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Boussingoside E, a New Triterpenoid Saponin from the Tubers of *Boussingaultia baselloides*

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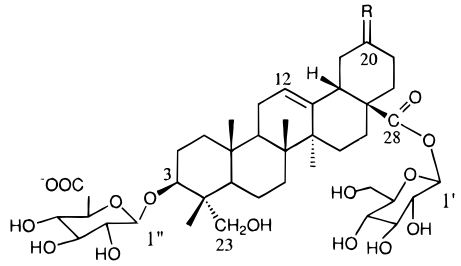
A new triterpenoid saponin, boussingoside E (**1**), 3-*O*-[*β*-D-glucuronopyranosyl]-20(29)-ene-30-norhederagenin 28-*O*-(*β*-D-glucopyranosyl) ester, along with quinoasaponin-9 (**2**) were isolated as sodium/choline salts from the methanolic extracts of the tubers of *Boussingaultia baselloides* H. B. K. (syn.: *Anredera baselloides* Baill.). Their structures were established on the basis of 1D and 2D NMR (DQFCOSY, HOHAHA, HSQC, HMBC, and ROESY spectra), FABMS experiments, and hydrolysis.

Some years ago we reported the isolation of several new triterpenoid saponins, the boussingosides, which exhibited very strong hypoglucaemic activity in rats,[1,2] from the polar extract of the leaves of *Boussingaultia baselloides* H. B. K. (syn.: *Anredera baselloides*), a member of the Basellaceae family. As part of our ongoing investigation for biologically active compounds, we decided to focus our attention in the tubers of this and other Basellaceae because preliminary tests showed the presence of saponins different from those found in the aerial parts.

In this paper, we describe the isolation, from the tubers of *B. baselloides*, of a new triterpenoid saponin, boussingoside E (**1**), along with quinoasaponin-9 (**2**), present as salts of sodium and choline as cation and whose structures were determined by NMR, (including DQFCOSY, HOHAHA, HSQC, HMBC, and ROESY spectra), FABMS, and hydrolysis.

The methanolic extracts of the tubers were defatted and then partitioned between *n*-BuOH and H_2O. The butanolic extract was chromatographed on a Sephadex LH-20 column to give a fraction containing the mixture of saponins. This fraction was first submitted to liquid−liquid chromatography (DCCC) and finally to HPLC to give pure compound **1**, named boussingoside E, along with quinoasaponin-9 (**2**).

Boussingoside E (**1**) was obtained as a solid that decomposes under heating. The ^{13}C-NMR spectrum suggested the presence of a triterpenoid aglycon with two olefinic bonds, an ester carbonyl group, four high-field methyls, and one hydroxymethyl group. DQF-COSY, HOHAHA, HSQC, HMBC, and ROESY maps allowed the assignment of all the proton and carbon resonances and the establishment of the relative stereochemistry of all chiral centers of the aglycon. Comparison with literature data[3,4] for related compounds identified the aglycon of **1** as 20(29)-ene-30-norhederagenin. The proton and carbon resonances corresponding to the sugar part of the molecule suggested the presence of two monosacharide units in **1**, clearly indicated by signals for two anomeric carbons at δ 95.8 and 105.2 ppm, and two anomeric protons at δ 5.35 and 4.42 ppm that resonate as doublets with coupling constants (J = 8.0, 7.8 Hz, respectively) indicative of β-stereochemistry. Analysis of the 2D NMR correlations allowed the identification of glucuronic acid and glucose as the sugar components of **1**. In keeping with these data, acid hydrolysis of **1** produced a 1:1 mixture of D-glucose and D-glucuronic acid (GC−MS). The points of attachment of these two monosaccharides to the aglycon were easily deduced from the HMBC spectrum of **1**, which showed cross-peaks between the anomeric signal of glucuronic acid (H-1″) and C-3 of the aglycon at δ 82.3 ppm and by the ROESY correlation between H-1″ and H-3 at δ 3.67 ppm. For its part, the HMBC experiment showed a correlation between the anomeric proton (H-1′) of the glucose unit and C-28 at δ 177.3 ppm, indicating that glucose is bonded to the carboxylic acid group of the aglycon. In accordance with that structure, the FABMS (positive ion mode) of **1** (magic bullet + NaCl as matrix) showed an ion $[M{\cdot}Na + Na]^+$ at *m/z* 839, in concordance with molecular formula $C_{41}H_{61}O_{15}Na_2$, and fragments at *m/z* 677 $[M{\cdot}Na - 162 + Na]^+$ and 631 $[M{\cdot}Na - 162 - CO_2H_2 + Na]^+$ due to the loss of the terminal glucose. Additionally, in the



1 R = CH_2

2 R = 

a cation = Na^+

b cation = $^+HOCH_2CH_2NMe_3$ Choline

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Table 1. ^{13}C- and ^{1}H-NMR Chemical Shifts (ppm) for **1** in CD_3OD

C	δ_C mult	δ_H mult (*J* In Hz)	C	δ_C mult	δ_H mult (*J* in Hz)
1	39.6 t	0.96 m, 1.60 m	28-COO-Glu		
2	26.2 t	1.75 m, 1.95 m	1′	95.8 d	5.35 d (8.0)
3	82.3 d	3.67 dd (11.5, 4.0)	2′	73.9 d	3.28 m
4	43.9 s		3′	78.3 d	3.36 m
5	48.1 d	1.25 m	4′	71.1 d	3.35 m
6	18.9 t	1.35 m, 1.48 m	5′	78.7 d	3.37 m
7	33.4 t	1.26 m, 1.62 m	6′	62.4 t	3.65 m, 3.80 dd (10.2, 1.0)
8	41.0 s				
9	49.2 d	1.64 br s			
10	37.7 s		3-O-GluA		
11	24.6 t	1.90 m	1″	105.2 d	4.42 d (7.8)
12	124.3 d	5.35 br t	2″	75.1 d	3.25 m
13	144.3 s		3″	78.2 d	3.38 (m)
14	43.0 s		4″	73.7 d	3.37 (m)
15	28.9 t	1.12 m, 1.82 m	5″	76.5 d	3.58 d (9.1)
16	24.2 t	1.82 m, 2.18 m	6″	176.4 s	
17	*a*				
18	48.6 d	2.73 dd (13.6, 6.5)			
19	42.6 t	2.05 dd (13.3, 6.5), 2.55 t (13.3)			
20	149.5 s				
21	38.5 s	1.55 t (13.3), 1.90 m			
22	30.9 t	2.12 m, 2.23 m			
23	64.9 d	3.24 d (3.3), 3.62 d (3.3)			
24	13.4 q	0.69 s			
25	16.5 q	0.97 s			
26	17.8 q	0.80 s			
27	26.4 q	1.27 s			
28	177.3 s				
29	34.6 q	4.60 brd			
30	107.3 s				

[a] Overlapped by the CD_3OD signal.

FABMS (positive ion mode) of **1** we noticed the presence of an ion at *m/z* 104 $[C_5H_{14}NO]^+$ and, in its NMR spectra, a set of signals at δ 3.29 (s, 9H)/54.7 (q), 3.47 (t, J = 9.0 Hz, 2H)/69.0 (t), and 3.99 (m, 2H)/57.1 (t) ppm (correlated by DQFCOSY and HSQC) that matched very well with those of choline ($C_5H_{14}NO$).[5] The 3:5 integration ratio of the choline signals with respect to those of the anion suggests that boussingoside E (**1**) has been isolated as a salt of sodium (**1a**) and choline (**1b**), and so boussingoside E (**1**) is the sodium/choline salt of 3-*O*-[β-D-glucuronopyranosyl]-20(29)-ene-30-norhederagenin 28-*O*-(β-D-glucopyranosyl) ester.

The second component isolated from the plant, compound **2**, presents ^{1}H- and ^{13}C-NMR spectra very similar to those of **1**, and in fact, the protons and carbons of the sugar part of **2** show chemical shifts practically identical to those of **1**. In accordance with this, acid hydrolysis produced a 1:1 mixture of D-glucuronic acid and D-glucose. The only differences between the NMR spectra of **1** and **2** are associated with the presence in **2** of two extra methyl singlets that resonate at δ 0.90 and 0.92 ppm (Me-29 and Me-30) and the lack of the $\Delta^{20(29)}$ olefinic bond of **1**. These spectroscopic data for **2** agree with those reported for quinoasaponin-9 isolated from *Chenopodium quinoa* (Chenopodiaceae).[6] Similar to **1**, saponin **2** is present in *B. baselloides* as the sodium/choline salt. This was indicated by the presence in its FABMS (positive ion mode) of ions $[M{\cdot}Na + Na]^+$ at *m/z* 855 (HRFABMS obsd 855.4112 calcd for $C_{42}H_{65}O_{15}Na_2$ 855.4118) and $[M{\cdot}choline]^+$ at *m/z* 913 along with a very intense ion at *m/z* 104 $[C_5H_{14}NO]^+$ and by signals for choline in the ^{1}H- and ^{13}C-NMR spectra. In this case a 4:4.5 integration ratio of choline with respect to the anion was observed.

The aerial parts of *B. baselloides* have been found in the past to be a good source of saponins with hypoglucaemic activity.[1,2] Boussingosides E (**1**) and quinoasaponin-9 (**2**) are absent in the aerial parts of the plant and are distinguished by the presence of choline as cation of the glucuronic acid part of the saponin. We have recently found a precedent of this situation in tuberosides A, B, and C, triterpenoid saponins isolated as salts of choline from the tubers of *Ullucus tuberosus*.[7] *B. baselloides* and *U. tuberosus* are both members of the Colombian Basellaceae, and curiously, enough choline was detected only in the saponins from the tubers but not from the aerial parts. The exact significance of this fact remains unknown.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-370 polarimeter. NMR spectra were recorded with a JEOL a-400 NMR spectrometer (399.65 for ^{1}H and 100 for ^{13}C) using CD_3OD as solvent. Mass spectra were recorded on Kratos MS-50, JEOL AMX505, and Hewlett-Packard HP 59970 spectrometers. The FABMS were obtained employing Xe atoms at 7−9 keV and glycerol + NaCl, glycerol/thioglycerol (1:1) + NaCl, or Magic bullet + NaCl, as matrix. The HPLC separations were performed on a Waters Model 6000A equipped with a differential refractometer. DCCC was carried out with an Eyela Model 300-S apparatus with 300 tubes.

Plant Material. Plants were collected in Tuluá, Colombia, in January 1989. 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. Air-dried tubers of the plant (670 g) were extracted successively with petroleum ether and MeOH. The MeOH extract (17.5 g) 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 *n*-BuOH extract (3.8 g) was dissolved in the 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 2.5 g of dry extract that was separated in five fractions on a Sephadex LH-20 column using methanol as eluant and TLC monitoring. The second fraction (430 mg) was subjected to ascending DCCC with 7:13:8 $CHCl_3$−MeOH−H_2O (flow rate = 12 mL/h) and the mixture of saponins (110 mg) was finally separated by reversed-phase HPLC on a C_{18} Partisil ODS-M9 column eluted with 40:60 MeOH−H_2O, affording 10 mg of compound **1** and 32 mg of compound **2** (flow rate = 2.5 mL/min, retention times 14.5 and 21 min, respectively).

Boussingoside E (1): $[\alpha]^{20}_D$ + 16° (*c* 0.5, MeOH); ^1H and ^{13}C-NMR (see Table 1); FABMS (positive ion mode) using glycerol/thioglycerol (1:1) + NaCl as matrix *m/z* (rel int) = 839 ([M·Na + Na]$^+$, 10), 677 ([M·Na − 162 + Na]$^+$, 4), 115 (100), 104 (choline, 17); FABMS (positive ion mode) using magic bullet + NaCl as matrix *m/z* (rel int) = 839 ([M·Na + Na]$^+$, 2), 677 ([M·Na − 162 + Na]$^+$, 1), 631 ([M·Na − 162 − CO_2H_2 + Na]$^+$, 1), 215 (100), 104 (choline, 30); FABMS (positive ion mode) using glycerol + NaCl as matrix *m/z* (rel int) = 839 ([M·Na + Na]$^+$, 3), 137 (100), 104 (choline, 30).

Quinoasaponin-9 (2): $[\alpha]^{20}_D$ + 28° (*c* 0.5, MeOH); HRFABMS (positive ion mode), glycerol + NaCl as matrix calcd for $C_{42}H_{65}O_{15}Na_2$ = [M·Na + Na]$^+$ 855.4125, found 855.4112; FABMS (positive ion mode), using glycerol + NaCl as matrix *m/z* (rel int) = 913 ([M + 104]$^+$, 7), 855 ([M·Na + Na]$^+$, 53), 693 ([M·Na − 162 + Na]$^+$, 43), 647 ([M·Na − 162 − CO_2H_2 + Na]$^+$, 50), 104 (choline, 100).

Acid Hydrolysis of 1 and 2. The saponin (1 mg in 1 mL of MeOH) was refluxed in 1 mL of 1 N HCl for 4 h and then extracted with CH_2Cl_2 (3 × 10 mL). The aqueous layer was neutralized and reacted with $NaBH_4$ (2 mg) at room temperature for 2 h. After addition of AcOH to eliminate excess $NaBH_4$, the mixture was concentrated to dryness and the AcOH codistilled with MeOH (2 × 5 mL). The resulting alditols were acetylated by refluxing for 12 h with Ac_2O/pyridine (1:1, 5 mL). After that time, 5 mL of H_2O was added, and the alditol acetates were extracted with CH_2Cl_2 and identified by GC−MS as those of glucose and glucuronic acid.

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