Hypoglucaemic triterpenoid saponins from *Boussingaultia baselloides*

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New saponins with hypoglucaemic activity (**1–4**) and momordin Ic (**5**) have been isolated from the methanolic extract of the leaves of *Boussingaultia baselloides*. They were shown by spectroscopy and chemical transformation to be derivatives of 12,20(29)-dien-30-noroleanic acid. Boussingoside A_1 (**1**) exhibits very strong hypoglucaemic activity in rats. Acid hydrolysis of the saponin **1** yielded five transformation products (**6–10**), though one of them (larreagenin A (**6**), which has been reported in the literature as a genuine plant metabolite) is considered to be an artifact formed by the acid treatment.

Key words: *Boussingaultia baselloides*, Basellaceae, boussingosides, triterpenoid saponins, hypoglucaemic activity.

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On a isolé de nouvelles saponines ayant une activité hypoglycémique (**1–4**) et la momordine Ic (**5**) à partir d'extraits méthanoliques des feuilles de *Boussingaultia baselloides*. Faisant appel à la spectroscopie et à des transformations chimiques, on démontre que ces composés sont des dérivés de l'acide (29)-12,20-diène-30-noroléanique. La boussingoside A_1 (**1**) exhibe une forte activité hypoglycémique chez les rats. L'hydrolyse acide de la saponine **1** conduit à cinq produits de transformation (**6–10**) dont l'un d'eux (la larréagénine A (**6**), qui est répertoriée dans la littérature comme un vrai métabolite végétal) est considéré comme un artefact résultant du traitement acide.

Mots clés : *Boussingaultia baselloides*, Basellaceae, boussingosides, saponines triterpénoïdes, activité hypoglycémique.
[Traduit par la revue]

Introduction

Two members of the herbaceous climbing plant family Basellaceae have been reported in Colombia (1, 2). One of these species, *Boussingaultia baselloides* H.B.K., known as insuline, is used in folk medicine (as a drink made by adding 4–6 fresh chopped *B. baselloides* leaves to approximately 150 mL of boiling water and reduction of the volume to a third), mainly for treatment of *diabetes* but also as an analgesic. In this paper we report the isolation of new triterpenoid glycosides responsible for the hypoglucaemic activity of the crude plant extract. This work constitutes the first research on an intact polar extract of a member of the Basellaceae family.

In the course of this study we have isolated and identified, by spectroscopic analysis (2D-NMR experiments and FAB-MS) and chemical transformation, four new nor-triterpenoid saponins with hypoglucaemic activity.

Results and discussion

The aerial parts of the plant, collected in Tuluá (Colombia), were extracted successively with petroleum ether and MeOH. The MeOH extract was then partitioned between *n*-BuOH and H_2O. Chromatographic separation of the BuOH soluble fraction, which showed hypoglucaemic activity, afforded a complex mixture of triterpenoid glycosides. This mixture was successively subjected to several separation techniques (Sephadex LH-20, DCCC, and HPLC) to give the pure compounds **1–5** (Fig. 1). We have named compounds **1–4** boussingosides A_1 (**1**), A_2 (**2**), B (**3**), and C (**4**); compound **5**, momordin Ic, has previously been reported in *Momordica cochinchinensis* (Cucurbitaceae) (3).

Boussingoside A_1 (**1**) was crystallized from methanol as a white microcrystalline powder (mp 213–215°C) that was subjected to fast atom bombardment MS (FAB-MS) (negative ion mode). A quasi-molecular ion was observed at *m/z* 615 $[M - H]^-$ indicating a molecular weight of *m/z* 616 and a molecular formula of $C_{35}H_{52}O_9$. The ^1H NMR spectrum of **1** showed a doublet at δ 4.93 (J = 7.9 Hz) attributable to the anomeric proton of a β-pyranoside. The ^{13}C NMR spectrum showed the anomeric carbon at δ 106.9, a carboxylic signal at δ 179.4 ppm, and four signals in the sugar region corresponding to glucuronic acid. The fragment at *m/z* 440 found in the FAB-MS, $[M - H - 175]^-$, clearly indicates the separation of this sugar; in fact, glucuronic acid was identified (GC–MS as alditol acetate) after hydrolysis of **1** (see Table 1).

Compound **1** also exhibited two olefinic bonds, one with signals at δ_H 5.43 and δ_C 122.9 and 144.1, indicating a $\Delta^{12(13)}$ double bond, and the other at δ_H 4.71 and 4.76 and δ_C 106.9 (CH_2 inferred from DEPT and XHCOR experiments) and δ 149.0, due to the C20—C29 double bond. The presence of five methyl singlets upfield in the ^1H NMR spectrum and comparison with the literature (4, 5) confirmed a nor-olean skeleton.

Further information about the structure of **1** was obtained from COSY and nOe experiments. Thus the double doublet at δ_H 3.34 (J = 11.9 and 4.0 Hz), H3α, was shown to be correlated in the ^1H–^1H COSY spectrum (Fig. 2) with two multiplets at 2.16 ppm (H2α) and 1.80 ppm (H2β). Furthermore, the double doublet at δ_H 3.17 (J = 13.7 and 4.9 Hz), H18, correlates with two signals at 2.20 (m) and 2.58 (t, J = 13.7 Hz) corresponding to protons H19β and H19α respectively. The stereochemistry at C18 was determined by irradiation of the signal at 3.17 (H18), which caused nOe on the broad singlet at 5.43 corresponding to H12. All these assignations were corroborated by a 2D-XHCOR experiment (see Fig. 3) and the assignation of the methyl groups was corroborated by a XHCORR-LR spectrum (Fig. 4) of compound **2**. We observe the following correlations: Me24/C4 (two bonds) (no correlation was observed with C3), Me23/C3 (three bonds), Me23/C4 (two bonds), Me27/C13, Me27/C8 (both three bonds), and Me27/C14 (two bonds), Me26/C8 (two bonds), Me26/C14 (three bonds), and finally Me25/C10 (two bonds).

On the basis of the above data, we concluded that boussingoside A_1 (**1**) is 3-*O*-[β-D-glucuronopyranosyl]-30-norolean-12,20(29)-dien-28-oic acid.

Boussingoside A_2 (**2**) was obtained as a microcrystalline white powder (mp 218–220°C), and presents a FAB-MS

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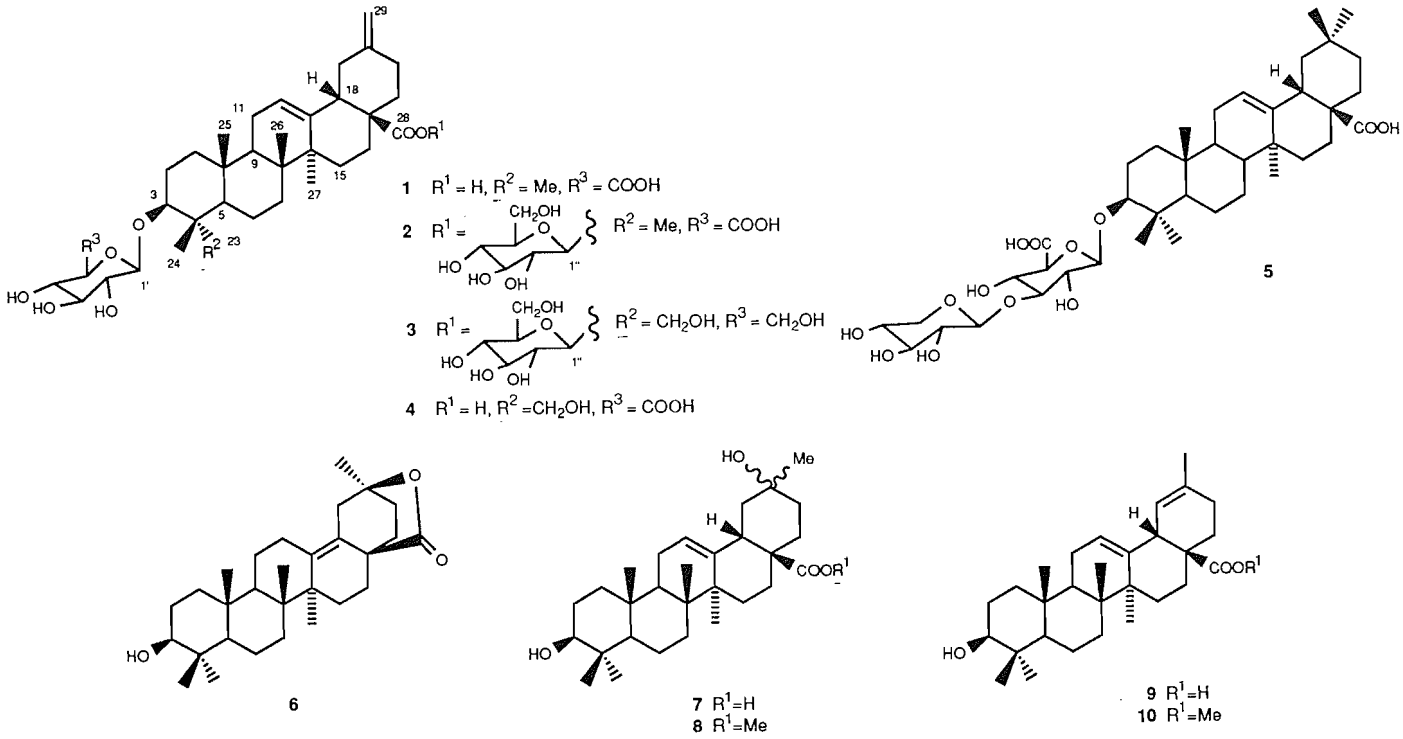

FIG. 1

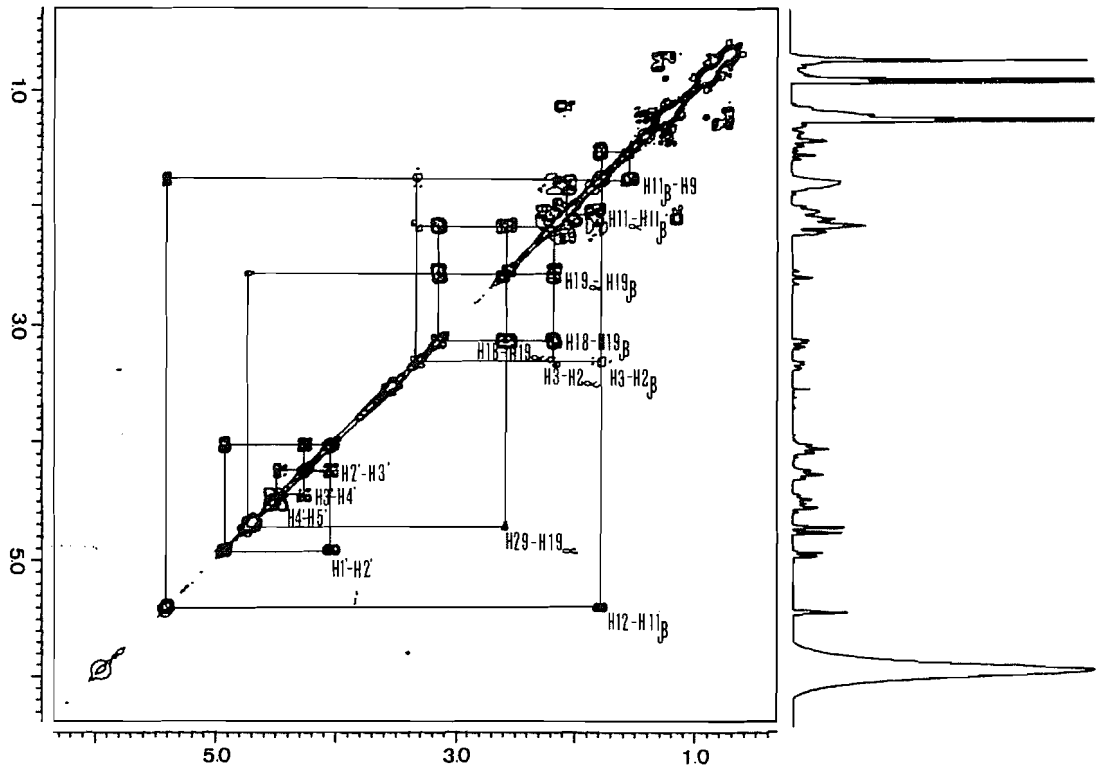

FIG. 2. ^1H–^1H COSY of boussingoside A_1.

(negative ion mode) spectrum showing a quasi-molecular ion at m/z 777 $[M - H]^-$, and thus a molecular weight m/z 778 and a molecular formula $C_{41}H_{62}O_{14}$. It also showed fragments corresponding to the cleavage of two sugars at m/z 614 $[M - H - \text{glucose}]^-$ and 439 $[M - H - \text{glucose} - \text{glucuronic acid}]^-$. The ^{13}C and ^1H NMR spectra are also similar to those of boussingoside A_1 (**1**) (see Table 2), but in addition present one upfield β-anomeric signal at δ_C 95.7 (δ_H 6.24, $J = 7.9$ Hz) and other peaks that correspond to a glucose unit attached to C28 of the aglycone through an ester bond. Thus boussingoside A_2 (**2**) is 3-*O*-[β-D-glucuronopyranosyl]-30-norolean-12,20(29)-dien-28-*O*-[β-D-glucopyranosyl] ester.

To confirm these structures and also to get information on the sugar unit bound through the ester bond at C28 or the acetal

TABLE 1. Selected ^{1}H NMR data of boussingosides A_1 (**1**) and A_2 (**2**) in C_5D_5N at 298 K

(a)

	δ	
	1	**2**
H-2α	2.16 m	2.20 m
H-2β	1.80 m	1.81 m
H-3	3.34 dd	3.36 dd
H-9	1.55 m	1.57 m
H-11α	2.04 m	2.03 m
H-11β	1.72 m	1.68 m
H-12	5.43 bs	5.39 bs
H-18	3.17 dd	3.08 dd
H-19α	2.58 dd	2.57 dd
H-19β	2.20 m	2.18 m
Me-23	1.27 s	1.28 s
Me-24	0.91 s	0.95 s
Me-25	0.74 s	0.78 s
Me-26	0.93 s	1.03 s
Me-27	1.25 s	1.22 s
H-29	4.71, 4.76 (bs each)	4.66 bs
H-1′	4.93 d	5.02 d
H-2′	4.05 dd	4.09 dd
H-3′	4.28 dd	4.66 m
H-4′	4.47 dd	4.60 dd
H-5′	4.55 d	3.99 m
H-1″		6.24 d
H-2″		4.12 dd
H-3″		4.37 dd
H-4″		4.31 dd
H-5″		4.45 m

(b)

	J (Hz)	
	1	**2**
$J_{3-2\alpha}$	4.0	4.9
$J_{3-2\beta}$	11.9	12.5
$J_{18-19\alpha}$	13.7	13.9
$J_{18-19\beta}$	4.9	4.2
$J_{19\alpha-19\beta}$	13.7	13.4
$J_{1'-2'}$	7.9	7.8
$J_{2'-3'}$	8.9	7.5
$J_{3'-4'}$	8.9	
$J_{4'-5'}$	9.4	8.8
$J_{1''-2''}$		7.9
$J_{2''-3''}$		8.5
$J_{3''-4''}$		8.5
$J_{4''-5''}$		8.5

bond at C3, we proceeded to study the acid and basic hydrolyses of **1** and **2**. Treatment of **2** with 0.5 N KOH gave a single sugar, glucose, and another product that was shown by HPLC and its spectroscopic data to be identical to boussingoside A_1 (**1**), implying that glucose must be bound to the C28 carboxylic group of the aglycone. Acid treatment of **2** gave, in turn, glucose and glucuronic acid (which were identified by GC–MS as alditol acetates) and a complex mixture of five lipophilic products which was identical in composition to that obtained from **1** (see below), thus proving that **1** and **2** have the same aglycone.

TABLE 2. ^{13}C NMR data of triterpenoid saponins of *Boussingaultia baselloides*

C	**1**[a]	**2**[a]	**3**[b]	**4**[a]	**5**[a]
1	39.5	39.3	39.6	39.7	38.5
2	26.3	26.4	26.2	25.9	26.5
3	90.0	88.9	82.2	81.9	89.2
4	38.1	39.7	43.9	43.3	39.4
5	55.6	55.6	—	47.9	55.6
6	18.2	18.3	18.8	18.0	18.3
7	32.9	32.9	33.3	32.7	33.1
8	38.4	41.5	40.8	39.7	39.6
9	47.7	47.7	49.0	47.4	47.9
10	36.7	37.4	37.7	36.7	36.8
11	23.5	23.4	24.5	23.6	23.6
12	122.9	123.5	124.4	123.1	122.5
13	144.1	143.3	144.4	144.1	144.9
14	41.8	41.9	43.0	41.8	41.9
15	28.0	28.0	28.9	28.2	28.2
16	23.5	23.4	24.1	23.6	23.6
17	46.9	47.1	48.7	46.9	46.4
18	47.7	47.4	49.2	47.8	42.1
19	41.9	41.9	42.6	42.0	46.6
20	149.0	148.4	149.8	148.1	30.8
21	39.3	38.5	38.4	38.5	34.2
22	30.2	29.9	30.9	30.2	33.1
23	28.0	28.0	64.9	64.4	28.0
24	16.7	16.7	16.5	15.9	16.8
25	15.2	15.3	13.4	13.5	15.3
26	17.1	17.2	17.8	17.3	17.3
27	26.0	25.8	26.3	26.2	26.1
28	179.4	175.7	177.5	179.4	180.2
29	106.9	107.1	107.4	107.2	34.1
30					23.6
1′	106.9	107.0	105.1	107.0	106.8
2′	75.2	75.3	75.1	75.3	74.6
3′	77.9	77.5	78.2	78.0	86.4
4′	73.3	73.2	71.2	73.3	71.4
5′	77.2	79.1	78.7	77.7	78.0
6′	179.4	172.8	62.5	172.8	172.1
1″		95.7	95.9		106.2
2″		73.8	74.0		75.2
3″		77.9	78.3		77.4
4″		71.0	71.2		70.9
5″		78.6	78.7		67.3
6″		62.1	62.5		

[a]In C_5D_5N.
[b]In CD_3OD.

Acid hydrolysis of **1** with HCl in methanol gave, as expected, glucuronic acid and the aforementioned mixture of five nortriterpenes (**6**–**10**; Fig. 4), which were separated by HPLC and identified by ^{1}H NMR and MS (6).

Boussingosides B (**3**) and C (**4**) were crystallized in MeOH. Their ^{1}H NMR spectra showed only four upfield methyl singlets instead of the five of **1** and **2**. In both **3** and **4** the methyl group at C23 of the aglycone is replaced by a -CH_2OH group, as indicated by the 6–8 ppm shift of C3, C4, and C5 (Table 2). The spectra of compound **3** showed two anomeric groups with signals at δ_C 95.9 and 105.1, and two doublets at δ_H 4.53 and 5.46. The remaining signals were assigned to two glucose units by comparison with published data (4). In keeping with this, the FAB-MS (negative mode) of **3** presents a quasi-molecular ion at *m/z* 797 $[M - H]^-$ (molecular weight 798, molecular formula

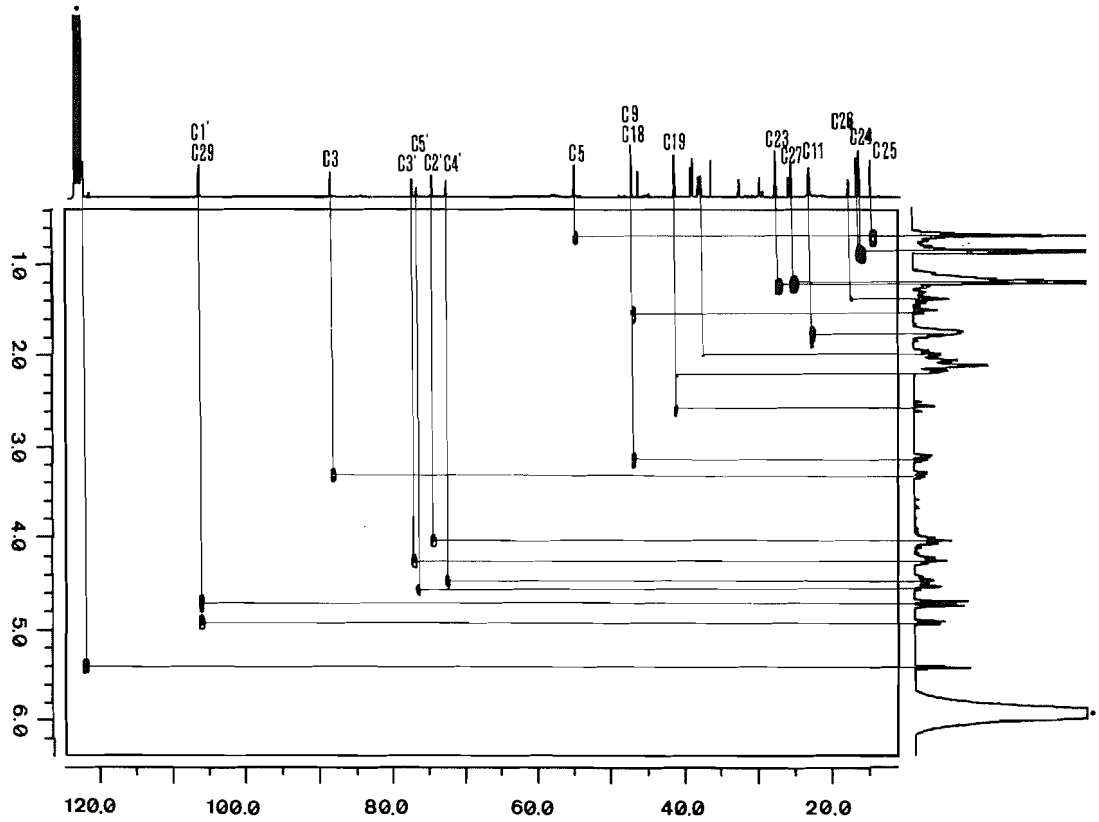

FIG. 3. XHCORR of boussingoside A_1 (J = 130 Hz).

$C_{41}H_{66}O_{15}$) and also shows fragments at m/z 634 [M − H − 163]$^-$ and 471 [M − H − 326]$^-$ corresponding to the successive elimination of two glucose units bound to a tetraoxygenated aglycone.

Thus boussingoside B (**3**) is a 3-*O*-[β-D-glucopyranosyl]-30-norolean-12,20(29)-dien-23-hydroxy-28-*O*-[β-D-glucopyranosyl] ester.

The ^{13}C NMR spectrum of boussingoside C (**4**) presents only one anomeric signal at 107.0 (δ_H 5.23 d, J = 7.5 Hz). Its FAB-MS (negative mode) shows an m/z 631 [M − H]$^-$ and a fragment at m/z 454 [M − H − 177]$^-$, indicating the loss of a glucuronic acid unit and proving that compound **4** is 3-*O*-[β-D-glucuronopyranosyl]-30-norolean-12,20(29)-dien-23-hydroxy-28-oic acid.

Compound **6**, larreagenin A, has been reported as a metabolite isolated from *Larrea divaricata* (7), but it has always been obtained after acid hydrolysis of the mixture of saponins (6). We suspected that compound **6** was an artifact generated during the acid treatment. This was confirmed by its appearing even when **1** was hydrolyzed under milder conditions (1 N HCl) than those described in the literature.

Preliminary pharmacological studies[2] have shown that the aqueous extract of the plant and boussingoside A_1 have hypoglucaemic activity.

[2]In rats with induced diabetes, intraperitoneal injection of a dose of aqueous extract equivalent to (10 g dried aerial parts)/kg significantly reduced glucose levels from over 399 mg/% to 60 mg/% after 8 h. A 20 mg/kg dose of boussingoside A_1 had a similar effect. R. Cadena and J. A. Otero, unpublished results.

Experimental

General

All melting points were taken on a Kofler Thermogeräte apparatus and are uncorrected. ^{1}H NMR spectra were taken on a Bruker WM-250 spectrometer. The ^{1}H NMR, ^{13}C NMR, and DEPT spectra of the glycosides were obtained in C_5D_5N and CD_3OD at 298 K. HPLC was performed with a Waters model 6000A chromatograph equipped with a pump and a model R401 differential refractometer. DCCC was carried out with an Eyela model 300-S apparatus equipped with 300 tubes. Conventional and FAB mass spectra were obtained on a Kratos MS50 mass spectrometer equipped with a Kratos FAB source. The FAB mass spectra were obtained by dissolving the samples in a glycerol matrix and bombarding with Xe atoms of 2–8 kV energy. GC–MS analyses were carried out on a Carlo Erba MFC 500 HRGC/MS chromatograph using a 0.25 mm × 10 m SP-2330 capillary column, column temperature 240°C, injection temperature 50°C for 1 min and 12°/min up to 240°C maintained for 2 min.

Two-dimensional experiments

The two-dimensional ^{1}H–^{1}H COSY spectra (64 × 1K) of the boussingosides were obtained by accumulating 32 scans per t_1; the relaxation delay was 3 s. The data were zero-filled to 512 in F_1 and subjected to Fourier transformation using Gaussian data manipulation in both dimensions and symmetrized.

The two-dimensional ^{1}H–^{13}C heteronuclear shift correlation (XHCORR) spectra (256 × 1K) of **1** and **2** for directly bonded protons and carbons were obtained by accumulating 80 scans per t_1; the relaxation delay was 3 s and the value of J_{CH} selected was 130 Hz. The long-range correlation XHCORR (256 × 2K) spectrum of boussingoside A_2 was obtained by accumulating 88 scans per t_1; the relaxation delay was 2 s and the value of J_{CH} selected was 9 Hz. Both XHCORR data (long range and direct correlation) were zero-filled to 512 in F_1 and subjected to Fourier transformation using Gaussian data manipulation in F_2.

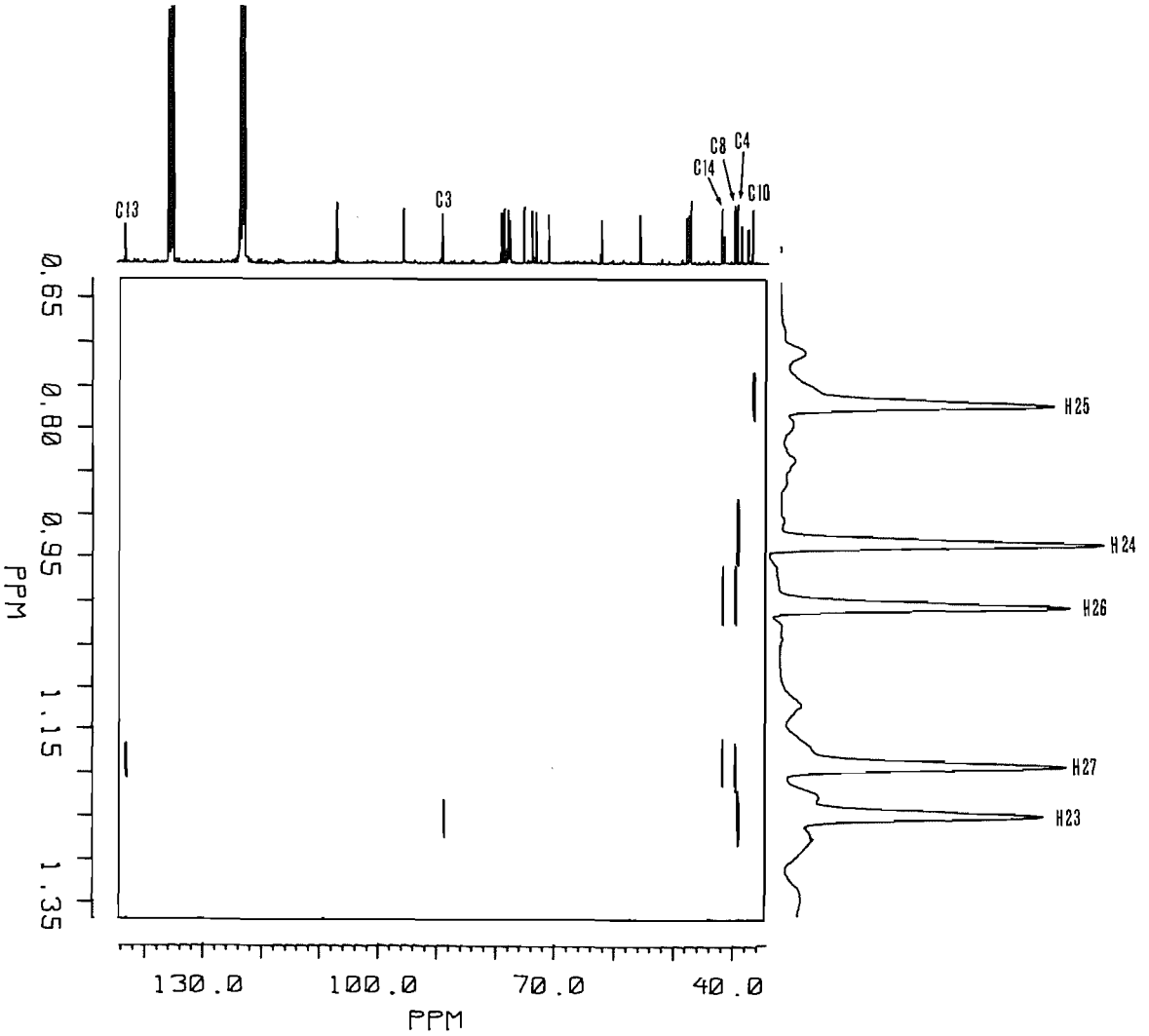

FIG. 4. XHCORR-LR of boussingoside A_2 (J = 9 Hz).

Plant material

Plants were collected in Tuluá, Colombia. Voucher specimens were deposited at the herbaria of the Universities of Santiago de Cali, Colombia, and Santiago de Compostela, Spain (SANT 18191).

Extraction and isolation

The ground leaves of the plant (200 g) were extracted successively with petroleum ether and MeOH giving respectively 30 g and 36.2 g of dry extract. The MeOH extract was defatted with *n*-hexane (2 × 200 mL), CCl_4 (200 mL), and CH_2Cl_2 (2 × 200 mL), and partitioned between *n*-BuOH and H_2O. The BuOH extract (12.6 g) was dissolved in a minimal amount of MeOH, and the solution dropped into Me_2CO and centrifuged. The precipitate was filtered off and the filtrate concentrated under reduced pressure to give 9.7 g of dry extract, which was separated in five fractions (A–E) on a Sephadex LH-20 column using MeOH as eluent and TLC monitoring. Fraction B (1.5 g) was purified by DCCC with $CHCl_3$–MeOH–H_2O (7:13:8) in ascending mode (flow rate 12 mL/h). Fractions 35–40 contained boussingoside A_2 (**2**) (110 mg) and fractions 80–95 boussingoside A_1 (**1**) (80 mg) in pure form. Fractions 40–60 (150 mg) were pooled and rechromatographed by DCCC with $CHCl_3$–MeOH–H_2O (7:13:8) in ascending mode (flow rate 8 mL/h), giving compound **5** (28 mg) and boussingoside C (**4**) (11 mg) in pure form and boussingoside B (**3**) (7 mg), which was repurified by HPLC with MeOH–H_2O 55% (reversed phase, Partisil ODS-M9 column).

Boussingoside A_1 (**1**): mp 213–215°C. ^1H and ^{13}C NMR as in Tables 1 and 2 respectively. FAB-MS (negative ion mode) m/z (relative int.): 615 ($[M - H]^-$, 100), 440 ($[M - H - 175]^-$, 70). FAB–MS (positive ion mode): 683 ($[M - 2H + 3Na]^+$, 80), 661, ($[M - H + 2Na - 162]^+$, 100), 507 ($[M - 2H + 3Na - 176]^+$, 40).

Boussingoside A_2 (**2**): mp 218–220°C. ^1H and ^{13}C NMR as in Tables 1 and 2. FAB-MS (negative ion mode) m/z (relative int.): 777 ($[M - H]^-$, 100), 614 ($[M - H - 163]^-$, 24), 569 ($[M - H - 163 - 44]^-$, 10), 439 ($[M - H - 163 - 175]^-$, 6). FAB-MS m/z (relative int.) (positive ion mode): 823 ($[M - H + 2Na]^+$, 76), 661 ($[M - 2H + 3Na - 162]^+$, 32).

Boussingoside B (**3**): mp 218–220°C. ^1H NMR (CD_3OD) δ_H: 0.80 (s, 3H, 25-Me), 0.90 (s, 3H, 26-Me), 1.08 (s, 3H, 24-Me), 1.30 (s, 3H, 27-Me), 4.53 (d, 1H, J = 7.7 Hz, H-1′), 4.71 (bs, 2H, H-29), 5.41 (bs, 1H, H-12), 5.46 (d, 1H, J = 7.8 Hz, H-1″). ^{13}C NMR, see Table 2. FAB-MS (negative ion mode) m/z (relative int.): 797 ($[M - H]^-$, 100), 634 ($[M - H - 163]^-$, 24), 471 ($[M - H - 163 - 163]^-$, 10). FAB-MS m/z (relative int.) (positive ion mode): 843 ($[M - H + 2Na]^+$, 76), 821 ($[M + Na]^+$, 32), 659 ($[M - H + Na - 162]^+$, 10).

Boussingoside C (**4**): mp 218–220°C. ^1H NMR (C_5D_5N) δ_H: 0.85 (s, 3H, 25-Me), 0.92 (s, 3H, 26-Me), 0.95 (s, 3H, 24-Me), 1.12 (s, 3H, 27-Me), 4.71–4.75 (bs each, 2H, H-29), 5.23 (d, 1H, J = 7.5 Hz, H-1′), 5.43 (bs, 1H, H-12). ^{13}C NMR, see Table 2. FAB-MS

(negative ion mode) m/z (relative int.): 631 ($[M - H]^-$, 100), 454 ($[M - H - 177]^-$, 29), 410 ($[M - H - 177 - 44]^-$, 17). FAB-MS m/z (relative int.) (positive ion mode): 699 ($[M - 2H + 3Na]^+$, 85), 675 ($[M - H + 2Na]^+$, 45), 523 ($[M - 2H + Na - 176]^+$, 18), 479 ($[M + Na - 176]^+$, 19).

Basic hydrolysis

Boussingoside A_2 (**2**) (25 mg) was refluxed in 0.5 N KOH (20 mL) for 2 h. The mixture was brought to pH 6 with 1 N HCl and extracted with *n*-BuOH (2 × 20 mL). The organic phase was washed with H_2O (3 × 20 mL), affording boussingoside A_1 (**1**).

Acid hydrolysis of 2

When **2** was treated with acid (see below) it gave two sugars and a mixture of triterpenes (**6**–**10**) identical to that obtained by similar treatment of **1**. The sugar fraction was dissolved in H_2O (10 mL), $NaBH_4$ (20 mg) was added, and the mixture was stirred at room temperature for 2 h. After addition of AcOH to eliminate excess $NaBH_4$, the mixture was concentrated to dryness and codistilled with MeOH (2 × 5 mL), and the resulting alditols were acetylated with Ac_2O–pyridine (1:1, 10 mL) by refluxing overnight. The solution was washed with H_2O and extracted with CH_2Cl_2, and the alditol acetates were then identified as those of glucose and glucuronic acid by GC–MS.

Acid hydrolysis of 1

To a solution of boussingoside A_1 (**1**) (20 mg in 10 mL of MeOH) was added 10 mL of 1 N HCl and the mixture was refluxed for 4.5 h. The mixture of aglycones was extracted with CH_2Cl_2 (3 × 20 mL) and subjected to HPLC on a μ-Porasil column (30 cm × 7.8 mm i.d.) with *n*-hexane–EtOAc (75:25) as eluent, affording compounds **6**–**10**.

Compound 6: ^{1}H NMR $CDCl_3$: 0.76 (s, 3H, H-25), 0.80 (s, 3H, H-26), 0.85 (s, 3H, H-24), 0.99 (s, 3H, H-27), 1.13 (s, 3H, H-23), 1.45 (s, 3H, H-29), 3.23 (m, 1H, H-3). EIMS (m/z, relative int.): 440 ($[M]^+$, 53); 422 ($[M - H_2O]^+$, 66); 407 ($[M - H_2O - Me]^+$, 20); 395 ($[M - CO_2]^+$, 40).

Compound 7: ^{1}H NMR $CDCl_3$: 0.76 (s, 3H, H-25), 0.78 (s, 3H, H-26), 0.91 (s, 3H, H-24), 0.99 (s, 3H, H-27), 1.14 (s, 3H, H-23), 1.25 (s, 3H, H-29), 2.81 (dd, 1H, J = 4.8, 13.6 Hz, H-18), 3.23 (m, 1H, H-3), 5.30 (m, 1H, H-12). EIMS (m/z, relative int.): 458 ($[M]^+$, 2); 440 ($[M - H_2O]^+$, 9); 425 ($[M - H_2O - Me]^+$, 2); 414 ($[M - CO_2]^+$, 4); 407 ($[M - 2H_2O - Me]^+$, 4); 232 (40); 207 (39); 187 (100), 173 (71).

Compound 8: ^{1}H NMR $CDCl_3$: 0.73 (s, 3H, H-25), 0.84 (s, 3H, H-26), 0.94 (s, 3H, H-24), 1.14 (s, 3H, H-27), 1.22 (s, 3H, H-23), 1.26 (s, 3H, H-29), 3.20 (m, 1H, H-3), 3.63 (s, 3H, COOMe), 5.35 (m, 1H, H-12). EIMS (m/z, relative int.): 472 ($[M]^+$, 1); 454 ($[M - H_2O]^+$, 28); 441 ($[M - OMe]^+$, 3); 436 ($[M - 2H_2O]^+$, 2); 413 ($[M - COOMe]^+$, 2); 405 ($[M - 2H_2O - OMe]^+$, 1); 395 ($[M - COOMe - H_2O]^+$, 12); 392 ($[M - OMe - H_2O]^+$, 2), 377 ($[M - 2H_2O - COOMe]^+$, 3).

Compound 9: ^{1}H NMR $CDCl_3$: 0.77 (s, 3H, H-25), 0.79 (s, 3H, H-26), 0.91 (s, 3H, H-24), 0.96 (s, 3H, H-27), 1.01 (s, 3H, H-23), 1.60 (s, 3H, H-29), 3.25 (m, 2H, H-3, H-18), 5.12 (bs, 1H, H-19), 5.40 (m, 1H, H-12). EIMS (m/z, relative int.): 440 ($[M]^+$, 10); 422 ($[M - H_2O]^+$, 14); 407 ($[M - H_2O - Me]^+$, 4); 396 ($[M - CO_2]^+$, 13); 381 ($[M - CO_2 - Me]^+$, 2); 378 ($[M - CO_2 - H_2O]^+$, 2); 363 ($[M - CO_2 - H_2O - Me]^+$, 1).

Compound 10: ^{1}H NMR $CDCl_3$: 0.78 (s, 3H, H-25), 0.80 (s, 3H, H-26), 0.92 (s, 3H, H-24), 0.98 (s, 3H, H-27), 1.05 (s, 3H, H-23), 1.61 (s, 3H, H-29), 3.29 (m, 2H, H-3, H-18), 3.63 (s, 3H, COOMe), 5.15 (m, 1H, H-19), 5.40 (t, J = 3.8 Hz, 1H, H-12). EIMS (m/z, relative int.): 454 ($[M]^+$, 28); 422 ($[M - H_2O]^+$, 13); 407 ($[M - H_2O - Me]^+$, 5); 396 ($[M - CO_2]^+$, 15); 381 ($[M - CO_2 - Me]^+$, 3); 378 ($[M - CO_2 - H_2O]^+$, 4); 363 ($[M - CO_2 - H_2O - Me]^+$, 1).

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