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Iridoid glucosides from *Randia spinosa* (Rubiaceae)

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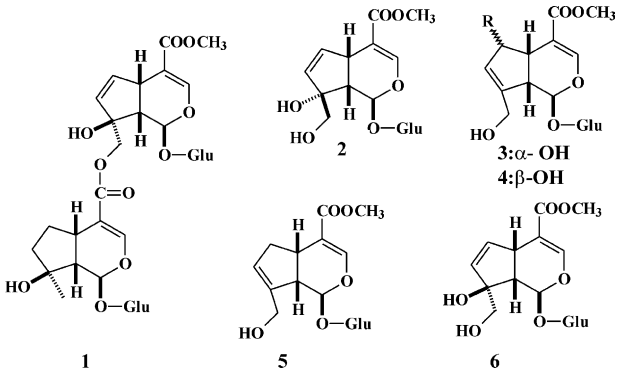
Abstract

An iridoid glucoside: randinoside, along with five known iridoids: galioside, deacetylasperulosidic acid methyl ester, scandoside methyl ester, geniposide and gardenoside, were isolated from the stems of *Randia spinosa*. The structures were determined by spectroscopic analysis, including 2D NMR techniques.

Keywords: *Randia spinosa*; Rubiaceae; Iridoid glucosides

1. Introduction

Randia spinosa belongs to the Ixoroideae subfamily in the Rubiaceae, these being known for the biosynthesis of iridoids as main secondary metabolites (Robbrecht et al., 1996). This genus is strictly neo-tropical, comprises about 80 species (Robbrecht, 1994) and some are known to display pharmacological properties such as anti-inflammatory (Baghdikian et al., 1997; Recio et al., 1994), antileishmanial (Tandon et al., 1991), and anti-tumor (Abdel-Kader et al., 1997) activities. *R. spinosa,* popularly known as "sacrifício de Cristo", is traditionally used in the Northeast of Brazil to treat inflammatory diseases (Correa, 1974). During continuing studies on Brazilian Rubiaceae plant species, the aqueous fraction from the EtOH extract of *R. spinosa*, collected at Pacatuba, Ceará State was investigated. This work resulted in the isolation of one iridoid glucoside, randinoside (**1**) together with the known iridoids galioside (**2**) (Karikas et al., 1987), deacetylasperulosidic acid methyl ester (**3**), (Ishiguro et al., 1983), scandoside methyl ester (**4**) (Miyagoshi et al., 1987; Inouye et al., 1974), geniposide (**5**) (Jensen, et al., 1973; Endo and Taguchi, 1973) and gardenoside (**6**) (Inouye et al., 1974; Bailleul et al., 1977).



1 2 5 6

2. Results and discussion

Randinoside (**1**) was obtained from the aqueous fraction of the EtOH extract of *R. spinosa* stems following semi-preparative HPLC, described in the Experimental section. The negative HRTOF–ESMS analysis of **1** established its molecular formula as $C_{33}H_{46}O_{20}$, which was deduced from the *quasi*-molecular ion peak at *m*/*z*

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779.2791 $[M+H_2O]^-$. MS/MS experiment on this *quasi*-molecular ion gave one fragment at *m/z* at 375.1255 [$(M+H_2O)$-404.1536] indicating the loss of one unit A (see below) from compound **1** (Fig. 1). The ES–MS spectrum showed a peak at *m/z* 763 assigned to $[M+H]^+$ and the intense peaks at *m/z* 601 and 163, which suggested loss of a glucose unit. In addition, peaks at *m/z* 404 and 387, as well as at *m/z* 359 were also observed, which might be formed from cleavage of ester linkages. The UV spectrum of this compound displayed an absorption maximum at 239 nm, which is characteristic of an iridoidal skeleton, and intense IR bands at 3450–3500 and 1708 cm^{-1}, which indicated the presence of hydroxyl and ester carbonyl functionalities, respectively. On the basis of its high resolution MS and NMR spectroscopic data (Table 1), the structure of an iridoid glucoside was proposed for this compound. The analysis of ^1H and ^{13}C NMR spectra indicated the presence of two distinct iridoid units, which are hereafter referred to as units A and B. The ^1H NMR spectrum showed signals at δ 7.37 (*d*, $J=1.4$ Hz), 5.78 (*d*, $J=2.5$ Hz), 6.14 (*dd*, $J=2.7$, 5.7 Hz), 5.72 (*dd*, $J=5.7$, 1.7 Hz), 3.62 (*d*, $J=11.3$ Hz) and 3.51 (*d*, $J=11.3$ Hz) which were assigned to H-3, H-1 H-6, H-7, H-10 of unit A, based on analysis of the HOMOCOSY, gHMQC and gHMBC spectra. Analysis of the PND and DEPT 135 ^{13}C NMR spectra revealed signal values similar to those reported for gardenoside (Inouye et al., 1974; Bailleul et al., 1977). The remaining spectral data revealed a second iridoid unit due to part B of the new iridoid. Signals at δ 7.33 (*s*), 5.43 (*d*, $J=4.0$ Hz), and 1.31 (*s*) in the ^1H NMR spectrum were assigned to H-3, H-1 and H-10 of unit B. One significant difference observed for this part was the lack of a methoxyl group ca. δ 3.70, which is a common feature in a large number of carbomethoxy iridoid structures. The ^{13}C NMR data of this moiety indicated signals identical to those of mussaenoside (Gardner et al., 1987; Takeda et al., 1977), which was confirmed by 2D NMR data analysis (Table 1). The absence of the ester methoxyl of unit B, when compared to mussaenoside, and observation of deshielding of C-10a (δ 67.1), when compared to the monomer gardenoside (δ C-10 = 63.0) (Bailleul et al., 1977), was the first indication for the attachment of units A and B between C-10a and C-11b. The partial structures A and B were reasonably connected to each other by following HMBC correlations. The correlations from H-3a (δ 7.37) with C-1a (δ 94.3), C-4a (δ 111.5), C-5a (δ 38.8) and C-11a (δ 168.9) and of OCH_3 (δ 3.69) with C-11a demonstrated that the carboxyl group in unit A is methoxylated. In addition, the correlations between H-7a (δ 5.72) with C-5a (δ 38.8), C-8a (δ 86.2), C-9a (δ 52.3) and C-10a (δ 67.1) corroborated the partial structure of A as similar to those of gardenoside (Inouye et al., 1974). Moreover, the downfield shift of H-10a (δ 3.62 *d*, $J=11.3$ Hz; δ 3.51 *d*, $J=11.3$ Hz) could be explained by an esterification at hydroxymethylene C-10a. Correlations between H-3b (δ 7.33) with C-1b (δ 95.9), C-4b (δ 108.9) and C-11b (δ 169.9) (Table 1 and Fig. 2) corroborated unit B and strongly indicated the connection between unit A and B through an ester linkage between C-10a and C-11b. These findings were also supported by ^{13}C chemical shift values analyzed for **1** in comparison with those observed for gardenoside and mussaenoside, model compounds used to establish the unit A and B, respectively. Confirmation of the stereochemistry of its stereogenic centers was achieved by analysis of *J* values and comparison with ^{13}C NMR literature data, especially those for chiral centers at C-5a, C-8a, C-9a and C-5b, C-8b and C-9b, which indicated the β-configuration of the hydroxyl group at C-8 in both fragments A and B, consistent with the configuration of this substituent in gardenoside (Inouye et al.,

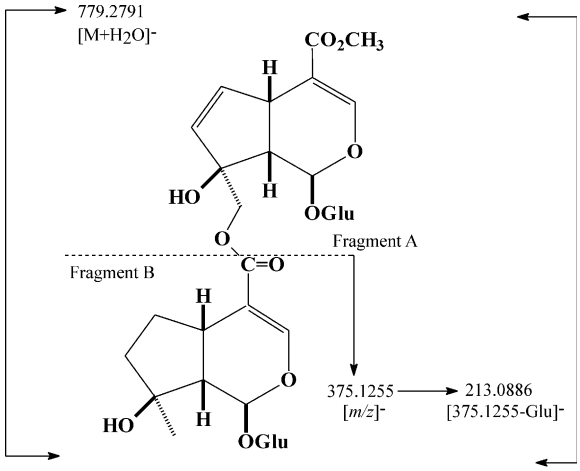

Fig. 1. Main fragmentary ions for compound **1**.

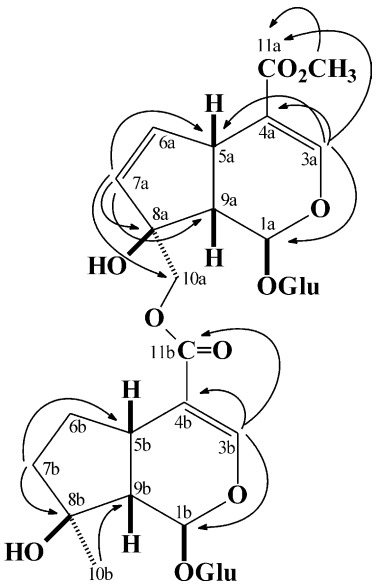

Fig. 2. Selected gHMBC correlations (H→C) for compounds **1** (Glu = glucose).

Table 1
NMR data for compound **1**[a,b]

Position	δ_C[c]	δ_H (Hz)	COSY	HMBC
1a	94.3 (*d*)	5.78 *d*, $J_{1,9}=2.5$	H-9a	C-3a, C-5a, C-1a′
3a	151.9 (*d*)	7.37 *d*, $J_{3,5}=1.4$	H-1a	C-1a, C-4a, C-5a, C-11a
4a	111.5 (*s*)	–	–	-
5a	38.8 (*d*)	3.69 *m*	H-6a, H-9a	C-1a, C-7a, C-11a
6a	135.6 (*d*)	6.14 *dd*, $J_{6,5}=2.7$; $J_{6,7}=5.7$	H-7a, H-5a	C-4a, C-9a
7a	135.8 (*d*)	5.72 *dd*, $J_{6,7}=5.7$; $J_{7,5}=1.7$	H-6a	C-5a, C8a, C-9a,C-10a
8a	86.2 (*s*)	–	–	-
9a	52.3 (*d*)	2.60 *dd*, $J_{9,1}=2.5$; $J_{9,5}=8.5$	H-1a, H-5a	C-7a, C-6a, C-10a
10a	67.1 (*t*)	3.62 *d*, $J_{10\alpha,10\beta}=11.3$ 3.51 *d*, $J_{10\beta,10\alpha}=11.3$	H-10a$_{(\beta)}$	C-7a, C-9a
11a	168.9 (*s*)	–	–	–
Ome	51.7 (*q*)	3.69 *s*	–	C-11a
1′	99.8 (*d*)	4.65 *d*, $J_{1',2'}=7.9$	H-2′	C-1a
2′	74.7 (*d*)	3.18 *dd*, $J_{1',2'}=7.9$, $J_{2',3'}=.9.0$	H-1′, H-3′	–
3′	78.3 (*d*)	3.28 *m*	H-2′, H-4′	–
4′	71.7 (*d*)	3.24 *t*, $J=8.8$	H-3′, H-5′	–
5′	78.0 (*d*)	3.35 *dd*, $J=2.1$, 9.0	H-4′, H-6′	–
6′	62.9 (*d*)	3.88 *dd*, $J=5.2$, 10.0 3.65 *dd* $J=5.2$, 10.0	H-5′	–
1b	95.9 (*d*)	5.43 *d*, $J_{1,9}=4.0$	H-9b	C-3b, C-1″
3b	150.0 (*d*)	7.33 *br*, *s*	H-1b	C-1b, C-4b, C-5b, C-11b
4b	108.9 (*s*)	–	–	–
5b	32.2 (*d*)	3.17 *m*	H-6b, H-9b	C-3b, C-7b, C-11b
6b	30.7 (*t*)	2.27 *m*, 1.47 *m*	H-5b, H-7b	C-4b
7b	40.8 (*t*)	1.70 *t*, $J=7.2$	H-6b	C-5b, C-8b, C-9b, C-10b
8b	80.5 (*s*)	–	–	–
9b	52.4 (*d*)	2.21 *dd*, $J_{9,1}=4.0$; $J_{9,5}=9.1$	H-1b, H-5b	C-4b, C-7b, C-10b
10b	24.6 (*q*)	1.31 *s*	–	C-7b, C-9b
11b	169.9 (*s*)	–	–	–
1″	99.8 (*d*)	4.64 *d*, $J_{1'',2''}=7.9$	H-2″	C-1b
2″	74.6 (*d*)	3.17 *dd*, $J_{1'',2''}=7.9$; $J_{2'',3''}=9.0$	H-1″, H-3″	–
3″	77.9 (*d*)	3.29 *m*	H-2″, H-4″	–
4″	71.5 (*d*)	3.25 *t*, $J=8.8$	H-3″, H-5″	–
5″	78.3 (*d*)	3.33 *dd*, $J=2.1$, 9.0	H-4″, H6″	–
6″	62.7 (*d*)	3.86 *dd*, $J=5.1$, 10.0 3.63 *dd* $J=5.1$, 10.0	H-5″	–

[a] CD_3OD.
[b] 500 MHz for ^1H and 125 for ^{13}C.
[c] Multiplicities obtained from DEPT 135° experiment.

1974; Bailleul et al., 1977) and mussaenoside (Gardner et al., 1987; Takeda et al., 1977, respectively). The *cis* junction between the two rings and the *O*-glycosyl residue at C-1 is biosynthetically supported with a β configuration (Bianco, 1990). According to these assumptions the new iridoid glucoside adopts the 1a/1b, 5a/5b, 8a/8b, and 9a/9b *S* configurations, similar to those iridoid models.

3. Experimental

3.1. General

Optical rotations were determined in MeOH using a Polamat A Carl Zeiss Jena polarimeter equipped with a sodium lamp operating at 546 and 548 nm. IR spectra were obtained using a Perkin Elmer 1600 (series FTIR) spectrometer, whereas UV spectra were performed using a Perkin Elmer Lambda 14P UV/vis spectrometer. ES-MS was conducted on a VG Platform Fisons instrument and HRTOF-ESMS was performed using a Q-Tof (Micromass) (40 eV). ^1H (500 MHz) NMR, ^{13}C (125 MHz) NMR and 2D NMR spectra were obtained on a Varian Inova 500 spectrometer. The NMR spectral data were recorded in CD_3OD or DMSO-d_6, and chemical shifts were expressed in δ (ppm), referring to TMS. Silica gel (230–400 mesh ASTM, particle size 0.040–0.063 μm, Merck), Amberlite XAD-7 nonionic polymeric adsorbent (20–60 mesh, surface area 450 m^2/g) or Sephadex LH-20 (Pharmacia) were used in the CC fractionations. Prep HPLC was performed using an ODS column (Phenomenex Luna ODS, 21.20 mm i.d.×250 mm) and UV detection at 254 nm.

3.2. Plant material

The stems of *R. spinosa* (4.5 kg) were colleted in Pacatuba, Ceará State, in March 1996. A voucher specimen (Andrade-19) was deposited at the herbarium of the Instituto de Botânica, at Universidade Federal do Ceará (UFCE).

3.3. Extraction and separation

Dried and powdered stems (1.9 kg) of *R. spinosa* were extracted with EtOH. The ethanolic extract (44.0 g) was partly dissolved in *n*-BuOH. Subsequent addition of water generated two phases (A and B), which were dried under reduced pressure separately. The *n*-BuOH fraction (A) was solubilized at 80% MeOH and partitioned with hexane, $CHCl_3$ and EtOAc. The aqueous fraction (B, 5.0 g) was subjected to XAD-7 column chromatography and successfully eluted with H_2O, MeOH/H_2O (1:1) and MeOH, affording thirteen fractions. Fraction 5 [MeOH/H_2O (1:1), 440 mg] was applied to a Sephadex LH-20 column eluted with EtOAc/MeOH (2:1), affording 23 fractions. Pooled fraction (14–16) (200 mg) was purified by prep HPLC [RP-18 column, MeCN–H_2O (6:94)], yielding pure compounds galioside **2**, (4.5 mg), deacetylasperulosidic acid methyl ester **3**, (35.3 mg), scandoside methyl ester **4**, (21.0 mg) and the new iridoid randinoside (**1**, 8.5 mg). Fraction 7 (MeOH, 300 mg) was subjected to silica gel column eluted with $CHCl_3$/MeOH/H_2O/HOAc (80:17.5:1.5:1), and afforded pure geniposide **5**, (21.0 mg) plus fraction C. Fraction C (35.0 mg) was further purified by prep HPLC [RP-18 column, MeCN–H_2O (6:94)], leading to pure gardenoside **6**, (4.0 mg). Compounds **2**–**6** were identified by comparison of their physical and spectral data with those of the literature.

3.4. Randinoside *1*

White powder; $[\alpha]_D^{20}$ −6.5 (*c* 1.7, MeOH); UV (MeOH) λ_{max} 239 nm; IR (KBr), 3500, 3399 and 1708 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD) data, see Table 1; ESMS *m*/*z* 763 $[M+H]^+$, 404, 387, 359, 331, 225 HRTOF-ESMS *m*/*z* 779.2791 $[M+H_2O]^-$, (requires $C_{33}H_{46}O_{20}+H_2O$ 779.2710).

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