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Spectral Assignments and Reference Data

Characterization of the fraction components using 1D TOCSY and 1D ROESY experiments. Four new spirostane saponins from *Agave brittoniana* Trel. spp. *Brachypus*

Francisco A. Macías,[1]* José O. Guerra,[2] Ana M. Simonet[1] and Clara M. Nogueiras[3]

[1] Grupo de Alelopatía, Departamento de Química Orgánica, Facultad de Ciencias, Universidad de Cádiz, C/República Saharaui, s/n, 11510 Puerto Real, Cádiz, Spain
[2] Departamento de Licenciatura en Química, Facultad de Química y Farmacia, Universidad Central ''Marta Abreu'' de Las Villas, Carretera a Camajuaní km 5.5, 54830 Santa Clara, Cuba
[3] Departamento de Química Orgánica, Facultad de Química, Universidad de La Habana, C/Zapata s/n entre G y Carlitos Aguirre, Vedado, Plaza de la Revolución, 10400, Ciudad de La Habana, Cuba

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A careful NMR analysis, especially 1D TOCSY and 1D ROESY, of two refined saponin fractions allowed us to determine the structures of four new saponins from a polar extract of the *Agave brittoniana* Trel. spp. Brachypus leaves. A full assignment of the ^{1}H and ^{13}C spectral data for these new saponins, agabrittonosides A–D (1–4), and one previously known saponin, karatavioside A (5) is reported. Their structures were established using a combination of 1D and 2D (^{1}H, ^{1}H-COSY, TOCSY, ROESY, *g*-HSQC, *g*-HMBC and *g*-HSQC-TOCSY) NMR techniques and ESI-MS. Moreover, the work represents a new approach to structural elucidation of saponins in refined fractions by NMR investigations. Copyright © 2007 John Wiley & Sons, Ltd.

KEYWORDS: NMR; ^{1}H; ^{13}C; 1D TOCSY; 1D ROESY; structural analysis; spirostane saponin; characterization of fraction components; *Agave brittoniana*

INTRODUCTION

The genus *Agave*, belonging to the family Agavaceae, has more than 300 species, with around 16 of them present with an even distribution throughout Cuba. Their leaves are used traditionally in the treatment of parasitic diseases.[1] Two endemic subspecies of *Agave brittoniana* Trel. (ssp. *Brachypus* and ssp. *Spirituana*) grow as endemic plants in the central region of Cuba.[2] The leaf extract of the first subspecies showed an interesting activity against the parasite *Fasciola hepatica*.[3]

The bioassay-guided isolation process resulted in a pure compound and two fractions with two saponins in each fraction, after repeated chromatography processes. The pure compound, karatavioside A, was isolated for the first time from *Allium karataviense* in 1978,[4] and herein is described a full assignment of the ^{1}H and ^{13}C spectral data.

The refined fractions had two saponins with the same aglycon and different sugar chains. The study of the NMR spectra, especially 1D TOCSY of the anomeric proton signals, led us to identify the nature of the sugar moiety and 1D ROESY, the sugar chain connection. These experiments showed a simple subspectrum, compared to a congested ^{1}H NMR. The utilization of *g*-HSQC-TOCSY spectra was especially useful for the complete assignment of ^{13}C spectral data for each saponin in the fraction spectrum, analyzing the anomeric proton signal correlations.

*Correspondence to: Francisco A. Macías, Grupo de Alelopatía, Departamento de Química Orgánica, Facultad de Ciencias, Universidad de Cádiz, C/República Saharaui, s/n, 11510 Puerto Real, Cádiz, Spain.
E-mail: famacias@uca.es

On the basis of these techniques, we elucidated four new steroidal saponins (Agabrittonosides A–D, Fig. 1).

The application of these methods represents a clear example in which the characterization of bioactive natural products from refined fractions could be achieved. Some authors propose alternative methods to simplify the identification of natural products.[5–8] This could be another reliable alternative to the unsolved problems that could appear by following classical isolation and structural determination procedures.

RESULTS AND DISCUSSION

The leaves of *A. brittoniana* ssp. *brachypus* were extracted exhaustively with EtOH-H_2O (7:3). This extract was partitioned (*n*-butanol/water), and the *n*-butanol-soluble portion was subjected to a bioassay-guided medium-pressure liquid chromatography (MPLC) separation using RP-18 to obtain five fractions. After multiple separation processes by reversed phase HPLC of the active fraction, 4 gave two refined fractions with two saponins in each one, and the pure known karatavioside A (**5**). This compound was isolated for the first time in *Agave* species and its NMR data are fully assigned.

The first refined fraction was obtained as the most efficient separation with two compounds in a 4:1 ratio, on the basis of their anomeric proton signals. From this mixture, the structure elucidation of the major compound **1** (Tables 1–3) was performed. The ESI-MS in negative mode of this fraction exhibited a pseudo-molecular ion peak at m/z 1179 $[M-H]^-$, and a peak in the positive mode at m/z 1203 $[M+Na]^+$, in accordance with an empirical molecular formula of $C_{55}H_{88}O_{27}$, which was supported by comparative analysis of the ^{13}C NMR and ^{13}C DEPT spectroscopic data. The NMR characteristics of the compound **1** were consistent with a saponin with yuccagenin as aglycon.[9] The observation of five anomeric signals, (δ_H 5.59, 5.19, 5.15, 5.05, 4.90; δ_C 105.9, 104.6, 104.1, 103.6, 103.1) in the ^{1}H and ^{13}C NMR spectra and related in the *g*-HSQC spectrum, suggested that this compound possesses five sugar moieties. The individual sugar units were identified on the basis of the analysis of the proton resonances in each sugar unit. These determinations were carried out by a combination of DQF-COSY and 1D TOCSY NMR experiments. 1D TOCSY subspectra were obtained from selective excitation of the anomeric protons. The selective TOCSY experiments for signals at δ 5.59 and 5.19 reveal two spin systems of the β-glucose units, and analogous experiments for the signals at δ 5.15 and 5.06 suggested the presence of two β-xylose units. Starting from the anomeric proton at δ 4.90, correlations for H-1/H-2 and H-2/H-3 in the 1D TOCSY spectrum, and a relatively small coupling constant of H-3/H-4, were observed. These observations together with the 1D ROESY correlation between H-3/H-5 indicated the presence of a β-galactopyrane sugar moiety. Analysis of the *g*-HSQC and *g*-HSQC-TOCSY experiments allowed the assignments of the signals of the ^{13}C NMR spectrum corresponding to the five sugar units. The sugar sequence of the five sugar units was deduced from the 1D ROESY and HMBC spectra, in which long-range correlations were observed from H-$1_{glc'}$ (δ 5.59) to H-2_{glc} (δ 4.31) and C-2_{glc} (δ 80.5), H-1_{glc} (δ 5.19) to H-4_{gal} (δ 4.57) and C-4_{gal} (δ 79.0), H-1_{xyl} (δ 5.15) to H-3_{glc} (δ 4.09) and C-3_{glc} (δ 86.8), H-$1_{xyl'}$ (δ 5.06) to H-$3_{glc'}$ (δ 4.08) and C-$3_{glc'}$ (δ 87.0), and H-1_{gal} (δ 4.90) to H-3 of the aglycon (δ 3.78) and C-3 of the aglycon (δ 84.5). Thus, the structure of **1** was established as yuccagenin-3-*O*-β-D-xylopyranosyl-(1 → 3)-[β-D-xylopyranosyl-(1 → 3)-β-D-glucopyranosyl-(1 → 2)]-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside. This compound has not previously been described and we propose the name 'agabrittonoside A'.

A second refined fraction was obtained after successive HPLC purifications, as a 1:1 mixture of compounds **2** and **3**. In the ^{1}H NMR spectrum, ten anomeric proton signals were observed and the signals corresponding to the aglycon moiety had double intensities. The ESI-MS of the mixture exhibited in the negative mode, two quasi molecular ion peaks at m/z 1163 and 1177 $[M-H]^-$, and in the positive mode showed sodiated molecular ion peaks at m/z 1187 and 1201 $[M+Na]^+$, in accordance with the molecular formulae $C_{55}H_{88}O_{26}$ and $C_{56}H_{90}O_{26}$, respectively. The NMR characteristics of the aglycone of the mixture of **2** and **3** (Tables 1–3) were consistent with the spectroscopic data of

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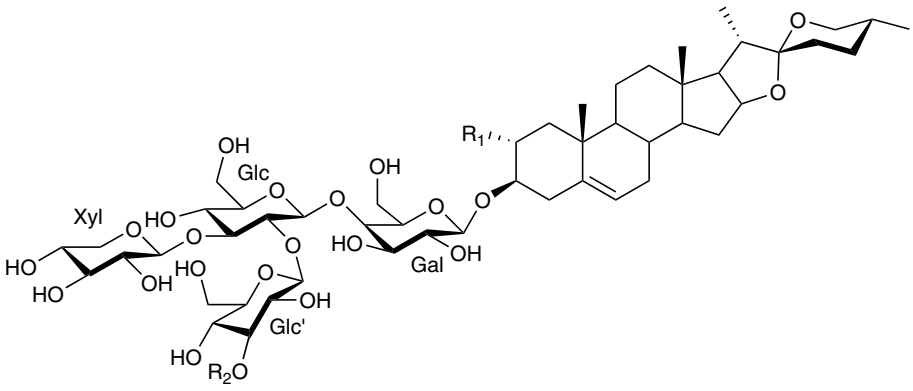

1 R_1=OH, R_2=Xyl, agabrittonoside A
2 R_1=H, R_2=Xyl, agabrittonoside B
3 R_1=H, R_2=Rha, agabrittonoside C
4 R_1=OH, R_2=Rha, agabrittonoside D
5 R_1=OH, R_2=H, karatavioside A

Figure 1. Structure of compounds **1**–**5**.

Table 1. ^{13}C and ^{1}H NMR spectroscopic data of the aglycon moieties of compounds **1**–**5** in pyridine-d_5[a]

	1, 4		**2, 3**		**5**	
position	δ_C	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)
1ax.	45.7	1.27 (dd, 12.7, 12.1)	37.4	0.93 (ddd, 14.0, 9.7, 4.7)	45.7	1.28 (dd, 13.0, 12.3)
1eq.		2.29 (dd, 12.7, 4.4)		1.65[b]		2.29 (dd, 13.0, 4.6)
2	70.0	4.06[b]	30.1	2.09 (m), 1.68[b]	70.0	4.06[b]
3	84.5	3.81[b]	78.2	3.87[b]	84.5	3,80 (dd, 11.5, 8.8, 5.4)
4ax.	37.6	2.53 (dd, 13.8, 11.9)	39.2	2.40 (m)	37.7	2.53 (ddd, 13.6, 11.5, 1.9)
4eq.		2.68 (dd, 13.8, 5.2)		2.64 (brd, 11.9)		2.68 (dd, 13.6, 5.4)
5	140.0	–	141.0	–	140.1	–
6	121.9	5.27 (brd, 5.2)	121.6	5.27 (m)	121.9	5.27 (brd, 5.4)
7	32.1	1.78,[b] 1.44[b]	32.2	1.81 (brd, 15.2), 1.44[b]	32.1	1.78,[b] 1.44[b]
8	31.0	1.45[b]	31.6	1.48[b]	31.1	1.45[b]
9	50.1	0.92[b]	50.2	0.85[b]	50.1	0.93[b]
10	37.9	–	37.0	–	37.9	–
11	21.1	1.44,[b] 1.35[b]	21.0	1.44,[b] 1.35[b]	21.2	1.44,[b] 1.36[b]
12	39.7	1.04 (ddd, 13.0, 13.0, 4.0)	39.8	1.05 (ddd, 12.3, 12.3, 4.1)	39.7	1.03 (ddd, 12.7, 12.7, 3.8)
		1.62[b]		1.66[b]		1.62[b]
13	40.4	–	40.4	–	40.4	–
14	56.4	1.01[b]	56.6	1.01[b]	56.4	1.00[b]
15	32.1	1.97 (m), 1.38[b]	32.1	1.99 (ddd, 11.3, 6.2, 1.8), 1.40[b]	32.1	1.97 (ddd, 7.3, 5.4, 1.4), 1.38[b]
16	81.1	4.49[b]	81.1	4.52[b]	81.1	4,50[b]
17	62.8	1.76 (dd, 8.4, 6.6)	62.8	1.77 (ddd, 8.6, 6.4, 1.6)	62.8	1.76 (dd, 8.4, 6.5)
18	16.3	0.78 (s)	16.3	0.80 (s)	16.3	0,78 (s)
19	20.4	0.91 (s) (**1**)/0.94 (s) (**4**)	19.4	0.85 (s) (**2**)/0.87 (s) (**3**)	20.4	0,91 (s)
20	41.9	1.91 (dq, 7.0, 6.6)	41.9	1.93 (dq, 7.0, 6.8)	41.9	1.90 (dq, 6.9, 6.5)
21	15.0	1.10 (d, 7.0)	15.0	1.12 (d, 7.0)	15.0	1,10 (d, 6.9)
22	109.2	–	109.2	–	109.2	–
23	31.8	1.66,[b] 1.61[b]	31.8	1.68,[b] 1.64[b]	31.8	1.66,[b] 1.61[b]
24	29.2	1.54[b]	29.2	1.54[b]	29.2	1.54[b]
25	30.6	1.55[b]	30.6	1.54[b]	30.6	1.55[b]
26ax.	66.8	3.47 (dd, 10.7, 10.7)	66.8	3.48 (dd, 10,7, 10,7)	66.8	3.47 (dd, 10.7, 10.7)
26eq.		3.56 (dd, 10.7, 2.9)		3.55[b]		3.56 (dd, 10.7, 3.1)
27	17.3	0.67 (d, 5.8)	17.3	0.67 (d, 5.8)	17.3	0,67 (d, 5.8)

[a] Assignments were from 2D COSY, 2D TOCSY, HSQC and HMBC experiments.
[b] Overlapped signals.

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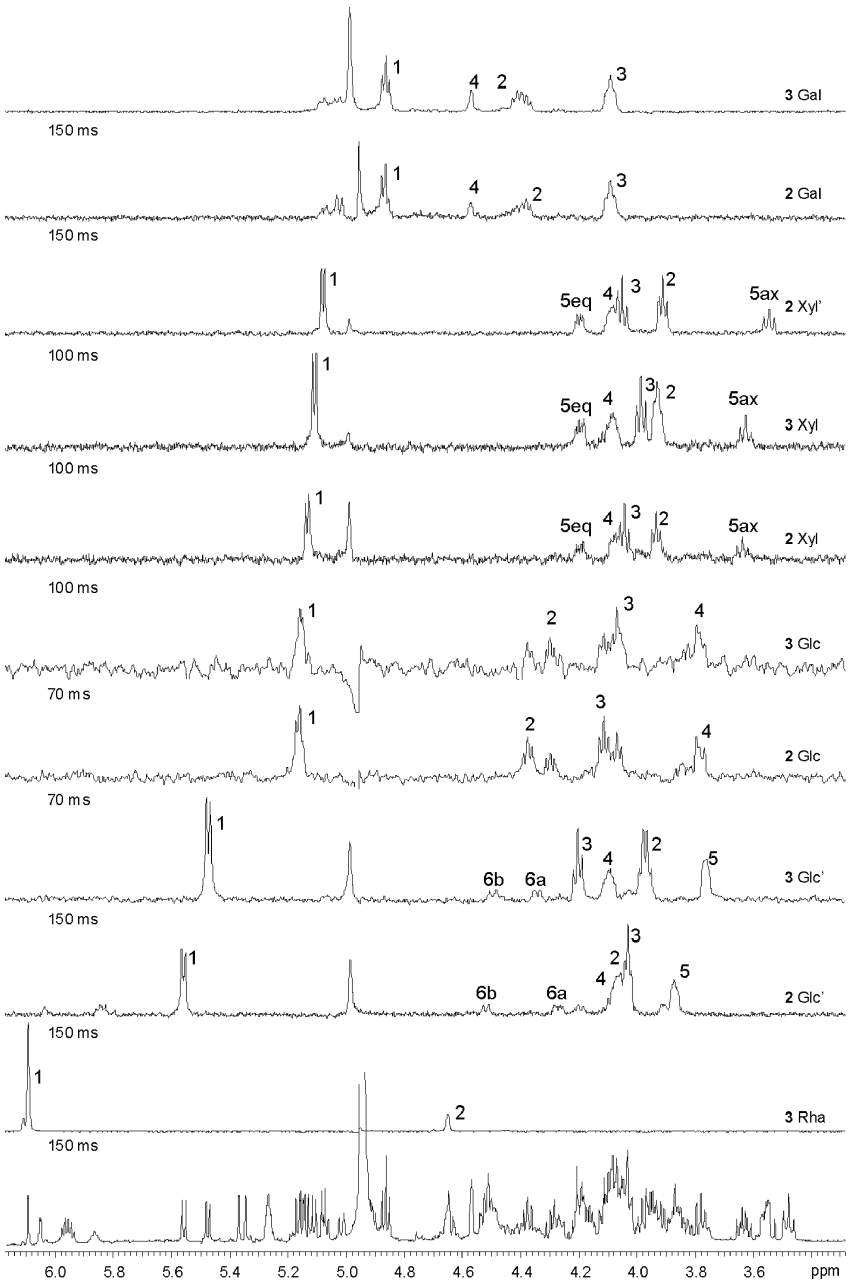

Figure 2. Selected 1D TOCSY NMR spectra of the ten anomeric signals of the refined fraction containing compounds **2** and **3**. The anomeric signals of Glc and Gal of both the compounds were too close to obtain independent subspectra, but a different ratio between the pair of signals could be observed. All showed signals were defined using the appropriate TOCSY 1D subspectra of an acquisition array of 15, 30, 55, 70, 100 and 150 ms. The rest of the signals were defined with additional 1D TOCSY on the rhamnose methyl and 1D ROESY for galactose.

diosgenin with the typical modifications due to a glycoside attached at C-3.[10]

Structure elucidation of the sugar portions was achieved by 1D TOCSY, DQF-COSY, *g*-HSQC and *g*-HMBC experiments. The 1D TOCSY subspectra obtained by irradiating the anomeric proton signals, allowed the determination of the nature of sugar units in the mixture (Fig. 2). The signals at δ 5.56 (d, J = 7.6 Hz), 5.47 (d, J = 8.0 Hz), 5.16 (d, J = 7.8 Hz), and 5.15 (d, J = 7.8 Hz) showed the typical spin system of β-glucose units, whereas the subspectra obtained by irradiating the signals δ 5.14 (d, J = 7.6 Hz), 5.08 (d, J = 7.4 Hz), and 5.11 (d, J = 7.6 Hz) allowed these protons to be established as belonging to the β-xylose units. The characteristic spin system for the two β-galactose units was obtained by irradiation of the signals at δ 4.87 (d, J = 7.4 Hz) and 4.86 (d, J = 7.6 Hz).

The broad singlet at δ 6.09 generated a 1D TOCSY subspectra with only a broad singlet at δ 4.65 due to H-2. A methyl doublet assigned to Me-C_6 of the deoxyhexose sugar at δ 1.62 (J = 6.3 Hz) was also evident. The TOCSY experiment conducted for this signal led to the identification of the H-6/H-2 sequences. The axial-axial couplings H-3/H-4 and H-4/H-5, and the axial-equatorial relationship between H-2 and H-3 as the resonances of C-3 and C-5 led to the determination of a L-rhamnose unit with α-configuration.[11,12]

The nature of the sugar moiety for both the compounds was deduced from the 1D ROESY and HMBC spectra, in which two sequences could be observed. The proposed sequences are confirmed after obtaining the ^{1}H NMR of minor aliquots from the chromatographic process with different intensities of anomeric

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Table 2. ^{1}H NMR spectroscopic data of sugar units of compounds **1**–**5** in pyridine-d_5[a]

Position	1	2	3	4	5
Gal					
1	4.90 (d, 7.7)	4.87 (d, 7.4)	4.86 (d, 7.6)	4.90[b]	4.93 (d, 7.7)
2	4.48 (dd, 9.1, 7.7)	4.38 (dd, 9.1, 7.4)	4.41 (dd, 9.3, 7.6)	4.48[b]	4.52 (dd, 9.2, 7.7)
3	4.11[b]	4.09[b]	4.09[b]	4.11[b]	4.11[b]
4	4.57 (brd, 2.7)	4.57 (brs)	4.57 (brs)	4.57[b]	4.58 (brd, 8.4)
5	3.99[b]	3.96 (m)	3.96 (m)	3.99[b]	4.00 (dd, 8.4, 5.7)
6a	4.13[b]	4.16 (m)	4.16 (m)	4.13[b]	4.16[b]
6b	4.53[b]	4.63 (m)	4.63 (m)	4.53[b]	4.58[b]
Glc					
1	5.19 (d, 8.1)	5.16 (d, 7.8)	5.15 (d, 7.8)	5.20 (d, 8.1)	5.21 (d, 8.1)
2	4.31 (dd, 8.8, 8.1)	4.38 (dd, 8.6, 7.8)	4.30 (dd, 7.8, 8.3)	4.23 (dd, 8.5, 8.1)	4.34 (dd, 8.8, 8.1)
3	4.09 (dd, 8.8, 8.0)	4.11 (dd, 9.2, 8.6)	4.07 (dd, 9.7, 8.3)	4.04 (dd, 8.5, 7.8)	4.13 (dd, 8.8, 8.8)
4	3.78 (dd, 8.8, 8.0)	3.78 (dd, 9.2, 8.8)	3.78 (dd, 9.7, 8.8)	3.78[b]	3.82 (dd, 8.8, 8.0)
5	3.82 (ddd, 8.0, 7.6, 2.3)	3.85[b]	3.84[b]	3.82[b]	3.86[b]
6a	4.04[b]	4.03 (m)	4.03 (m)	4.04[b]	4.04[b]
6b	4.47 (brd, 10.9)	4.50 (m)	4.48 (m)	4.47[b]	4.50[b]
Glc′					
1	5.59 (d, 7.5)	5.56 (d, 7.6)	5.47 (d, 8.0)	5.47 (d, 7.9)	5.56 (d, 7.7)
2	4.04 (dd, 8.8, 7.5)	4.05[b]	3.96[b]	3.94[b]	4.03[b]
3	4.07 (dd, 8.8, 8.4)	4.03[b]	4.20 (dd, 9.0, 9.2)	4.20 (dd, 9.2, 8.8)	4.12 (dd, 8.8, 8.4)
4	3.93 (dd, 8.4, 8.3)	4.07[b]	4.09 (m)	3.96[b]	4.08 (dd, 8.8, 8.4)
5	3.84 (ddd, 8.3, 5.2, 2.2)	3.87[b]	3.76 (m)	3.73 (m)	3.89 (ddd, 8.8, 5.4, 2.3)
6a	4.30[b]	4.23[b]	4.32[b]	4.35[b]	4.39 (brd, 11.4)
6b	4.47 (brd, 8.4)	4.50[b]	4.50[b]	4.45[b]	4.55 (brd, 11.4)
Xyl					
1	5.15 (d, 7.9)	5.14 (d, 7.6)	5.11 (d, 7.6)	5.10 (d, 7.7)	5.24 (d, 7.7)
2	3.93 (dd, 8.6, 7.9)	3.93 (dd, 8.8, 7.6)	3.93 (dd, 8.2, 7.6)	3.92 (dd, 8.6, 7.7)	3.94 (dd, 7.7, 9.2)
3	4.04 (dd, 8.6, 8.9)	4.04 (dd, 9.2, 8.8)	3.98 (dd, 8.2, 8.2)	3.98 (dd, 8.6, 8.4)	4.07 (dd, 9.2, 8.8)
4	4.08[b]	4.06[b]	4.09[b]	4.09[b]	4.08[b]
5ax.	3.62 (dd, 11.1, 10.3)	3.64 (dd, 11.0, 10.7)	3.63 (dd, 10.9, 10.7)	3.61 (dd, 11.1, 10.3)	3.64 (dd, 11.1, 10.0)
5eq.	4.18 (dd, 11.1, 5.2)	4.20 (dd, 11.0, 5.0)	4.19 (dd, 10.9, 4.8)	4.18 (dd, 11.1, 5.6)	4.20 (dd, 11.1, 5.0)
Xyl′/Rha					
1	5.06 (d, 7.3)	5.08 (d, 7.4)	6.09 (brs)	6.08 (brs)	
2	3.91 (dd, 8.5, 7.3)	3.91 (dd, 8.4, 7.4)	4.65 (brs)	4.65 (brs)	
3	4.01 (dd, 8.9, 8.5)	4.05 (dd, 9.0, 8.4)	4.45 (brd, 9.5)	4.45 (dd, 9.5, 2.8)	
4	4.08[b]	4.08[b]	4.27[b]	4.27 (dd, 9.2, 9.2)	
5ax./5	3.46 (dd, 11.4, 10.7)	3.54 (dd, 11.0, 10.4)	4.93 (dq, 9.5, 6.3)	4.87 (dq, 9.2, 6.3)	
5eq./6	4.16 (dd, 11.4, 5.2)	4.20 (dd, 11.0, 5.0)	1.62 (d, 6.3)	1.60 (d, 6.3)	

[a] Assignments were from 1D TOCSY, 2D COSY and 2D TOCSY experiments, (mult., J in Hz). J values determined by 1D TOCSY spectra, except for the anomeric, isolated proton signals and methyl resonances, which were determined directly from the ^{1}H spectrum.
[b] Overlapped signals.

signals. The sequence of the compound **2** was the same as for the compound **1** and the ^{1}H and ^{13}C chemical shifts are in good agreement. The analysis of the long-range correlations of the compound **3** showed it to differ from **2** only in the substitution of the terminal β-xylopyranoside′ unit, occurring in **2,** with an α-L-rhamnose unit in **3**. Thus, the structure of the new compound **2** was characterized as diosgenin-3-*O*-β-D-xylopyranosyl-(1 → 3)-[β-D-xylopyranosyl-(1 → 3)-β-D-glucopyranosyl-(1 → 2)]-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside and compound **3** as diosgenin-3-*O*-β-D-xylopyranosyl-(1 → 3)-[α-L-rhamnopyranosyl-(1 → 3)-β-D-glucopyranosyl-(1 → 2)]-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside, being named, respectively, agabrittonoside B and agabrittonoside C.

The sugar sequence pattern of the compound **3** allowed the comparison of a similar signal system in the minor component **4** of the first refined fraction containing compounds **1** and **4** in the ratio 4 : 1. In the ^{1}H NMR spectrum of this fraction, the anomeric proton signals corresponding to **4** were at δ 6.09 (brs), 5.47 (d, J = 7.9 Hz), 5.20 (d, J = 8.2 Hz), 5.10 (d, J = 7.7 Hz), 4.89 (d, J = 7.7 Hz). Analysis of 1D TOCSY spectra allowed the assignments of all the proton resonances, whereas evaluation of the coupling constants was used to elucidate the nature of the monosaccharides. The signals δ 5.47 and 5.20 showed the typical spin system of β-glucose units, whereas the subspectra obtained by irradiating the signals δ 5.10 allowed these protons to be established as belonging to β-xylose units. The signal at δ 6.09 and the doublet at δ 1.60 (3H, J = 6.3 Hz) were characteristic

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of an α-L-rhamnose unit, as in the compound **3**. The 1D TOCSY subspectra of these signals confirmed this inference. The ^{1}H NMR spectrum did not show galactose unit signals for the compound **4** because they were superimposed with the ones belonging to the compound **1**.

Minor quasi molecular ion peaks were observed in the MS ESI of the combined fraction containing **1** and **4** at m/z 1193 in negative mode and m/z 1217 in positive mode. These were in agreement with a molecular formula $C_{56}H_{90}O_{27}$ for the compound **4**, and supported yuccagenin as aglycone and the sugar units proposed. The complete assignments of the ^{13}C NMR signals of compound **4**, observed as minor ones in the fraction spectrum, were carried out by comparing with the signals of the compound **3** and from the *g*-HSQC and *g*-HSQC-TOCSY spectra. The sugar sequence was confirmed with 1D ROESY experiments, in which correlations from H-1$_{rha}$ (δ 6.08) to H-3$_{glc'}$ (δ 4.20), H-1$_{glc'}$ (δ 5.47) to H-2$_{glc}$ (δ 4.23), H-1$_{glc}$ (δ 5.20) to H-4$_{gal}$ (δ 4.57) and H-1$_{xyl}$ (δ 5.10) to H-3$_{glc}$ (δ 4.04) were observed. The structure of compound **4** was determined as Yuccagenin-3-*O*-β-D-xylopyranosyl-(1 → 3)-[α-L-rhamnopyranosyl-(1 → 3)-β-D-glucopyranosyl-(1 → 2)]-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside, and named agabrittonoside D.

The purification of complex saponins is usually very dificult and tedious. In this article, unambiguous structure elucidation, including complete relative stereochemistry and sugar connectivities, of the components of a refined saponin fraction (not only with instrumental methods alone) has been carried out. This contribution represents an efficient approach for the structural elucidation of partially purified saponins, which implies saving time and laboratory work.

Table 3. ^{13}C NMR spectroscopic data of sugar units of compounds **1**–**5** in pyridine-d_5[a]

Carbon	1	2	3	4	5
Gal					
1	103.3	102.7	102.7	103.3	103.3
2	72.6	73.1	73.1	72.6	72.7
3	75.4	75.4	75.4	75.6	75.5
4	79.0	79.6	79.6	79.2	79.3
5	75.6	76.4	76.4	75.8	75.7
6	60.5	60.6	60.6	60.6	60.5
Glc					
1	104.4	104.7	104.8	104.8	104.7
2	80.6	80.7	80.9	80.7	81.2
3	86.9	86.7	86.6	87.2	86.9
4	70.3	70.4	70.3	70.3	70.4
5	77.5	77.5	77.5	77.5	77.6
6	62.8	62.9	62.8	62.8	62.9
Glc′					
1	103.9	103.9	104.3	104.1	104.8
2	75.0	75.9	76.4	76.3	76.1
3	87.0	87.1	83.1	83.4	78.1
4	69.3	69.1	69.1	69.4	71.3
5	77.9	78.3	78.3	77.9	78.4
6	62.3	62.1	62.2	62.4	62.7
Xyl					
1	104.8	104.9	104.9	104.8	104.9
2	75.1	75.3	75.3	75.2	75.1
3	78.4	78.4	78.5	78.5	78.7
4	70.7	70.7	70.7	70.6	70.7
5	67.2	67.2	67.2	67.2	67.3
Xyl′/Rha					
1	106.1	106.2	102.7	102.7	
2	75.4	75.4	72.3	72.3	
3	77.7	77.7	72.5	72.5	
4	70.7	70.6	74.1	74.1	
5	67.0	67.1	69.7	69.7	
6			18.6	18.7	

[a] Assignments were from HSQC, HSQC-TOCSY and HMBC experiments.

EXPERIMENTAL

Plant material

Leaves of *Agave brittoniana* Trel. ssp *Brachypus* were collected at the protected zone 'Cubanacán', bordering the city of Santa Clara, in January 2003 and identified by botanists Drs Alfredo Noa and Jesús Matos. A voucher specimen (number 07 869) was deposited in the Herbarium of the Universidad Central 'Marta Abreu' de Las Villas, Cuba.

Extraction and isolation

Dried and powdered leaves (1 kg) were extracted with ethanol-water (7 : 3) three times by maceration at room temperature. The solvent was removed under reduced pressure and the syrupy extract was suspended in distiller water, defatted with *n*-hexane, and extracted with water-saturated *n*-butanol. After solvent removal, the *n*-butanol extract (10 g of 157 g total) was purified using LiChrospher RP-18 and eluting with a MeOH-H_2O gradient to give five fractions (Fr 1–5). Fr 4 (3.43 g) was chromatographed with MPLC on a Büchi 861 apparatus using LiChrospher RP-18 and MeOH-H_2O (4 : 1) and purified further by HPLC equipped with an ODS column (LiChrospher 100 RP-18 5 μm; flow rate, 1 min ml^{-1} with MeOH-H_2O (4 : 1)) to afford refined fractions: with **1** and **4** (4 : 1), 11 mg;, with compounds **2** and **3** (1 : 1), 17 mg; and the pure compound **5**, 15 mg.

NMR measurements

All experiments were performed on a Varian INOVA-600 spectrometer equipped with 5 mm ^{1}H (^{15}N–^{31}P) PFG high-field indirect detection *z*-gradient probe. ^{1}H (599.775 MHz) and ^{13}C (150.831 MHz) NMR spectra were recorded in pyridine-d_5 at 25 °C. Chemical shifts are given on the δ scale and were referenced to residual pyridine, δ ^{1}H 8.70, 7.55, 7.18 and δ ^{13}C 149.84, 135.50, 123.48.

Varian pulse sequence using gradient were applied. All 2D spectra, except for HMBC, were recorded in the phase-sensitive mode.

One-dimensional proton spectra were acquired using the following parameters: 40 K data points, zero filled to 64 K, sweep width 6746 Hz, 90° pulse, 32 scan, no relaxation delay, no exponential multiplication. One-dimensional carbon spectra were acquired using the following parameters: 82 K data points, zero filled to 128 K, sweep width 31 595 Hz, 70° pulse, 50 000 scan, no relaxation delay, 0.5 exponential multiplications.

For heteronuclear experiments (multiple edited HSQC, HMBC, HSQC-TOCSY), the parameters were as follows: 2 K × 512 data points, processed to 2 K × 2 K points using linear prediction in F_1, sweep widths 6746 Hz in F_2 and 30 165 Hz in F_1. The *g*-HSQC and *g*-HSQC-TOCSY were set to j1 × h = 140 Hz, mult = 2 and Gaussian function processing in both dimensions (*g*-HSQC-TOCSY mixing time = 80 ms). *g*-HMBC was set to j1 × h = 140 Hz, jn × h = 3 Hz and sinebell function processing in both dimensions.

The parameters for homonuclear experiments (TOCSY, DQCOSY, ROESY) were 2 K × 200 data points, zero filled to 2 K × 2 K points, sweep width 6 746 Hz. Gaussian functions were applied in both dimensions (for DQCOSY, shifted squared sine window function). A 150 ms MLEV17 mixing time was used for TOCSY and for ROESY experiments, a 200 ms spin lock mixing time.

The 1D TOCSY and 1D ROESY spectra were recorded using a PFG selective excitation. For 1D TOCSY, the MLEV17, mixing time was set with an acquisition array of 15, 30, 55, 70, 100, 150 ms and for 1D ROESY, 200 ms spin lock mixing time.

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Spectral Assignments and Reference Data

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