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Aragoside and iridoid glucosides from *Aragoa cundinamarcensis*

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Dedicated to the memory of Professor Jeffrey B. Harborne

Abstract

From the water-soluble part of an extract of *Aragoa cundinamarcensis* were isolated seven iridoid glucosides, namely aucubin, catalpol, rehmannioside D, globularin, gardoside methyl ester, epiloganin and mussaenoside. The main glycoside isolated, however, was a new caffeoyl phenylethanoid triglycoside, named aragoside, containing two β-gluco- and one α-arabinopyranosyl moieties which constituted almost 5% of the dry weight of the plant. Finally, sorbitol was found to be the main carbohydrate constituent of the plant. This distinctive combination of compounds is very similar to that reported from some species of *Plantago*. The present findings therefore support the results from a recently published molecular phylogenetic study of plastid and nuclear ribosomal DNA sequences, where *Aragoa* was found to be the closest relative to *Plantago* so far discovered.
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Keywords: Chemotaxonomy; *Aragoa cundinamarcensis*; Plantaginaceae; Iridoid glucosides; Caffeoyl phenylethanoid glycosides; Aragoside

1. Introduction

Aragoa Kunth comprises 19 species of woody perennial shrubs endemic to the páramos of Colombia and Venezuela. Most authors consider it part of Scrophulariaceae s.l., but the systematic position of the genus has been controversial due to its peculiar morphological characteristics (Fernández-Alonso, 1995; Bello et al., 2002). Scrophulariaceae as usually circumscribed has recently been shown to be polyphyletic and some of the genera are more closely related to *Veronica* and *Plantago* in an expanded Plantaginaceae (*sensu lato*) than to the core Scrophulariales (Olmstead et al., 2001).

In the first molecular analysis of the relationship of *Aragoa*, based on plastid *rbcL* and nuclear ribosomal ITS sequence data, Bello et al. (2002) found *Aragoa* to be the sister of *Plantago* in a clade also including *Veronica, Hemiphragma, Digitalis* and *Globularia* (Fig. 1). A similar clade has been found in other studies and includes *Campylanthus* (Hjertson, 1997), *Ourisia, Sibthorpia, Lagotis, Isoplexus, Erinus*, and a number of genera included in the Veroniceae tribe (Albach and Chase, 2001), and more genera will probably be assigned to this clade in the future when they are subjected to molecular analysis. The sister relationship of *Aragoa* and *Plantago* was completely unexpected. *Plantago* is a genus of over 200 species of annual and perennial herbs and semi-shrubs with a worldwide distribution, but a number of similarities with *Aragoa* can be found (Fernández-Alonso, 1995; Rahn, 1996). Most remarkably, all the 19 species of *Aragoa* are woody shrubs while most species of *Plantago* are herbaceous, although there are some woody insular species, e.g. *P. arborescens* Poir. and *P. famarae* Svent. from the Canary Islands and *P. princeps* Cham. & Schltdl. from Hawaii. The wood of *Aragoa* is rayless (Mennega, 1975), as is the wood of the woody *Plantago* species (Carlquist, 1970). Rayless wood has also been reported from *Veronica* and *Digitalis* (Mennega, 1975). According to Carlquist (1992) rayless wood is characteristic of groups in which woodiness increases from a herbaceous habit, which is likely to be the case if *Aragoa* and *Plantago* were derived from a common herbaceous ancestor.

In the only previous study of the chemistry of *Aragoa*, Corothie and Lilja (1975) reported that β-sitosterol and some methyl esters of substituted *trans*-cinnamic acids were obtained after boiling the crude extract from

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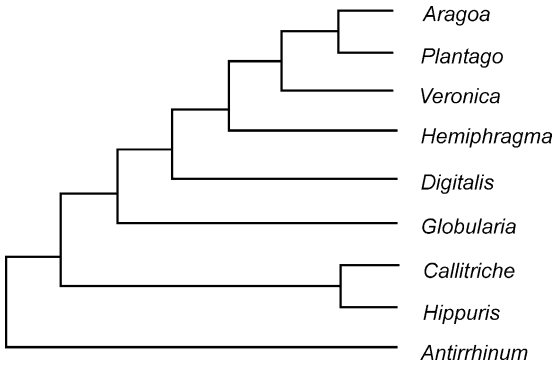


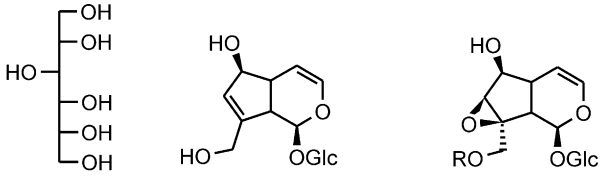
Fig. 1. Clade of *Aragoa* and closest known relatives as obtained by phylogenetic analysis of *rbcL* sequence data. Redrawn from Bello et al., 2002.

A. lucidula S. F. Blake with methanolic sulfuric acid. In order to evaluate the relationships of *Aragoa* suggested by molecular data, we here present an investigation of the water-soluble fraction of an extract of *Aragoa cundinamarcensis* Fernández-Alonso.

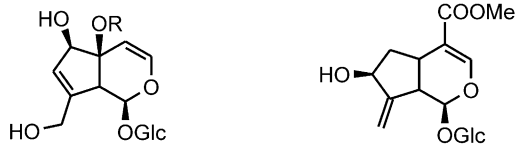
2. Results and discussion

A. cundinamarcensis was collected in March 2002 and quickly dried. After extraction with ethanol, the water-soluble part was subjected to reversed phase column chromatography to give a sugar fraction consisting mainly of sorbitol (**1**). Aucubin (**2**) was the main iridoid glucoside present, with minor amounts of catalpol (**3**), rehmannioside D (**4**) and globularin (**5**), as well as gardoside methyl ester (**6**), epiloganin (**7**), and mussaenoside (**8**). The main glycoside isolated, however, was a new caffeoyl phenylethanoid glucoside (CPG), which we have named aragoside (**9**) and which constituted almost 5 per cent of the dry weight of the plant.

Aragoside (**9**) was isolated as an amorphous solid $[\alpha]_D^{20}$ −51° with the molecular formula $C_{34}H_{44}O_{20}$, as established by HRESIMS. The ^{13}C NMR spectrum (Table 1) showed 34 signals some of which were typical for a CPG-like compound (Jensen, 1996). Thus, eight signals from the 3,4-dihydroxyphenylethyl moiety and nine signals from the caffeoyl moiety could be sorted out, leaving 17 signals. These represented three sugar moieties as seen by the three signals at δ 103–105, obviously arising from anomeric carbon atoms. Comparison with similar compounds, namely persicoside (**10**) from *Veronica persica* Poiret (Harput et al., 2002) and ehrenoside (**11**) from *V. bellidioides* L. (Lahloub et al., 1986) and *V. pectinata* L. (Saracoglu et al., 2002) showed the presence of a central β-glucopyranosyl moiety (Glc-1) substituted with additional sugar units at the oxygens of C-2′ and C-3′, as seen by their characteristic absorptions at δ 82.7 and 81.4, respectively. Further comparison with **10** allowed assignment of the six signals from a β-glucopyranosyl moiety (Gly-3) appended at the oxygen of C-3′. This left five signals to be assigned and a final comparison with **11** showed that these belonged to an α-arabinopyranosyl moiety (Gly-2) attached to the oxygen at the 2′-position. COSY and HSQC spectra allowed an almost complete analysis of the ^1H NMR spectral data (Table 1), and also proved that the glucopyranosyl moiety (Gly-3) was attached to Glc-1 at the oxygen of C-3′. Thus, when comparing (Table 1) the set of ^1H signals arising from the Gly-3 with those of the two glucopyranosyl units in **10**, almost complete overlap (no more than 0.03 ppm in difference) was seen with Gly-3 of **10**, while it was rather different from that of the other glucopyranosyl moiety (Gly-2), probably due to the close proximity of the caffeoyl group to the former. Similarly, the signals from the α-arabinopyranosyl moiety (Gly-2) of **9** were almost identical to those reported (not shown) for ehrenoside (**11**). This established the structure of **9** to be that shown in the formula.

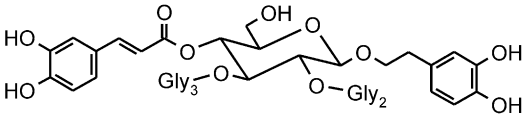


1 **2** **3** R = H
5 R = *E*-Cinnamoyl



4 R = Sophoroside **6**

7 **8**



9 Gly_2 = α-Ara, Gly_3 = β-Glc
10 Gly_2 = Gly_3 = β-Glc
11 Gly_2 = α-Ara, Gly_3 = α-Rha

Table 1
^{1}H (500 MHz) and ^{13}C NMR (125 MHz) spectra of aragoside (**9**) and model compounds in MeOH-d_4

Atom	Aragoside (**9**)[a]		Persicoside (**10**)[b]		Ehrenoside (**11**)[c]
	^{1}H-δ (mult. Hz)	^{13}C	^{1}H	^{13}C	^{13}C
Aglucone					
1		132.0		131.7	131.9
2	6.75 (*d* 2.1)	117.5	6.72	117.4	117.5
3		146.1		146.2	146.1
4		144.7		144.7	144.8
5	6.69 (*d* 8.1)	116.3	6.67	116.4	116.4
6	6.57 (*dd* 8.1/2.1)	121.5	6.58	121.4	121.5
α	3.70/4.08 (*m*'s)	72.1	3.76/4.07	72.3	72.2
β	2.78 (*m*)	36.7	2.79	36.6	36.8
Central Glc					
1′	4.51 (*d* 8.1)	103.4	4.54	103.3	103.1
2′	3.71 (*dd* 8.1/9.4)	82.7	3.74	81.5	82.7
3′	4.11 (*t* 9.4)	81.4	4.13	82.1	81.4
4′	4.93 (*t* 9.4)	70.5	4.92	70.6	70.9
5′	3.58 (*obsc*)	75.4	3.59	75.8	75.9
6′	3.55/3.65 (*obsc*)	62.3	3.54/3.66	62.4	62.5
Gly-2					
1‴	4.55 (*d* 7.2)	104.9	4.73	104.1	104.2
2‴	3.56 (*obsc*)	73.3	3.20	75.9	73.1
3‴	3.49 (*dd* 3.4/9.0)	74.1	3.32	77.8	74.6
4‴	3.75 (*m*)	69.8	3.27	71.6	69.6
5‴	3.18 (*dd* 1/12.5)	67.3	3.42	78.7	66.9
	3.79 (*br d* 12)				
6‴	–	–	3.66/3.80	63.2	–
Gly-3					
1″	4.65 (*d* 8.1)	104.3	4.65	104.2	103.3
2″	3.11 (*dd* 7.8/9.4)	75.6	3.09	75.5	72.2
3″	3.30 (*t* 9.3)	78.0	3.27	78.0	72.0
4″	3.05 (*t* 9.4)	72.0	3.02	70.6	73.9
5″	3.23 (*ddd* 2.5/6.5/9.4)	78.0	3.20	78.1	70.7
6″	3.46 (*dd* 6.4/11.5)	63.2	3.44	62.8	18.5
	3.78 (*dd* 2.5/12)		3.78		
Caffeoyl					
1⁗		127.8		127.7	127.7
2⁗	7.09 (*d* 2.1)	115.3	7.07	115.3	115.3
3⁗		146.8		146.9	146.9
4⁗		149.6		149.7	149.8
5⁗	6.79 (*d* 8.1)	116.6	6.78	116.6	116.9
6⁗	6.99 (*dd* 8.1/2.1)	123.0	6.98	123.1	123.2
α⁗	6.35 (*d* 15.8)	115.5	6.34	115.5	114.9
β⁗	7.57 (*d* 15.8)	147.2	7.56	147.2	148.0
CO⁗		168.5		168.6	168.4

[a] Signals were assigned using COSY and HSQC spectra.
[b] Data from Harput et al. (2002).
[c] Data from Saracoglu et al. (2002). *obsc*: Signals were obscured.

The main sugar detected in the carbohydrate fraction of *A. cundinamarcensis* consists of the sugar alcohol sorbitol (**1**), which as a natural substance is uncommon. Sorbitol normally occurs in all subfamilies of the Rosaceae, except Rosoideae (Wallaart, 1980); it has also been reported from the small family Tetrachondraceae (Jensen, 2000), and from all *Plantago* species investigated for it (Wallart, 1981; Rønsted et al., 2000) as well as from *Campylanthus* (Rønsted and Jensen, 2002). Finally, a disaccharide ester containing sorbitol has recently been reported from *Globularia* (Calis et al., 2002a). In chemotaxonomic reports, the sugar fraction is not usually investigated, but it is known that mannitol is present in some species of *Veronica* (Shimada et al., 1971).

The iridoid glucosides found in *Aragoa* are typical for Plantaginaceae (Rønsted et al., 2000). Thus, aucubin (**2**), catalpol (**3**), gardoside methyl ester (**6**), epiloganin (**7**) and mussaenoside (**8**) are found widespread in this family. Rehmannioside D (**4**) is a less common iridoid

so far only reported from *Plantago maritima* L. (Rønsted et al., 2000) and from *Rehmannia glutinosa* (Gaertn.) Libosch. ex Fisch. & C. A. Mey. (Oshio and Inouye, 1982). Finally, globularin (**5**) is fairly widespread, but it is interesting that it is present in some species of *Plantago* in subgenus *Albicans* (Rønsted et al., 2000), in most species of *Globularia* (e.g. Calis et al., 2002a; 2002b), in *Hemiphragma heterophyllum* Wall. (Ma et al., 1995) and *Lagotis stolonifera* Maxim. (Calis et al., 1991). It has so far not been reported from *Veronica* (Taskova et al., 2002).

CPGs are common in all Lamiales families (Harborne, 1966; Jensen, 1992). The only CPG isolated from *A. cundinamarcensis* is aragoside with a β-glucopyranosyl and an α-arabinopyranosyl moiety, respectively, attached to the oxygen atoms of C-3 and C-2 of the central sugar unit. Similar compounds are so far limited to the genus *Veronica* (Harput et al., 2002; Saracoglu et al., 2002; Aoshima et al., 1994). However, a related CPG, namely plantamajoside (which lacks the α-arabinopyranosyl unit present in **9**) is common in *Plantago* (Rønsted et al., 2000), and it has also been reported from *Veronica fuhsii* Freyn & Sint. (Ozipek et al., 1999), *Hemiphragma heterophyllum* (Ma et al., 1995), *Lagotis stolonifera* (Calis et al., 1991) and *Digitalis purpurea* L. (Matsumoto et al., 1987), and is only known from a few other sources.

In conclusion, the chemical data fit very well with molecular results since the compounds found in *Aragoa* are most similar to those reported from some species of *Plantago*, but as might be expected, a close relationship with the other genera in the clade is also found.

3. Experimental

3.1. General

^{1}H and ^{13}C NMR spectra were recorded on a Varian Inova-500 instrument in MeOH-d_4 using the solvent peak (δ 3.31 and 49.0, respectively) as the internal standard. LC-HR ESIMS was performed on a Agilent HP 1100 Liquid Chromatograph equipped with a BDS-C18 reversed phase column running a water-acetonitrile (50 ppm TFA in water) gradient. The LC was coupled to a LCT of a TOF MS (Micromass, Manchester, UK) operated in the positive electrospray ion mode using 5-leucineenkephalin as lock mass.

3.2. Plant material

A. cundinamarcensis was collected in the Municipio of Villapinzón, Cundinamarca, Colombia in March 2002. The plant material was authenticated by one of us (M. A. B.) and the voucher specimen (MAB-243) has been deposited at Herbario Nacional Colombiano (COL).

3.3. Extraction and isolation

Dry aerial parts (40 g) were pulverized in a blender with EtOH, brought to boiling and left for extraction for three days. The extract was filtered, concentrated and partitioned in Et_2O–H_2O. Evaporation of the aq. phase followed by treatment with a little activated C in MeOH gave a crude extract (6.2 g) of which 3.6 g was applied to a C-size Lobar C-18® reversed phase column. Elution with H_2O–MeOH (1:0 to 2:1) gave first a sugar fraction (400 mg) mainly consisting of sorbitol (**1**; ca. 75% of total sugars, as seen by ^{13}C NMR spectral analysis) and glucose (ca. 20%), followed by catalpol (**3**; 15 mg, 0.07%), aucubin (**2**; 390 mg, 1.7%), rehmannioside D (**4**; 25 mg, 0.1%), gardoside methyl ester (**6**; 20 mg, 0.1%), epiloganin (**7**; 45 mg, 0.2%), mussaenoside (**8**; 60 mg, 0.3%), aragoside (**9**; 1.11 g, 4.8%) and globularin (**5**; 25 mg, 0.1%). Rechromatography gave a pure sample of aragoside (**9**) as a foam; $[\alpha]_D^{20} = -51^\circ$ (*c* 0.34, MeOH); LC-HR ESIMS *m*/*z*: 790.2273 $[M+NH_4]^+$ ($C_{34}H_{48}NO_{20}$ requires 790.2770) and *m*/*z*: 795.2324 $[M+Na]^+$ ($C_{34}H_{44}O_{20}Na$ requires 795.2324); NMR data in Table 1.

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