MIC **w**as determined by a microdilution technique using the Mueller Hinton (MHB-DIFCO) broth. The dilutions were prepared from a solution of 2 mg/ml where we added a bacterial population of $6x10^6$ ufc/ml of each microorganism, placing in the microplate controls of bacterial growth (solvent and antibiotic). The plates were incubates at 37 °C for16-18 hrs, after this period the plates was examined visually. The MIC is considered the lowest concentration of the sample that prevents visible growth.

Minimal Bactericidal Concentration (MBC) and Minimal Bacteriostatic Concentration (MbtaticaC) were determined by sub-culturing the negative samples of the previous technique. Since the bactericidal substances not always sterilize totally a bacterial population, the minimal concentration of the bacteriological agent that allows surviving less than 0.1% of original inocule is denominates Minimal Bactericidal Concentration (MBC). MBstaticC is defined as the lowest concentration that avoid the grow of the bacteria without sterilize the bacterial population [17].

ACKNOWLEDGEMENTS

Considerando que algunas xantonas presentaron una interesante actividad antibacteriana [11]. Se evaluó la actividad antibacteriana del extracto crudo y los compuestos aislados frente a cuatro especies de bacterias: *Staphylococcus aureus, Bacillus subtilis, Shigella flexneri* y *Escherichia coli* (Tabla 3)*.* Determinándose una alta actividad bacteriostática, MbtaticaC= 1.9 μg/ml, contra *S. aureus* y *B. subtilis* en el compuesto **(1),** mayoritario en el extracto diclorometánico de *R. acuminata* (Tabla 4).

REFERENCES

[1] Kiesling, R.; Manzini, P. M. (1994) *Advances in Legume Systematics*, Buenos Aires Volumen 1, p. 274-275.

[2] Irwin, H. S.; Barneby, R. C. Memorias of the New Cork Botanical Garden, Editorial Borrad, Vol. 35, part. 11 1982.

[3] Olivera C.M., Schenkel, E. P., Gosmann, G., Palazzo, J.C:, Mentz, L. A., Petrovick, P. R., Farmacognosia da planta ao Medicamento, Editorial DA UFSC, Editora da Universidade, Brasil, 1999, 445-570.

[4] Valencia, C. , fundamentos de Fitoquimica, 1ra edicion, editorial Trillas Mexico, Argentina, españa Colombia, Puerto Rico, Venezuela, 1995.

[5Steinergger, E.; Hansel, R.; "Lejrbuch der Pharmakonosie und Phytopharmazie" $. Aufl. Bgerlin: Springer, 1998 Roach.

[6] Ito, A.; Yasumoto, K.; Kasai, R.; Yamasaki, K. 1994A sterol with an unusual side chain from Anoectochilus koshunensis; *Phytochemistry* 36 6 1465-1467.

[7] Geiger, H. In The Flavonoids, Harbone, J. B., Ed., Chapman & Hall: London, (1994), pp 95-115.

[8] Wamer I.; Zielinska A., 13C CP/MAS NMR studies of flavobnoids" MaGNETIC Resonance in Chemistry, 2001, 374-380