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ISOLATION AND CHARACTERIZATION OF STIGMASTEROL FROM HIGHLY CONSUMED LEAVES BY THE LARGE FRUIT-EATING BAT, *Artibeus amplus* (Chiroptera: Phyllostomidae)

Juan M. Amaro-Luis, Adriana Duque-Márquez, Paolo Ramoni-Perazzi, and Mariana Muñoz-Romo

SUMMARY

Folivory in bats is still not well understood in functional and evolutionary contexts, partially because it has not been thoroughly studied. The large fruit-eating bat, Artibeus amplus *(Chiroptera: Phyllostomidae) frequently includes leaves of* Aspidosperma desmanthum *(Apocynaceae) in its diet but maximizes its consumption prior to reproduction. The functional and evolutionary understanding of these folivorous habits requires further research, focused on the biological properties of leaves. Species of* Aspidosperma *exhibit a wide range of biological activities and biosynthesize secondary metabolites. The aim of this study was to purify and isolate the predominant secondary metabolite present in* A. desmanthum *leaves to identify a candidate compound to explore its potential role in the stimulation or inhibition of* A. amplus*' reproductive activity. A methanolic extract was prepared with dried leaves of* A. desmanthum. *Using conventional chromatographic methods, a unique compound was isolated and purified, and structurally characterized by spectroscopic methods including IR, EI-MS and uni- and two-dimensional NMR techniques. It was identified as stigmasterol, a metabolite widely distributed in plants, but reported in only two species of* Aspidosperma. *Stigmasterol has shown a variety of biological activities, including regulation of reproductive activity in several mammals. We found a target component and thus suggest testing it in future controlled experiments aimed to study the regulation of reproductive activity in* A. amplus. *Our study is the first in the Neotropics to isolate a potential hormonal precursor from leaves consumed by bats; as such, it is a relevant contribution to the understanding of folivory in frugivorous bats.*

Introduction

Folivory in bats was discovered more than sixty years ago (Greenhall, 1957; Van der Pijl, 1957), and researchers are still trying to understand the functional and evolutionary significance of chewing leaves of specific plant species by some frugivorous bats (Kunz and Díaz, 1995). In most cases of folivory, bats chew pieces of leaves to swallow the liquid portion presumably containing micronutrients, vitamins, and proteins, usually scarce in fruits, and drop the resulting fibrous mass (i.e., pellets) formed in their mouths while chewing (Kunz and Ingalls, 1994). In some bats with folivorous habits this strategy presumably allows them to obtain essential biological components by squeezing them from leaves without modifying and extending their digestive tracts to metabolize cellulose and/or process fiber (Kunz and Ingalls, 1994).

Folivory in bats has remained poorly studied especially in terms of the chemical properties of leaves, and their meticulous selection and processing. Kunz and Ingalls (1994) proposed several questions to address an explanation for folivory besides nutrition: Do bats select specific plant leaves? Do secondary compounds from leaves stimulate or inhibit reproductive activity? In particular, these last questions have been repeatedly postulated in the past, but the

KEYWORDS / *Artibeus amplus* / *Aspidosperma desmanthum* / Folivory / Reproductive Activity / Secondary Metabolite / Stigmasterol /

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Juan M. Amaro-Luis. Doctor in Chemistry, Universidad de La Laguna, España. Professor, Universidad de Los Andes (ULA), Venezuela.

Adriana Duque-Márquez. Biologist, ULA, Venezuela.

Paolo Ramoni-Perazzi. Biologist, ULA, Venezuela. M. Sc. in Systematics, Instituto de Ecología A.C., México. Professor, ULA, Venezuela.

Mariana Muñoz-Romo (Corresponding author). Biologist, ULA, Venezuela. M.Sc. in Biology, Universidad Simón Bolívar, Venezuela. Ph.D. in Biology, Boston University, USA. Professor, ULA, Venezuela. Address: Laboratorio de Zoología Aplicada, Facultad de Ciencias, ULA. Núcleo Universitario Pedro Rincón Gutiérrez, Edificio A, 3er piso, La Hechicera, Mérida 5101, Venezuela. e-mail: munozromo@gmail.com

AISLAMIENTO Y CARACTERIZACIÓN DEL ESTIGMASTEROL DE HOJAS MUY CONSUMIDAS POR EL MURCIÉLAGO FRUGÍVORO GRANDE, *Artibeus amplus* (Chiroptera: Phyllostomidae)

Juan M. Amaro-Luis, Adriana Duque-Márquez, Paolo Ramoni-Perazzi, y Mariana Muñoz-Romo

RESUMEN

La folivoría en murciélagos aún no se comprende bien en contextos funcionales y evolutivos, en parte por no haberse estudiado a fondo. El murciélago frugívoro grande, Artibeus amplus *(Chiroptera: Phyllostomidae) frecuentemente incluye hojas de* Aspidosperma desmanthum *(Apocynaceae) en su dieta, pero maximiza su consumo antes de la reproducción. La comprensión funcional y evolutiva de la folivoría requiere investigación adicional sobre las propiedades biológicas de las hojas. Las especies de* Aspidosperma *exhiben actividad biológica variada y biosintetizan metabolitos secundarios. Nuestro objetivo fue purificar y aislar el metabolito secundario predominante en las hojas de* A. desmanthum *para identificar un compuesto candidato para explorar su papel potencial en la estimulación o inhibición de la actividad reproductiva de* A. amplus*. Se preparó un extracto metanólico con hojas secas de* A. desmanthum*. Se aisló y purificó por cromatografía convencional un compuesto único presente en este extracto, y se le caracterizó estructuralmente mediante métodos espectroscópicos que incluyen IR, EI-MS y RMN unidimensional y bidimensional. El compuesto fue identificado como estigmasterol, un metabolito ampliamente distribuido en plantas, pero reportado en solo dos especies de* Aspidosperma*. El estigmasterol ha mostrado una amplia variedad de actividades biológicas, incluyendo la regulación de la actividad reproductiva en varios mamíferos. Encontramos un componente específico y sugerimos probarlo en futuros experimentos controlados destinados a estudiar la regulación de la actividad reproductiva en* A. amplus*. Nuestro estudio es el primero en el Neotrópico en aislar un potencial precursor hormonal de hojas consumidas por murciélagos; como tal, es una contribución relevante a la comprensión de la folivoría en murciélagos frugívoros.*

ISOLAMENTO E CARACTERIZAÇÃO DO ESTIGMASTEROL A PARTIR DE FOLHAS ALTAMENTE CONSUMIDAS PELO MORCEGO FRUGÍVORO GRANDE, *Artibeus amplus* (Chiroptera: Phyllostomidae)

Juan M. Amaro-Luis, Adriana Duque-Márquez, Paolo Ramoni-Perazzi, e Mariana Muñoz-Romo

RESUMO

A folivoria em morcegos ainda não é bem compreendida em contextos funcionais e evolutivos, em parte porque não tem sido amplamente estudada. O grande-morcego-comedor-de-frutas, Artibeus amplus *(Chiroptera: Phyllostomidae) frequenemente inclui folhas de* Aspidosperma desmanthum *(Apocynaceae) como parte de sua dieta, mas maximiza o seu consumo antes da reprodução. A compreensão funcional e evolutiva desses hábitos folívoros requer mais pesquisas focadas nas propriedades biológicas das folhas. Espécies de* Aspidosperma *exibem uma ampla gama de atividades biológicas e biosintetizam metabólitos secundários. O objetivo deste estudo foi purificar e isolar o metabólito secundário predominante presente nas folhas da* A. desmanthum *para identificar um composto candidato para explorar seu potencial papel na estimulação ou inibição da atividade reprodutiva do* A. amplus*. Um extrato metanólico foi preparado com folhas secas de* A. desmanthum*. Com métodos cromatográficos convencionais um composto único presente neste extrato foi isolado e purificado, e estruturalmente caracterizado por métodos espectroscópicos, incluindo técnicas de IV, EI-MS e RMN uni e bidimensional. O composto foi identificado como estigmasterol, um metabólito amplamente distribuído em plantas, mas relatado em apenas duas espécies de* Aspidosperma*. O estigmasterol tem mostrado uma grande variedade de atividades biológicas, incluindo a regulação da atividade reprodutiva em vários mamíferos. Encontramos um componente químico alvo e sugerimos testá-lo em futuros experimentos controlados com o objetivo de estudar a regulação da atividade reprodutiva em* A. amplus*. Nosso estudo é o primeiro dos Neotrópicos em isolar um potencial precursor hormonal das folhas consumidas por morcegos; assim, é uma contribuição relevante para compreensão da folivoria em morcegos frutíferos.*

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last one has not been experimentally tested, confirmed, or thoroughly investigated. Regarding this poorly explored hypothesis, Kunz and Díaz (1995) postulated that the frequent use of leaves by several bat species could allow males to obtain secondary metabolites from the liquid fraction from leaves (Janeczko and Skoczowski, 2005; Krishnarathi *et al*., 2014) that would function as hormone precursors affecting reproductive regulatory steps, and/or females could obtain secondary metabolites from the liquid fraction from leaves that would function as critical hormone precursors for pregnancy and lactation (Wickler and Seibt, 1976; Janeczko and Skoczowski, 2005; Krishnarathi *et al*., 2014).

Duque-Marquez *et al*. (2019) determined that leaves of *Aspidosperma desmanthum* (Apocynaceae) (Figure 1A) were frequently consumed by individuals of the large fruit-eating bat, *Artibeus amplus* (Chiroptera: Phyllostomidae). However, the highest consumption of leaves from this plant species was observed in August (Duque-Marquez *et al*., 2019), shortly before males showed maximum size of testes, and three months before all females were observed with advanced pregnancy (Ruiz-Ramoni *et al*., 2017). The clear and unequivocal predominance of leaves of *A. desmanthum* (Ruiz-Ramoni *et al*., 2011; Duque-Marquez *et al*., 2019) previous to important changes in reproductive status of males and females (Ruiz-Ramoni *et al*., 2017) made us wonder whether leaves of *A. desmanthum* have an abundant

secondary metabolite (Pagare *et al*., 2015; Tarkowská, 2019), candidate to be a hormone or hormonal precursor (Janeczko and Skoczowski, 2005; Tarkowská, 2019). Thus, we hypothesized that *A. desmanthum* contains a secondary metabolite that could potentially become a hormonal precursor to be tested in future experiments. The aim of this study was to isolate and purify the predominant secondary metabolite in *A. desmanthum* through chromatographic analytical procedures with the hope of finding a target chemical component to test in future controlled experiments on the regulation of reproductive phenomena in *A. amplus*. Here we report the isolation and purification of stigmasterol from highly consumed leaves of *A. desmanthum* in the search of key elements to improve our understanding of folivory in frugivorous bats.

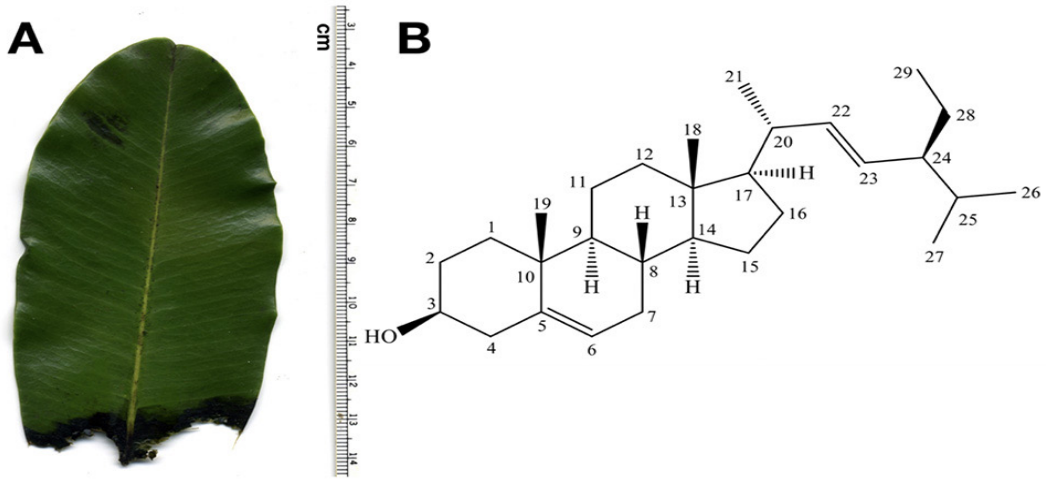

Figure 1. Partially consumed leaf of *Aspidosperma desmanthum* (A); structure of stigmasterol found in leaves (B).

Materials and Methods

Plant material, collection and identification

Aspidosperma is a genus of moderate size in the family Apocynaceae, with over 50 species distributed in the Neotropical region from Mexico and the Antilles to Argentina, with a higher diversity in Brazil (Woodson, 1951; Endress *et al*., 2018). *Aspidosperma desmanthum* Benth. ex Müll. Arg. is a tree 13-25 m tall, and its exact distribution in Venezuela is unknown especially to the north of the Orinoco river. It seems to be a rare genus in the Venezuelan Andes, since it is neither included in detailed inventories (Ataroff and García-Nuñez, 2013), nor was abundant in the study area. Plant material (fresh mature leaves of *A. desmanthum*; Figure 1A) was collected near the cave where *Artibeus amplus* lives (08°00'N, 71°43'W), at an altitude of 1320masl in May 2010. The collection place is known as '*Parque Las Escaleras*', near the village of Pregonero, Municipio Autónomo Uribante, Estado Táchira, Venezuela. A voucher specimen (J.M. Amaro-Luis, Nº 1652) was deposited at Herbarium MERF, Faculty of Pharmacy and Bioanalysis, Universidad de Los Andes (ULA), and its identity was confirmed by molecular (Ruiz-Ramoni *et al*., 2011) and morphological (J. Carmona Arzola, Department of Pharmacognosy and Organic Medicaments, Faculty of Pharmacy and Bioanalysis, ULA) procedures. Previous mentions of this species in Duque-Marquez *et al*. (2019) were made using the synonym *A. cruentum*, replaced here by the current valid name, *A. desmanthum*.

Extraction and isolation of the chemical compound from leaves

The main goal of our study was the isolation, purification, identification and structural characterization of the predominant secondary metabolite present in the leaves of *A. desmanthum*. To achieve this goal, we followed a methodology that included conventional procedures typical in phytochemical investigations: chromatographic techniques for isolation and purification, such as column (CC) and thin layer (TLC) chromatography; and spectroscopic methods for identification and structural characterization: infrared spectroscopy (IR), uni- (^{1}H- and ^{13}C-NMR) and two-dimensional (^{1}H,^{1}H-COSY, HMQC, HMBC and NOESY) nuclear magnetic resonance and mass spectrometry (MS).

General experimental procedures

Melting point was determined with a Fisher-Johns apparatus and it has not been corrected. Optical activity was measured on 60Hz-Steeg & Reuter polarimeter using methanol as solvent. IR spectra were recorded on a Perkin-Elmer FT-1725X spectrophotometer as KBr pellets. ^{1}H-, ^{13}C- and two-dimensional NMR spectra were run with a Bruker-Avance DRX-400 instrument, using $CDCl_3$ as solvent. HRMS were acquired on a VG Micromass ZAB-2F. TLC was carried out on 0.25mm layers of silica gel PF 254 (Merck); spots were visualized using UV light (254 and 365nm) and subsequently by spraying with 'oleum', an CH_3COOH-H_2O-H_2SO_4 mixture (20:4:1 v/v) and then heating with air flow at 100°C for a few minutes.VCC was performed with silica gel 60 (70-230 mesh).

Extraction and separation

Partially consumed mature leaves of *A. desmanthum* were always observed below the colony of bats. Mature leaves were sampled and analyzed. Air dried crushed leaves (≈390g) were extracted with methanol in a Soxhlet to give, after evaporation under reduced pressure at 40ºC in a rotary evaporator, 26.5g of crude extract. This extract was pre-adsorbed on silica gel 60 and chromatographed on the same adsorbent, using the Coll and Bowden (1986) vacuum liquid chromatography technique. The column was eluted with hexane, dichloromethane and methanol in binary mixtures of increasing polarity. Fractions of 0.5L were collected, concentrated *in vacuo*, and combined according to TLC similarity to afford twelve major fractions (A-L; Table I and Figure 2A). The combined major fraction 'D' (≈5.18g) was re-chromatographed on a silica gel column using hexane-dichloromethane (3:2; Figure 2B) to furnish an impure crystalline solid (≈0.418g; 1.57%), that was purified by preparative TLC (silica gel, three developments in hexane-ethyl acetate 4:1; Figure 2C). Crystallization from methanol provided pure white needles (≈78mg; 0.29%) detected in TLC plates as a red-brown spot. These yields, from the phytochemical point of view, are acceptable for a metabolite that can be considered abundant.

TABLE I
DEVELOPMENT DATA OF THE GENERAL COLUMN CHROMATOGRAPHY OF THE METHANOLIC EXTRACT FROM LEAVES OF *Aspidosperma desmanthum*

Collection	Fractions	Mass (g)	Eluent (proportion)	Nº of significant spots on TLC
A	1-14	3.22	Hexane	3
B	15-24	2.25	Hexane-Dichlorometane (9:1)	4
C	25-41	4.40	Hexane-Dichlorometane (4:1)	2
D	42-53	5.18	Hexane-Dichlorometane (7:3)	1
E	54-58	3.20	Hexane-Dichlorometane (3:2)	5
F	59-62	2.70	Hexane-Dichlorometane (1:1)	4
G	63-69	1.03	Hexane-Dichlorometane (2:3)	2
H	70-73	1.21	Hexane-Dichlorometane (1:4)	2
I	74-77	0.80	Dichlorometane (100 %)	3
J	79-81	0.24	Dichloromethane-Methanol (3:2)	2
K	82-85	----	Dichloromethane-Methanol (2:3)	----
L	86-87	----	Methanol (100%)	----

Stigmasterol identification

White needles; m.p.= 165-168ºC; $[\alpha]_D$ -54.0º (MeOH, c 0.45). HR EI-MS: m/z: 478.3663 [M^+] $C_{29}H_{48}O$ (requires 412.3705); LW EI-MS: m/z: 412.4 (53), 394.4 (17), 369.4 (10), 351.3 (20), 300.3 (29), 271.2 (46), 255.2 (60), 229.2 (13), 213.2 (28), 159.1 (53), 143.5 (49), 133.1 (52), 105.1 (50), 93.1(81), 65.3 (62), 55.1 (100); IR (KBr), ν_{max} (cm^{-1}): 3442 (O-H), 2938-2865 (C-H), 1605 (C=C), 1458 and 1375 (C-H), 1056 (C-O), 967 (=C-H). ^{1}H NMR (400MHz, $CDCl_3$), δ_H (ppm): 0.69 (3H, s, H-19), 0.79 (3H, d, J= 7.0Hz, H-26), 0.80 (3H, t, J= 7.0Hz, H-29), 0.84 (3H, d, J= 7.0Hz, H-27), 1.01 (3H, s, H-18), 1.03 (3H, d, J= 7.0Hz, H-21), 3.52 (1H, tdd, J= 4.5, 4.0 and 3.9Hz, H-3), 5.01 (1H, dd, J= 9.0 and 15.0Hz, H-23), 5.14 (1H, dd, J= 9.0 and 15.0Hz, H-22) and 5.35 (1H, d, J= 5.0Hz, H-6). ^{13}C NMR (100 MHz $CDCl_3$), δ_C (ppm): 12.1 (CH_3, C-19), 12.3 (CH_3, C-29), 19.0 (CH_3, C-26), 19.5 (CH_3, C-18), 21.1 (CH_3, C-27), 21.1 (CH_2, C-11), 21.3 (CH_3, C-21), 24.4 (CH_2, C-15), 25.5 (CH_2, C-28), 29.0 (CH_2, C-16), 31.7 (CH_2, C-2), 31.7 (CH_2, C-7), 31.9 (CH, C-8), 31.9 (CH, C-25), 36.6 (>C<, C-10), 37.3 (CH_2, C-1), 39.7 (CH_2, C-12), 40.5 (CH, C-20), 42.3 (CH_2, C-4), 42.4 (>C<, C-13), 50.2 (CH, C-9), 51.3 (CH, C-24), 56.0 (CH, C-17), 56.9 (CH, C-14), 71.9 (CH-O-, C-3), 121.8 (=CH-, C-6), 129.3 (=CH-, C-23), 138.4 (=CH-, C-22), 140.8 (=C<, C-5).

Results

The followed methodology led to the isolation and purification of a single compound, whose identity was established by IR, EI-MS and 1D- and 2D-NMR studies: stigmasterol (Figure 1B), the most abundant secondary metabolite present in the leaves of *A. desmanthum* (Figure 1A).

Stigmasterol was obtained as white needles (m.p.= 165-168 ºC; $[\alpha]_D$: - 54.0°). It showed in TLC on silica gel GF_{254} plates revealed with 'oleum' a red-brown spot typical of an isoprenoid derivative. It is evident from the molecular formula deduced by EI-MS, $C_{29}H_{48}O$ [m/z: 412 (M^+)] and from the NMR data, that the product is a phytosterol. The presence in the molecule of six methyl groups, assigned through the HMQC and HMBC spectra (Figures 3A, B) {[δ_H: 1.01 (H-18) ↔ δ_C: 19.5 (C-18)]; [δ_H: 0.69 (H-19) ↔ δ_C: 12.1 (C-19); [δ_H: 1.03 (H-21) ↔ δ_C: 21.3 (C-21)]; [δ_H: 0.79 (H-26) ↔ δ_C: 19.0 (C-26)]; [δ_H: 0.84 (H-27) ↔ δ_C: 21.1 (C-27)]; [δ_H: 0.80 (H-29) ↔ δ_C: 12.3 (C-29)]}, unequivocally confirms this assumption. The fact that, according to ^{1}H NMR spectrum data, two methyl signals are singlets (angular methyl C-18 and C-19), three correspond to doublets (secondary methyl C-21 and isopropyl group C-26 and C-27), and one a triplet (primary methyl C-29), confirms that phytosterol belongs to the stigmastane series; this is also consistent with the presence in the molecule of seven sp^3 non-oxygenated methines (>CH, C-8, C-9, C-14, C-17, C-20, C-24 and C-25) and two sp^3 quaternary carbons (>C<, C-10 and C-13) as it is confirmed by the DEPT-135 and DEPT-90 traces in the ^{13}C NMR spectrum (Figure 3A). The functional groups present in the molecule were identified from the spectral data as a trisubstituted double bond [δ_H: 5.35, d, J= 5.0Hz, =C<u>H</u>-(H-6) ↔ δ_C: 121.8, =C<u>H</u>-(C-6); δ_C: 140.8, =C<(C-5)], an 1,2-disubstituted 'trans' double bond [δ_H: 5.01 and 5,14, dd, J= 9.0 and 15.0Hz, =C<u>H</u>-(H-23 and H-22)

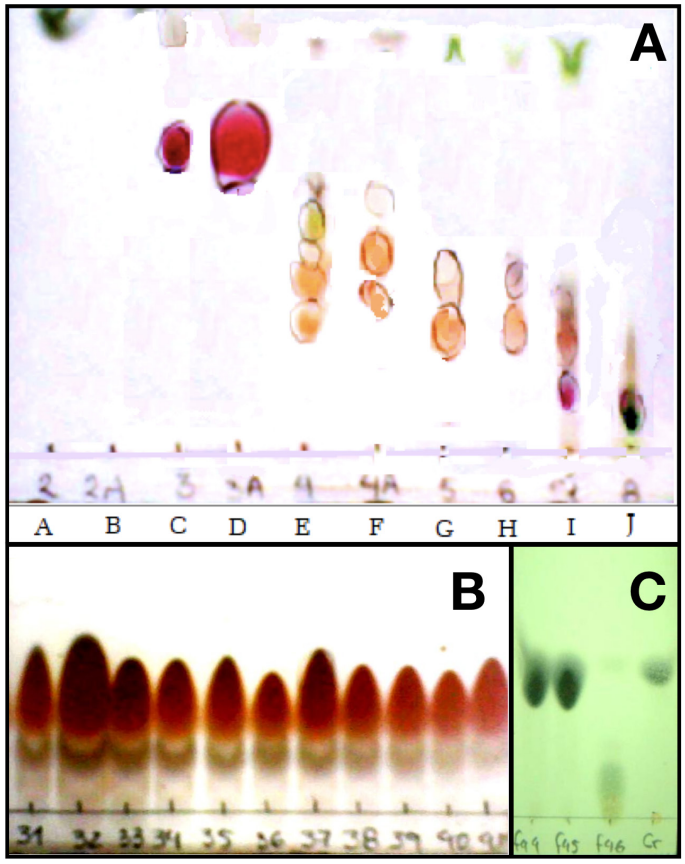

Figure 2. TLC in the different chromatographic processes of the methanolic extract from leaves of *Aspidosperma desmanthum*. A: TLC [Silicagel; Hex-EtOAc (4:1)] of collections (A-J) of general column chromatography; B: TLC [Silicagel; (hexane-dichloromethane 3:2)] of fractions obtained by chromatography of combined fraction 'D'; C: TLC [Silicagel; (Hex-EtOAc 4:1)] of fractions obtained by extraction of scraped bands in preparative TLC of impure stigmasterol.

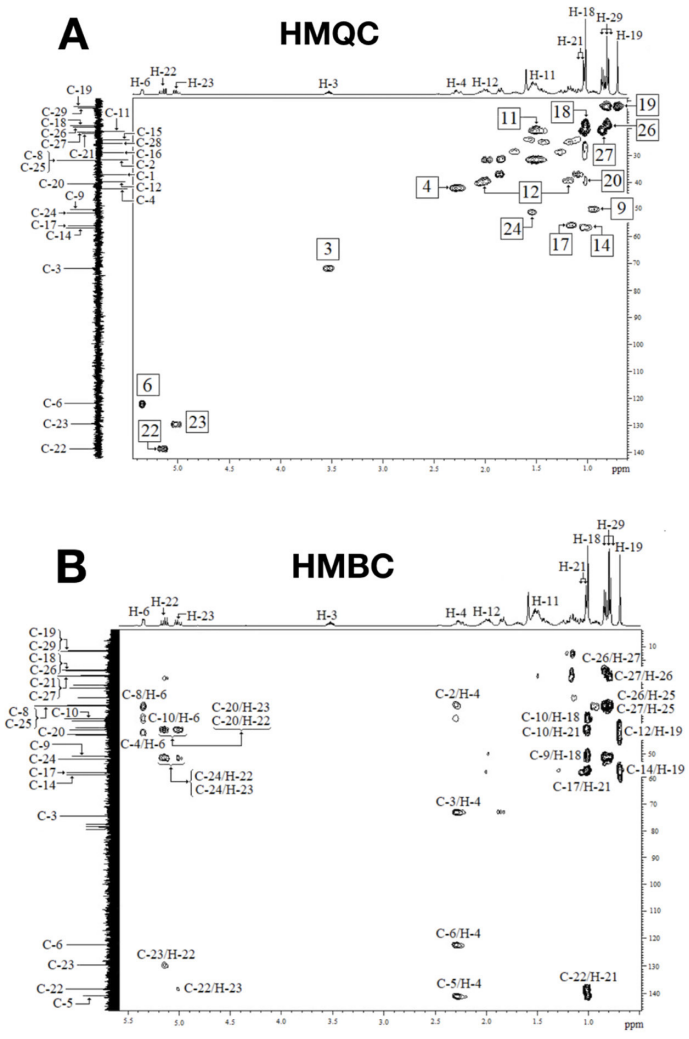

Figure 3. Heteronuclear Multiple Quantum Coherence (HMCQ; A) and Heteronuclear Multiple Bond Correlation (HMBC; B) spectra of stigmasterol.

↔ δ_C: 129.3 and 138.4, =CH (C-23 and C-22)], and a secondary alcohol [IR, ν_{max}: 3442 (OH) and 1056 (C-O); EI-MS, m/z: 394 [M^+-H_2O], RMN, δ_H: 3.52, tdd, J= 4.5, 4.0 and 3.9Hz, >C<u>H</u>-O-(H-3) ↔ δ_C: 71.9, ><u>C</u>H-O-(C-3)]. The configuration of the hydroxyl group (3βec.) of the secondary alcohol was deduced from the multiplicity (tdd) of the signal assigned to its geminal proton (>C<u>H</u>-O-) and from the values of its coupling constants ($J_{3\alpha ax.,\ 2\beta ax.}$= $J_{3\alpha ax.,\ 4\beta ax.}$= 4.5Hz; $J_{3\alpha ax.,\ 2\alpha ec.}$= 4.0Hz and $J_{3\alpha ax.,\ 2\alpha ec.}$= 3.9Hz). Similarly, the 'trans' stereochemistry of the double bond $\Delta^{22,23}$ was consistent with the value of its highest coupling constant ($J_{22,23}$= 15.0Hz).

The position of the three functional groups in the steroidal skeleton was unequivocally assigned by C-H interactions in the HMBC spectrum (Figure 3B). Thus, the H-3 ↔ C-4 correlation places the secondary hydroxyl group at C-3, which is consistent with biogenetic considerations; the position $\Delta^{5,6}$ of the trisubstituted double bond was secured by the crossed peaks of H-6 with C-4, C-8 and C-10 and H-4 with C-5 and C-6; the location $\Delta^{22,23}$ of the 1,2-disubstituted double bond, is consistent for the following correlation sequences: H-22 ↔ C-24 ↔ H-23 ↔ C-20 ↔ H-22 ↔ C-23 and H-21 ↔ C-22 ↔ H-23. The position in the molecule of all methyl groups was also fixed through the HMBC spectrum (Figure 3B).

Discussion

The preceding analysis indicates that the structure of the compound found in leaves of *A. desmanthum* corresponds to 3β, 22*E*-stigmasta-5,22-dien-3-ol, named in the literature as stigmasterol. This compound is widely distributed in plants, it is efficiently used as a starting material for the synthesis of sex hormones, and has a wide range of biological and pharmacological activities (Janeczko and Skoczowski, 2005; Kaur *et al*., 2011; Tarkowská, 2019). Stigmasterol is often present in plants mixed with β-sitosterol and campesterol, but there are numerous reports in the literature (Kaur *et al*., 2011) of detection and isolation of only one of these phytosterols. In TLC these three phytosterols have very similar Rf and our NMR data indicate that there are no traces of β-sitosterol or campesterol in our isolated sample. The presence of this phytosterol in species of the genus *Aspidosperma* has not been well studied as it has only been reported in two species, *A. nitidum* Benth ex Müll. Arg. (Pereira *et al*., 2006) and *A. parvifolium* A. DC. (Jacome *et al*., 2004).

Several biological activities reported for stigmasterol would specifically affect the mammalian reproductive system. For example, it is a precursor of the sex hormones (Janeczko and Skoczowski, 2005; Kaur *et al*., 2011; Wang *et al*., 2011; Janeczko, 2012; Tarkowská, 2019), it has shown androgenic activity as it can regulate the metabolism, secretion, and growth of the prostate under specific concentrations (Hirano *et al*., 1994), and it also affects the ability to regenerate spermatogenic cells in mammalian testes, so that spermatogenesis could occur earlier, and sperm production is promoted (Alfiah, 2011). Stigmasterol was also found to be a key factor in increased levels of prolactin, critical for the initiation and maintenance of breast milk production in mammals (Anjaria *et al*., 1975). Wickler and Seibt (1976) were the first researchers that suggested leaves could be providing bats hormonal precursors, although this field (Tarkowská, 2019) remained neglected for years. These authors found that leaves of *Balanites wilsoniana* were the source of steroidal sapogenins for the African fruit bat, *Epomophorus wahlbergi* (Wickler and Seibt, 1976), although this plant species also contains stigmasterol (Sofowora and Hardman, 1973). Since steroid hormones play a vital role as androgenes, gestagenes, glucocorticoids, and as mineralocorticoids (Wickler and Seibt, 1976; Krishnarathi *et al*., 2014), the complete understanding of ecological and evolutionary implications of folivory in bats will be based on further field and laboratory studies.

Several functions of stigmasterol reported in the available literature support the idea of its potentially important role in mammalian reproductive systems (Tarkowská, 2019). Thus, we would suggest testing stigmasterol as a potential regulator of reproduction in the Neotropical frugivorous bat *A. amplus*, based on the highest consumption of leaves of *A. desmanthum* (Ruiz-Ramoni *et al*., 2011; Duque-Marquez *et al*., 2019) before the exhibition of unequivocal signs of reproductive activity in both sexes, such as maximum testes size and advanced pregnancy (Ruiz-Ramoni *et al*., 2017). The identification of stigmasterol in this study provides a target chemical compound that could function as potential regulator of individuals' reproductive condition, which allows its experimental testing in future follow up studies.

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