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Article

Saponins and Sapogenins from *Brachiaria decumbens* Stapf

Viviane S. Pires[a], Alexandre T. C. Taketa[b], Grace Gosmann[a] and Eloir P. Schenkel*[a]

[a]*Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul (UFRGS), Av. Ipiranga, 2752, 90610-000 Porto Alegre, RS - Brazil*

[b]*Kekulé-Institut für Organische Chemie und Biochemie der Rheinischen Friedrich-Wilhelms-Universität, Bonn, Germany*

Quatro saponinas esteroidais e três sapogeninas foram identificadas das partes aéreas de *Brachiaria decumbens*. As estruturas desses compostos foram estabelecidas através de métodos químicos e espectroscópicos (RMN de ^{1}H e ^{13}C, HMBC, HMQC) como 3β-metóxi-lanost-9(11)-eno (**1**), diosgenina (**2a**), iamogenina (**2b**), 3-O-β-D-glicopiranosil-24(*S*)-etil-22*E*-deidrocolesterol (**3a**), 3-O-β-D-glicopiranosil-24(*R*)-etil-22*E*-deidrocolesterol (**3b**), dioscina (**4a**) e 3-O-{α-L-ramnopiranosil-(1→4)-[α-L-ramnopiranosil-(1→2)]-β-D-glicopiranosil}-25(*S*)-espirost-5-en-3β-ol (**4b**). Esses compostos foram isolados pela primeira vez em *B. decumbens*, sendo que o composto **4b** não foi ainda descrito na natureza conforme revisão realizada.

Four steroidal saponins and three sapogenins were identified from aerial parts of *Brachiaria decumbens*. Their structures were established by chemical and spectroscopic means (^{1}H and ^{13}C NMR, HMBC, HMQC) as 3β-methoxy-lanost-9(11)-ene (**1**), diosgenin (**2a**), yamogenin (**2b**), 3-O-β-D-glucopyranosyl-24(*S*)-ethyl-22*E*-dihydrocholesterol (**3a**), 3-O-β-D-glucopyranosyl-24(*R*)-ethyl-22*E*-dihydrocholesterol (**3b**), dioscin (**4a**) and 3-O-{α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranosyl}-25(*S*)-spirost-5-en-3β-ol (**4b**). All these compounds were isolated for the first time from *B. decumbens* and compound **4b** is described for the first time as far as we know.

Keywords: *Brachiaria decumbens*, steroidal saponins, dioscin, diosgenin, yamogenin, triterpene.

Introduction

Brachiaria decumbens Stapf belongs to a group of plants capable of inducing hepatogenous photosensitizations[1-4] similar to those described for *Panicum* spp.,[5-8] *Tribulus terrestris*,[8,9] *Agave lecheguilla*[10] and *Narthecium ossifragum*[8]. These species are all known to contain steroidal saponins which have been associated with deposition of crystalloid material within the biliary system and photosensitization. In our previous paper,[11] we reported the isolation and structural determination of steroidal sapogenins present in rumen contents of lambs intoxicated with *B. decumbens*. In this paper we describe the structural elucidation of triterpene and steroidal compounds isolated from the aerial parts of *B. decumbens* as free aglycones or as saponins. The latter compounds were isolated as epimeric pairs.

* e-mail: schenkel@ccs.ufsc.br

Experimental

Plant material and extraction

Aerial parts from *B. decumbens* Stapf were collected in Teutônia, State of Rio Grande do Sul, Brazil, in January 2000. A herbarium specimen is on deposit in the Herbarium of the Botany Department of the Federal University of Rio Grande do Sul (Herbarium ICN, Porto Alegre, Brazil, voucher nº 121456). Air-dried powdered plant material (500 g) was extracted during 7 days at room temperature with ethanol. The solvent was evaporated and the residual alcoholic phase was suspended in water. This suspension was partitioned with dichloromethane and the main dichloromethane compounds were isolated using column chromatography.

Isolation

Part of the dichloromethane extract (5 g) was chromatographed on a silica gel column and eluted with dichloro-

methane:ethanol (99:1; 90:10; 80:20 and 70:30, v/v). Fractions were pooled according to thin-layer chromatography (TLC) analysis. Fractions 2-10 (250 mg) containing compound **1** as main component, were further purified by silica gel column chromatography, eluting with hexane and resulting in pure compound **1** (12.5 mg). Fractions 30-40 (500 mg), 50-60 (350 mg) and 68-81 (500 mg) containing compounds **2**, **3** and **4** as main components were submitted to successive silica gel column chromatography, eluting with hexane:ethyl acetate (3:1, v/v), dichlorometane:ethanol (9:1, v/v) and dichlorometane: ethanol (7:3, v/v), respectively. By these procedures compounds **2** (33 mg), **3** (15 mg) and **4** (40 mg) were obtained.

General procedures

Dichloromethane extract and fractions were chromatographed on a silica gel column (Merck, particle size: 230-400 mesh) at atmospheric pressure. TLC was carried out on silica gel (Merck, GF_{254}) using hexane:ethyl acetate (3:1, v/v) and dichlorometane:ethanol (7:3, v/v). Compounds were visualized by heating (120 °C) using the anisaldehyde-sulfuric acid-spray. FAB-MS spectrum was performed on a MS50 spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker AMX 500 spectrometer.

*3β-methoxy-lanost-9(11)-ene (**1**).* ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.83 (H-1), 1.91, 1.75 (H-2), 2.70 (H-3), 0.89 (H-5), 1.67, 1.72 (H-6), 1.37 (H-7), 2.19 (H-8), 5.25 (H-11), 1.93, 2.09 (H-12), 1.28 (H-15), 1.65, 1.71 (H-16), 1.62 (H-17), 0.67 (H-18), 0.91 (H-19), 1.41 (H-20), 0.77 (H-21), 1.38 (H-22), 1.38 (H-23), 1.16 (H-24), 1.54 (H-25), 0.89 (H-26), 0.90 (H-27), 1.06 (H-28), 1.00 (H-29), 0.83 (H-30), 3.40 (OCH_3).^{13}C NMR (125 MHz, $CDCl_3$) δ 36.4 (C-1), 22.9 (C-2), 89.0 (C-3), 39.4 (C-4), 53.4 (C-5), 21.6 (C-6), 34.3 (C-7), 42.2 (C-8), 149.0 (C-9), 37.5 (C-10), 115.2 (C-11), 37.5 (C-12), 44.5 (C-13), 47.4 (C-14), 30.1(C-15), 28.5 (C-16), 51.4 (C-17), 14.8 (C-18), 18.8 (C-19), 36.6 (C-20), 18.9 (C-21), 36.9 (C-22), 24.5 (C-23), 39.9 (C-24), 28.4 (C-25), 22.9 (C-26), 23.2 (C-27), 22.6 (C-28), 28.6 (C-29), 16.8 (C-30), 57.9 (OCH_3).

*Compound **2a**.* diosgenin. ^{1}H NMR and ^{13}C NMR data ($CDCl_3$): same as in references 15 and 17.

*Compound **2b**.* yamogenin. ^{1}H NMR and ^{13}C NMR data ($CDCl_3$): same as in references 15 and 17.

*3-O-β-D-glucopyranosyl-24(S)-ethyl-22E-dihydrocholesterol (**3a**).* ^{1}H NMR data (500 MHz, C_5D_5N): δ 1.71, 0.98 (H-1), 2.12, 1.74 (H-2), 3.94 (H-3), 2.72, 2.46 (H-4), 5.34 (H-6), 1.88, 1.54 (H-7), 1.36 (H-8), 0.89 (H-9), 1.45, 1.45 (H-11), 1.97, 1.09 (H-12), 0.92 (H-14), 1.02, 1.53 (H-15), 1.26, 1.84 (H-16), 1.08 (H-17), 0.88 (H-18), 0.95 (H-19), 2.03 (H-20), 0.90 (H-21), 5.20 (H-22), 5.06 (H-23), 1.56 (H-24), 1.54 (H-25), 1.07 (H-26), 0.85 (H-27), 0.95 (H-28), 0.87 (H-29), 5.05 (glc H-1), 4.05 (glc H-2), 4.28 (glc H-3), 4.28 (glc H-4), 3.97 (glc H-5), 4.41, 4.56 (glc H-6); ^{13}C NMR (125 MHz, C_5D_5N): δ 37.6 (C-1), 30.4 (C-2), 78.2 (C-3), 39.5 (C-4), 141.1 (C-5), 122.1 (C-6), 32.3 (C-7), 32.2 (C-8), 50.5 (C-9), 37.1 (C-10), 21.5 (C-11), 40.0 (C-12), 42.5 (C-13), 57.0 (C-14), 24.7 (C-15), 28.7 (C-16), 56.2 (C-17), 12.3 (C-18), 19.2 (C-19), 41.0 (C-20), 19.6 (C-21), 139.0 (C-22), 129.6 (C-23), 51.6 (C-24), 32.4 (C-25), 21.7 (C-26), 19.4 (C-27), 25.9 (C-28), 12.7 (C-29), 102.7 (glc C-1), 75.5 (glc C-2), 78.8 (glc C-3), 71.8 (glc C-4), 78.7 (glc C-5), 63.0 (glc C-6).

*3-O-β-D-glucopyranosyl-24(R)-ethyl-22E-dihydrocholesterol (**3b**).* ^{1}H NMR (500 MHz, C_5D_5N): δ 1.71, 0.98 (H-1), 2.12, 1.74 (H-2), 3.94 (H-3), 2.72, 2.46 (H-4), 5.34 (H-6), 1.88, 1.54 (H-7), 1.36 (H-8), 0.89 (H-9), 1.45, 1.45 (H-11), 1.97, 1.09 (H-12), 0.92 (H-14), 1.02, 1.53 (H-15), 1.26, 1.84 (H-16), 1.08 (H-17), 0.88 (H-18), 0.95 (H-19), 2.03 (H-20), 0.87 (H-21), 5.20 (H-22), 5.06 (H-23), 1.56 (H-24), 1.54 (H-25), 0.85 (H-26), 0.92 (H-27), 1.28 (H-28), 0.66 (H-29), 5.05 (glc H-1), 4.05 (glc H-2), 4.28 (glc H-3), 4.28 (glc H-4), 3.97 (glc H-5), 4.41, 4.56 (glc H-6).^{13}C NMR (125 MHz, C_5D_5N): δ 37.6 (C-1), 30.4 (C-2), 78.2 (C-3), 39.5 (C-4), 141.1 (C-5), 122.1 (C-6), 32.3 (C-7), 32.2 (C-8), 50.5 (C-9), 37.1 (C-10), 21.5 (C-11), 40.1 (C-12), 42.6 (C-13), 57.1 (C-14), 24.7 (C-15), 28.7 (C-16), 56.4 (C-17), 12.3 (C-18), 19.2 (C-19), 41.0 (C-20), 20.2 (C-21), 139.0 (C-22), 129.6 (C-23), 51.6 (C-24), 32.4 (C-25), 19.4 (C-26), 21.5 (C-27), 23.6 (C-28), 12.2 (C-29), 102.7 (glc C-1), 75.5 (glc C-2), 78.8 (glc C-3), 71.8 (glc C-4), 78.7 (glc C-5), 63.0 (glc C-6).

Compound **4**. FAB-MS (positive mode) *m/z* 907.6 $[M+K]^+$, 891.6 $[M+Na]^+$, 869.5 $[M+H]^+$, 723.3 $[(M+H)-146]^+$, 415.3 $[(M+H)-146-146-162]^+$, 413.3 [aglycone-H]$^+$, 395.3 [aglycone-H_3O]$^+$.

Compound **4a**. ^{1}H NMR and ^{13}C NMR data (C_5D_5N): same as in references 17 and 22.

*3-O-{α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranosyl}-25(S)-spirost-5-en-3β-ol (**4b**).* ^{1}H NMR (500 MHz, C_5D_5N): δ 1.72, 0.97 (H-1), 2.06, 1.84 (H-2), 3.86 (H-3), 2.75, 2.75 (H-4), 5.30 (H-6), 1.84, 1.45 (H-7), 1.55 (H-8), 0.89 (H-9), 1.42, 1.42 (H-11), 1.66, 1.07 (H-12), 1.03 (H-14), 2.01, 2.01 (H-15), 4.48 (H-16), 1.77 (H-17), 1.06 (H-18), 1.05 (H-19), 1.87 (H-20), 1.13 (H-21), 1.44, 1.87 (H-23), 2.13, 1.34 (H-24), 1.80 (H-25), 4.03, 3.34 (H-26), 1.12 (H-27), 4.95 (glc H-1), 4.22 (glc H-2), 4.18 (glc H-3), 4.39 (glc H-4), 3.62 (glc H-5), 4.08, 4.21 (glc H-6), 6.39 (rha H-1), 4.84 (rha H-2), 4.67 (rha H-3), 4.37 (rha H-4), 4.95 (rha H-5), 1.75 (rha H-6), 5.85 (rhaI H-1), 4.62 (rhaI H-2), 4.53 (rhaI H-3), 4.33 (rhaI H-4), 4.93 (rhaI H-5), 1.62 (rhaI H-6).

^{13}C NMR (125 MHz, C_5D_5N): δ 37.6 (C-1), 30.3 (C-2), 78.1 (C-3), 39.1 (C-4), 140.9 (C-5), 121.9 (C-6), 32.4 (C-7), 31.8 (C-8), 50.4 (C-9), 37.2 (C-10), 21.2 (C-11), 39.9 (C-12), 40.5 (C-13), 56.7 (C-14), 32.3 (C-15), 81.2 (C-16), 63.0 (C-17), 16.4 (C-18), 19.5 (C-19), 42.5 (C-20), 15.0 (C-21), 109.4 (C-22), 26.5 (C-23), 26.3 (C-24), 27.6 (C-25), 65.2 (C-26), 15.2 (C-27), 100.4 (glc C-1), 77.9 (glc C-2), 78.2 (glc C-3), 78.6 (glc C-4), 77.0 (glc C-5), 61.3 (glc C-6), 102.1 (rha C-1), 72.6 (rha C-2), 72.7 (rha C-3), 74.2 (rha C-4), 69.6 (rha C-5), 18.8 (rha C-6), 103.0 (rhaI C-1), 72.9 (rhaI C-2), 72.8 (rhaI C-3), 74.0 (rhaI C-4), 70.5 (rhaI C-5), 18.6 (rhaI C-6).

Results

Solvent partition and chromatographic procedures allowed the isolation of the main compounds **1-4** (Figure 1) from the aerial parts of *B. decumbens*.

Acid hydrolysis of **3** yielded only one sugar identified as glucose (glc), and acid hydrolysis of **4** yielded two sugars identified as rhamnose (rha) and glucose (glc). In both cases, the sugars were identified by co-TLC using authentic samples.

The ^{13}C NMR spectrum of compound **1** displayed 31 signals, whereas the DEPT spectrum revealed 9 methyl, 10 methylene, 7 methine and 5 quartenary carbon atoms. The presence of an insaturation can be observed in the ^{13}C NMR spectrum by the signals at δ_c 149.0 (Cq) and δ_c 115.2 (CH) and, by one-proton absorbance at δ_H 5.25 (H-11) in the ^{1}H NMR spectrum. These data suggest a $\Delta^{9(11)}$-lanostene skeleton. The ^{1}H NMR spectrum of **1** showed the presence of a singlet at δ_H 3.40 (3H) correlated with the carbon at δ_c 57.9 (OCH_3), demonstrating the presence of a methoxyl group. Long range correlation peaks were detected between the methoxyl signal and C-3 (δ_c 89.0). The others signals of **1** were established by HMBC, HMQC and ^{1}H,^{1}H COSY experiments. Through detailed comparison of these data with those from literature,[12-14] **1** was identified as 3β-methoxy-lanost-9(11)-ene.

Compound **2** presented the same Rf value of an authentic sample of diosgenin on silica gel plates (TLC).

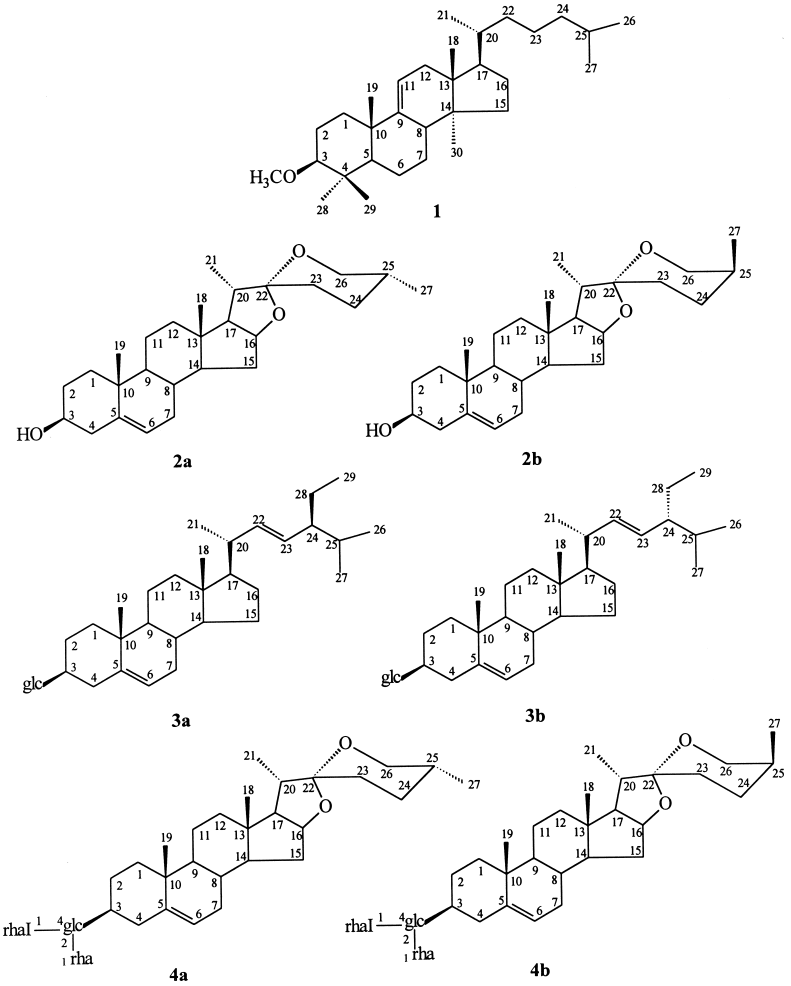

Figure 1. Compounds **1, 2a, 2b, 3a, 3b, 4a, 4b** isolated from aerial parts of *Brachiaria decumbens*

The IR spectrum showed an hydroxy group (3445 cm^{-1}). The ^{13}C NMR spectrum showed some duplicated signals indicating the presence of an epimeric mixture. All the signals of **2** and their connectivity were established by HMBC, HMQC and $^{1}H,^{1}H$ COSY experiments. It was possible to demonstrate a spiroketal ring system through the presence of carbon quaternary signals at δ_c 109.7 (C-22, **2a**) and 110.1 (C-22, **2b**), -OCH_2 group at δ_c 67.2 (C-26, **2a**) and 65.5 (C-26, **2b**), and OCH group at δ_c 81.2 (C-16, **2a**) and 81.3 (C-16, **2b**). The epimeric mixture was also characterized through the ^{1}H NMR spectrum by the different multiplicity of the H-26 methylene protons caused by the methyl group at C-25. In this way, compound **2a** showed signals at δ_H 3.40 (*t*, *J* 11.0, H-26α) and at δ_H 3.49 (*ddd*, *J* 10.9, 4.1, 2.1 Hz, H-26β), and compound **2b** presented signals at δ_H 3.98 (*dd*, *J* 10.7, 2.5 Hz, H-26α) and δ_H 3.32 (*d*, *J* 10.7 Hz, H-26β), demonstrating the equatorial and axial protons. Regarding the ^{13}C NMR spectrum, the configuration 25*R* or 25*S* can be differentiated by the chemical shifts for C-23, C-24, C-25, C-26 and C-27. Careful comparison of ^{1}H NMR and ^{13}C NMR spectral data of **2** with those of the literature[15-18] allowed to establish **2** as the 25-epimeric mixture of diosgenin [(25*R*)-spirost-5-en-3β-ol] **2a** and yamogenin [(25*S*)-spirost-5-en-3β-ol] **2b**.

The ^{13}C NMR of compound **3** displayed 46 signals including, as determined from the DEPT spectrum, 13 methyl, 11 methylene, 18 methine and 4 quaternary carbon atoms, indicating an epimeric mixture through the presence of some duplicated signals. All signals of **3** and their connectivity were established by HMBC, HMQC and $^{1}H,^{1}H$ COSY experiments. The NMR data showed the presence of one sugar identified as glucose (δ_H 5.05, *d*, *J* 7.6, δ_c 102.7) which signals have correlation to H-3. Two insaturations at δ_c 141.1 (Cq, C-5) and 122.1 (CH, C-6), and δ_c 139.0 (CH, C-22) and 129.6 (CH, C-23) together with the corresponding olefinic protons at δ_H 5.34 (H-6), 5.20 (H-22) and 5.06 (H-23) are characteristic for $\Delta^{5,22}$-3β-hydroxysterols. Comparing to the literature data[19,20] it was possible to verify that **3** is 24α- and 24β-stigmastane mixture that can be differentiated by the chemical shifts for C-26 and C-27, which vary about δ 2 ppm. Therefore, it was concluded that **3** is the epimeric mixture of 3-O-β-D-glucopyranosyl-24(*S*)-ethyl-22*E*-dihydrocholesterol (**3a**) and 3-O-β-D-glucopyranosyl-24(*R*)-ethyl-22*E*-dihydrocholesterol (**3b**, stigmasterol glucoside).

FAB-MS (positive mode) of compound **4** exhibited ions at *m/z* 869.5 $[M+H]^+$, 891.6 $[M+Na]^+$ and 907.6 $[M+K]^+$ indicating the molecular mass $C_{45}H_{72}O_{16}$. Fragments ions at *m/z* 723.3 and *m/z* 415.3 indicated the elimination of one methylpentose, and two methylpentoses and one hexose, respectively.[21] The ^{13}C NMR spectrum displayed 57 signals, determined from the DEPT spectrum as 8 methyl, 15 methylene, 28 methine and 5 quaternary carbon atoms. Careful comparison of ^{13}C NMR spectral data of **4** with that of **2** showed that **4** has same aglycone moieties (an epimeric mixture) and that **4** differs structurally from **2** only by the presence of sugar signals. This assumption was confirmed by HMBC, HMQC and $^{1}H,^{1}H$-COSY experiments. Comparing the chemical shifts of sugars with the literature data[7,17,22] it was possible to identify them as two rhamnoses and one glucose. Using the HMBC, HMQC and $^{1}H,^{1}H$-COSY spectra it was possible to identify the interglycosidic linkage. Through all these data we could identify **4** as dioscin (**4a**) and 3-O-{α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranosyl}-25(*S*)-spirost-5-en-3β-ol (**4b**). The latter compound was not described in the literature, as far as we know.

Discussion

Over the past years photosensitization in ruminants grazing *B. decumbens* had been associated with sporidesmin from *Pithomyces chartarum*. However, hepatogenous photosensitization was detected without the presence of *P. chartarum*,[1-4] proving the association of the disease with the ingestion of plant saponins as, demonstrated by Miles et al.[8] Such compounds are metabolized by microbial flora in ruminants to β-D-glucuronide insoluble salts of episarsasapogenin and epismilagenin.

In the case of *B. decumbens*, the presence of saponins was detected and the aglicone was characterized by GC-MS after acid hydrolysis from butanolic extract,[2] but the chemical structures of these saponins were not elucidated. In the present investigation it was possible to isolate and identify the following compounds: 3β-methoxy-lanost-9(11)-ene (**1**), diosgenin (**2a**), yamogenin (**2b**), 3-O-β-D-glucopyranosyl-24(*S*)-ethyl-22*E*-dihydrocholesterol (**3a**), 3-O-β-D-glucopyranosyl-24(*R*)-ethyl-22*E*-dihydrocholesterol (**3b**), dioscin (**4a**) and, 3-O-{α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranosyl}-25(*S*)-spirost-5-en-3β-ol (**4b**). This last compound is the dioscin 25-epimer and, to the best of our knowledge, has not been previously described. All these compounds were isolated for the first time from *Brachiaria decumbens*. Diosgenin and yamogenin were already isolated from the acid hydrolysis of *B. decumbens* butanolic extract,[2] but were not isolated in the free form from this plant. These results together with our previous work[11,23] demonstrated that *B. decumbens*, as *Panicum* spp., *T. terrestris*, *A. lecheguilla* and *N. ossifragum*, is capable of producing photosensitization in animals due the presence of steroidal saponins and sapogenins.

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