Passibiflorin, α-hydroxy- and α-β-D-glucopyranosyloxy-amides from *Passiflora punctata* L. leaves

Passibiflorina, α-hidroxi- y α-β-D-glucopiranosiloxi-amidas de las hojas de *Passiflora punctata* L.

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Abstract

A cyanogenic glycoside, passibiflorin (**1**) and two primary amides, (1*S*,1*R*)-1-hydroxy-4-(6-deoxy-β-D-glucopyranosyloxy)-2-cyclopentene-1-carboxamide (**2**) and (1*S*,4*R*)-1-(β-D-glucopyranosyloxy)-4-(6-deoxy-β-D-gulopyranosyloxy)-2-cyclopentene-1-carboxamide (**3**), were isolated from the leaves of *Passiflora punctata* L. The carbohydrates D-antiarose (**4**), glucose (**5**) and sucrose (**6**) were also obtained. The structures were established by IR, NMR and MS data and by comparison of these data against values reported in the literature. All the compounds are reported for the first time in *P. punctata*. In addition, the antimicrobial activities of the amides were evaluated with the diffusion method on agar plates by measuring the zone of inhibition against selected test microorganisms *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Pseudomonas aeruginosa*, while antifungal activity was assessed against *Candida tropicalis*. The amides (**2** and **3**) exhibited activity against all microorganisms and the yeast evaluated. This activity has not been reported prior to this work.

Key words: *Passiflora punctata*; cyclopentanoid cyanogenic glycosides; passibiflorin; α-hydroxyamides and α-β-D-glucopyranosyloxyamides; antibacterial activity

Resumen

Un glicósido cianogénico, passibiflorina (**1**) y dos amidas primarias, (1*S*,1*R*)-1-hidroxi-4-(6-desoxi-β-D-glucopiranosiloxi)-2-ciclopenteno-1-carboxamida (**2**) y (1*S*,4*R*)-1-(β-D-glucopiranosiloxi)-6-desoxi-β-D-gulopiranosiloxi)-2-ciclopenteno-1-carboxamida (**3**) fueron aislados de las hojas de *Passiflora punctata* L. También se encontraron los carbohidratos: D-antiarosa (**4**), glucosa (**5**) y sacarosa (**6**). Las estructuras químicas fueron establecidas mediante el análisis de los espectros de IR, RMN y EM y comparando los datos obtenidos con los reportados en la literatura. Todos los compuestos son reportados por primera vez en la planta. También se evaluó la actividad antibacteriana de las amidas utilizando el método de difusión en placas de agar midiendo el halo de inhibición. Los compuestos 2 y 3 fueron activos contra los microorganismos *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* y *Pseudomonas aeruginosa* y la levadura *Candida tropicalis*. Esta actividad no había sido previamente reportada.

Palabras claves: *Passiflora punctata*; glicósidos cianogénicos pentacíclicos; passibiflorina; α-hidroxiamidas, α-β-D-glucopiranosiloxiamidas; actividad antibacteriana

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Introduction

The family Passifloraceae has long been documented as being cyanogenic. Several species of the genus *Passiflora* have previously been reported to contain cyclopentanoid cyanogenic glycosides. A subclass of these glycosides contains an additional sugar residue. Passibiflorin belongs to this group (Jaroszewski *et al.*, 2002). Moreover, α-hydroxy- and α-glucopyranosyloxyamides corresponding to the cyanogenic glycosides have been found to occur in the genus *Passiflora*. These amides are considered artifacts formed during processing of the plant material (Jaroszewski *et al.*, 1987; Olafsdottir *et al.*, 1989; Adsersen *et al.*, 1993; Nahrstedt and Rockenbach, 1993; Jaroszewski *et al.*, 2002; Hungeling *et al.*, 2009). The species *Passiflora punctata* L., native of Panamá, Colombia, Ecuador, Perú and Bolivia, grows in dry and humid areas between 50 and 1750 m (Kingma and Moerman, 2001). This twining vine has trilobed leaves, much wider than long and produces small black fruits of 3-4 cm diameter (Van den Eynden *et al.*, 1999). In this work we report on the isolation of passibiflorin and their corresponding α-hydroxy- and α-glucopyranosyloxyamides from the leaves of *P. punctata*, as well as antimicrobial and antifungal activity of these amides.

Material and method

The leaves of *P. punctata* L. were collected in San Diego de Los Altos, Edo. Miranda, as cultivated material maintained from seed collected in Lima, Perú, in 1967 (Tillett 674-378, and 985-7); voucher specimens are deposited in the Herbarium "Víctor Manuel Ovalles" (School of Pharmacy, Universidad Central de Venezuela) under the accession numbers 7408 and 19989, respectively.

Solvents used: methanol (Merck), n-hexane (Burdick & Jacson), chloroform (Riedel de Haën), dichloromethane (Merck), CD_3OD (Aldrich) and D_2O (Aldrich). Analytical TLC was conducted on chromatoplates with silica gel 60 F_{254} (20 x 20 cm, 0.25 mm, Merck). Solvent system used for TLC: CH_3Cl-MeOH (75:25). The spots were visualized with p-anisaldehyde-sulfuric acid reagent (0.5 mL p-anisaldehyde (Sigma) mixed with 10 mL glacial acetic acid (Merck), followed by 85 mL methanol (Merck) and 5 mL concentrated sulphuric acid (Merck), in that order). The TLC plate was sprayed with about 10 mL, heated at 100°C for 5-10 min, then evaluated in VIS (Wagner *et al.*, 1984). Column chromatography was performed with Sílicagel 60 F_{254}, (0.2-0.5 mm; 35-70 mesh ASTM, Merck) and Sephadex LH-20 (Pharmacia). 1- and 2-D NMR spectra (^1H-^1H COSY, HETCOR and FLOCK) were recorded on an Eclipse-JOEL (270 MHz) spectrometer. Chemical shifts were reported in ppm (δ), and coupling constants were reported in Hz. EIS-MS spectra were recorded with a Perkin-Elmer API 100 spectrometer. The IR spectra were recorded on a 470 Shimadzu spectrometer.

Isolation of compound **1**

The fresh cut leaves (33 g) of *P. punctata* were extracted by maceration with EtOH (600 ml) for 8 days. The solvent was removed by evaporation under reduced pressure to give a crude extract (3 g) which was examined with silica gel TLC. The extract was re-dissolved in a MeOH-H_2O (9:1) mixture and partitioned with n-hexane. The MeOH-H_2O phase was adjusted with water to a 1:1 ratio, and extracted with CH_2Cl_2. The MeOH-H_2O (1:1) phase was concentrated under reduced pressure to a solvent-free residue (2.35 g). The residue was subjected to

silica gel (90 g) column chromatography and eluted with a CH_2Cl_2-MeOH gradient (8:2, 7:3, 6:4 v/v). The fractions (60 x 20 mL) were collected and monitored by silica gel TLC. The similar fractions were pooled. The fractions 22-26, 13-15, 27-31 and 35-41 were concentrated under reduced pressure, re-chromatographed on Sephadex and eluted with methanol to obtain compound **1** (28 mg), antiarose (24 mg), α- and β-glucose (40 mg) and sucrose (43 mg), respectively.

Passibiflorin [(1S,4R)-1-(β-D-glucopyranosyloxy)-4-(6-deoxy-β-D-gulopyanosyloxy)-2-cyclopentene-1-carbonitrile] (**1**). Pale yellow syrup (28 mg). Amber brown spot, R_f = 0.52. ^{1}H-NMR δ ppm (270 MHz, CD_3OD): **Table I**. ^{13}C-NMR δ ppm (67.9 MHz, CD_3OD): **Table II**.

D-Antiarose (6-desoxy-β-D-gulopyranose), (6-desoxy-β-D-gulose), (β-D-gulomethylose) (**4**). Pale yellow syrup (24 mg). Yellow spot, R_f = 0.70. ^{1}H-NMR δ ppm (270 MHz, CD_3OD): 4.75 (H-1, d, 8.2 Hz), 4.00 (H-5, dq, 6.5, 1.5 Hz), 3.94 (H-3, t, 3.2, 1.4 Hz), 3.50 (H-2, dd, 8.3, 2.6 Hz), 3.44 (H-4, d, 3.5, Hz) y 1.18 (3H-6, d, 6.5, Hz). ^{13}C-RMN δ ppm (67.9 MHz, CD_3OD): 94.4 (C-1), 72.4 (C-4), 72.1 (C-3), 69.5 (C-2), 68.8 (C-5), 15.0 (C-6).

α- and β-D-Glucose (α- and β-D-glucopyranose)(**5**). Pale yellow syrup (40 mg). Gray spot, R_f = 0.35. ^{1}H-NMR δ ppm (270 MHz, CD_3OD): 5.09 (H-1α, d, 4.3 Hz), 4.46 (H-1β, d, 7.6 Hz), 3.86-3.60 (m), 3.38-3.26 (m), 3.12 (H-2β, t, 16.5, 8.15 Hz). ^{13}C-NMR δ ppm (67.9 MHz, CD_3OD): 96.8 (C-1β), 92.6 (C-1α), 76.7 (C-5β), 76.6 (C-3β), 74.9 (C-2β), 73.5 (C-3α), 72.5 (C-2α), 71.6 (C-5α), 70.5 (C-4α), 70.4 (C-4β), 61.5 (C-6α), 61.4 (C-6β). ^{1}H-RMN δ ppm (270 MHz, D_2O): 3.88-3.64 (m), 3.53-3.31 (m), 3.20 (1H, t, 16.0, 8.04 Hz). ^{13}C-NMR δ ppm (67.9 MHz, D_2O): 96.3 (C-1β), 92.5 (C-1α), 76.3 (C-5β), 76.2 (C-3β), 74.6 (C-2β), 73.2 (C-3α), 72.0 (C-2α), 71.8 (C-5α), 70.1 (C-5α, C-4α), 61.2 (C-6α), 61.1 (C-6β).

Sucrose (α-D-glucopyranosyl-(1→2)-β-D-fructofuranoside)(**6**). Crystalline powder (43 mg). Gray spot, R_f = 0.19. ^{1}H-NMR δ ppm (270 MHz, CD_3OD): 5.39 (H-1´, m), 4.11-3.29 (m). ^{13}C-NMR δ ppm (67.9 MHz, CD_3OD): 104.0 (C-2´), 92.3 (C-1), 82.4 (C-5´), 78.0 (C-3´), 74.5 (C-4´), 73.4 (C-3), 73.0 (C-5), 71.9 (C-2), 70.1 (C-4), 62.8 (C-6´), 62.0 (C-1´), 61.0 (C-6). Signals of compounds **4**, **5** and **6** were assigned by comparing the spectroscopic data with those reported in the literature (Olafsdottir *et al.*, 1989; Neil, 2007; Roslund *et al.*, 2008).

ISOLATION OF COMPOUNDS **2** AND **3**.

Leaves (50 g) were dried (oven) at 55°C (72 h), milled and extracted by Soxhlet (24 h) with EtOH (600 mL). The solvent was removed as above. The extract (2 g) was chromatographed on a silica gel (100 g) column and eluted with CH_2Cl_2-MeOH gradient (8:2, 6:4, 4:2, 2:8 v/v). The fractions (60 x 20 mL) were collected and monitored as above. The analogous fractions were united. The fractions 13-19 and 48-54 were concentrated and re-chromatographed on Sephadex and eluted with methanol to yield compounds **2** (123.6 mg) and **3** (82 mg), respectively.

(1S,1R)-1-hydroxy-4-(6-deoxy-β-D-gulopyranosyloxy)-2-cyclopentene-1-carboxamide (**2**). Pale yellow syrup (123.6 mg). Amber brown spot, R_f = 0.77. IR cm-1: 3680-3120 (s), 1670 (s), 1587 (m). ^{1}H-NMR δ ppm (270 MHz, CD_3OD): **Table I**. ^{13}C-NMR δ ppm (67.9 MHz, CD_3OD): **Table II**. MS (m/z, relative intensity (%)): 288 (100), 324 (65), 351 (45).

Table I

^{1}H-NMR (270 MHz) spectral data for compounds 1-3 in CD_3OD, δ in ppm (multiplicities, *J* in Hz)

Moiety	Proton	Compounds		
		1	2	3
Cyclopentanoid	H-2	6.14	5.77	5.97
		(dd, 5.6, 0.9)	(dd, 5.6, 1.0)	(dd, 5.5, 1.2)
	H-3	6.35	6.10	6.19
		(dd, 5.6, 1.9)	(dd, 5.6, 2.0)	(dd, 5.5, 2.0)
	H-4	4.92	4.91	4.94
		(m)	(s)	(s)
	H-5	2.45	1.82	2.10
		(dd, 14.8, 3.9)	(dd, 13.6, 4.4)	(dd, 14.3, 3.7)
		2.96	2.78	2.83
		(dd, 14.8, 7.1)	(dd, 13.6, 7.1)	(dd, 14.3, 7.1)
Glucose	H-1´	4.59	-	4.49
		(d, 7.6)		(d, 7.6)
	H-2´-H-5´	3.2-3.34	-	3.2-3.4
		(m)		(m)
	H-6´	3.62	-	3.61
		(dd, 12.1, 4.4)		(dd, 12.0, 5.6)
		3.82		3.78
		(dd, 12.1, 2.0)		(dd, 12.0, 2.4)
Antiarose	H-1´´	4.67	4.67	4.68
		(d, 8.1)	(d, 7.9)	(d, 8.1)
	H-2´´	3.52	3.56	3.57
		(dd, 8.1, 3.2)	(dd, 8.2, 3.5)	(dd, 8.1, 3.4)
	H-3´´	3.95	3.97	3.96
		(t, 3.2, 1.5)	(t, 3.2, 1.4)	(t, 3.4, 1.6)
	H-4´´	3.45	3.47	3.47
		(d, 3.4)	(dd, 3.5, 1.3)	(d, 3.6)
	H-5´´	4.02	4.01	4.04
		(dq, 6.6, 1.3)	(dq, 6.6, 1.3)	(dq, 6.6, 1.3)
	H-6´´	1.22	1.21	1.22
		(d, 6.4)	(d, 6.2)	(d, 6.6)

(1S,4R)-1-(β-D-glucopyranosyloxy)-4-(6-deoxy-β-D-gulopyranosyloxy)-2-cyclopentene-1-carboxamide (**3**). Pale yellow powder (82 mg). Amber brown spot, R_f = 0.23. IR cm-1: 3632-3104 (s), 1675 (s), 1590 (m). ^{1}H-NMR δ ppm (270 MHz, CD_3OD): **Table I**. ^{13}C-NMR δ ppm (67.9 MHz, CD_3OD): **Table II**. MS [m/z, relative intensity (%)]: 450 (100), 486 (43), 513 (32).

Detection of cyanogenic glycosides

Cyanogenic glycoside was detected using the technique of the picrate-impregnated paper (Castro and Rodríguez, 2012). Strips (10 x 1 cm) of filter paper (Whatman No. 1) were cut and immersed in a solution 1% of picric acid (Merck), and allowed to dry in a dark environment. The strips adquired a deep yellow color. One of these strips was fixed with adhesive tape at the center

Table II
^{13}C-NMR (67.9 MHz) spectral data for the compounds 1-3 in CD_3OD, δ in ppm

Moiety	Carbon	Compounds		
		1	2	3
Cyclopentanoid	C-1	80.5	84.4	91.6
	C-2	131.2	135.6	133.6
	C-3	140.5	136.9	138.8
	C-4	80.5	82.3	81.7
	C-5	47.3	44.8	41.5
	$CONH_2$	-	178.8	176.5
	CN	118.9	-	-
Glucose	C-1´	99.2	-	97.8
	C-2´	73.3	-	73.4**
	C-3´	76.6*	-	76.3**
	C-4´	70.0*	-	70.1**
	C-5´	76.7*	-	76.7**
	C-6´	61.3	-	61.1
Antiarose	C-1´´	100.4	100.0	100.1
	C-2´´	68.0	68.2	68.1
	C-3´´	71.9	72.0	72.0
	C-4´´	72.2	72.2	72.2
	C-5´´	68.9	68.9	68.9
	C-6´´	14.9	15.0	15.0

*, ** : Signals are interchangeable

of the top of a narrow mouth flask. Fresh leaves were carefully cut (1-3 mm), and then crushed in a mortar so that there is rupture of cell walls. The processed leaves were placed slightly compressed into the flask, filling up a third of the volume and covered to prevent leakage of hydrocyanic gas. Then two drops of a solution 10% of sodium carbonate (Riedel de Haën) was added to the strip fixed to the cap. The flask was covered, making sure that the strip did not touch the plant material or walls of the flask. Then the flask was placed in a water bath at 40 °C for 30-40 min until a color change of the paper from yellow to red was observed.

ANTIMICROBIAL ACTIVITY

The disc-diffusion method was employed for the determination of the antimicrobial activity of compounds **2** and **3** (Janssen *et al.*, 1987). Four bacteria, *Staphylococcus aureus* (ATCC 25963), *Bacillus cereus* (ATCC 14579), *Escherichia coli* (ATCC 35218) and *Pseudomonas aeruginosa* (ATCC 10145) and one yeast, *Candida tropicalis* (MLDM 345611) were chosen as test organisms. Strains were grown on LB agar plates (Sambrook *et al.*, 1989) and adjusted to 0.5 McFarland standards with NaCl (Merck) solution (0.85% v/v). An aliquot (100 µL) of the bacterial and yeast suspension was spread on Mueller-Hinton agar (Merck 1.05437) and YPD agar (Sigma-Aldrich), respectively. Discs (5 mm diameter, Macherey-Nagel) were impregnated with 7 µL of compounds (50 mg/mL in H_2O/EtOH/MeOH (50:30:20)) and placed on the inoculated agar. Amikacin (30 µL),

Table III
Antimicrobial activity (expressed as zone of inhibition) of amides 2 and 3 against selected microbial strains

Compounds (5mg/mL)	Zone of inhibition (mm)				
	Test microorganism				
	Bacillus cereus	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Candida tropicalis*
2	13	15	12	14	12
3	12	12	14	16	12
Controls					
Amikacin (30mg)	15	16	18	15	16
Ampicilin/ Sulbactam (20mg)	16	--	16	12	--
Ofloxacin (5mg)	24	22	24	22	20
Tilmicosin (15mg)	28	26	29	18	23
Polimixin B (300 UI)	--	--	--	--	--
Solvents	--	--	--	--	--

-- : no zone of inhibition

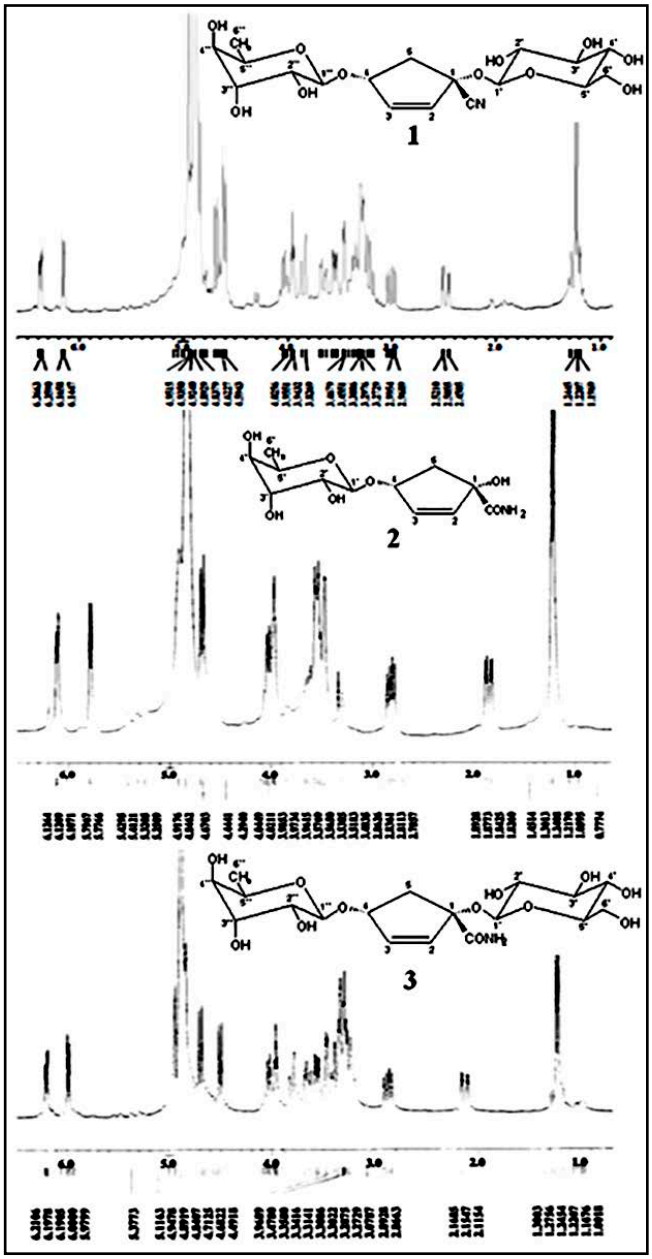

Figure 1. 1H-NMR spectra of compounds **1-3**

ampicilin-sulbactam (20 µL), ofloxacin (5 µL), tilmicosin (15 µL) and polimixin B (300UI) (BBL-Sensi-Disc) were used as positive reference standards. Negative controls were prepared using the same solvents. The inoculated plates were incubated at 30°C for *Bacillus cereus*, *Staphylococcus aureus* and *Candida tropicalis*, and at 37°C for *Escherichia coli* and *Pseudomonas aeruginosa*. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test microorganisms. The assay was performed in triplicate and average values reported.

Results and Discussion

P. punctata, was found to be cyanogenic using the technique of the picrate-impregnated paper (Castro and Rodríguez, 2012). Extraction and fractionation of fresh leaves in the usual way yielded the compounds: a cyanogenic glycoside, passibiflorin (**1**) and the carbohydrates, D-antiarose, α- and β-D-glucose and sucrose. ^{1}H-NMR spectrum of **1** (**figure 1**) showed the signals consistent with the cyclopentanoid moiety. Two vinyl proton doublets centred at δ 6.35 (1H, *J* = 5.6, 1.9 Hz, H-3) y 6.14 (1H, *J* = 5.6, 0.9 Hz, H-2), their coupling constant indicates an asymmetrically substituted five membered ring; a multiplet at δ 4.92 (1H) arises from the proton on the oxygen bearing carbon (C-4), and two further pairs of doublets centred at δ 2.96 (1H, *J* = 14.8, 7.1 Hz, H-5 b) and 2.45 (1H, *J* = 14.8, 3.9 Hz, H-5a), assigned to the nonequivalent geminal protons (C-5) of the cyclopentene ring. The spectrum also showed the spin systems of the β-D-glucopyranosyloxy unit: δ 4.59 (1H, d, *J* = 7.6 Hz, H-1´), 3.82 (1H, dd, *J* = 12.1, 2.0, Hz, H-6b´), 3.62 (1H, dd, *J* = 12.1, 4.4 Hz, H-6a´), 3.34-3.20 (4H, m, H-2´, H-3´, H-4´, y H-5´), and of the second sugar moiety correspond to the 6-desoxy-β-D-gulopyranosyl (antiarosyl) group: δ 4.67 (1H, d, *J* = 8.1 Hz, H-1´´), 4.02 (1H, dq, *J* = 6.6, 1.3 Hz, H-5´´), 3.95 (1H, t, *J* = 3.2, 1.5 Hz, H-3´´), 3.52 (1H, dd, *J* = 8.1, 3.2 Hz, H-2´´) 3.45 (1H, d, *J* = 3.4, Hz, H-4´´) y 1.22 (3H, d, *J* = 6.4, Hz, H-6´´). ^{13}C-NMR spectrum (**figure 2**), DEPT 135 and the HETCOR experiment revealed the existence a cyano group (δ 118.9, C-1), and confirm the presence of the β-D-glucopyranose (97.8, 73.4, 76.3, 70.1, 76.7 and 61.1) and a β-D-antiarose

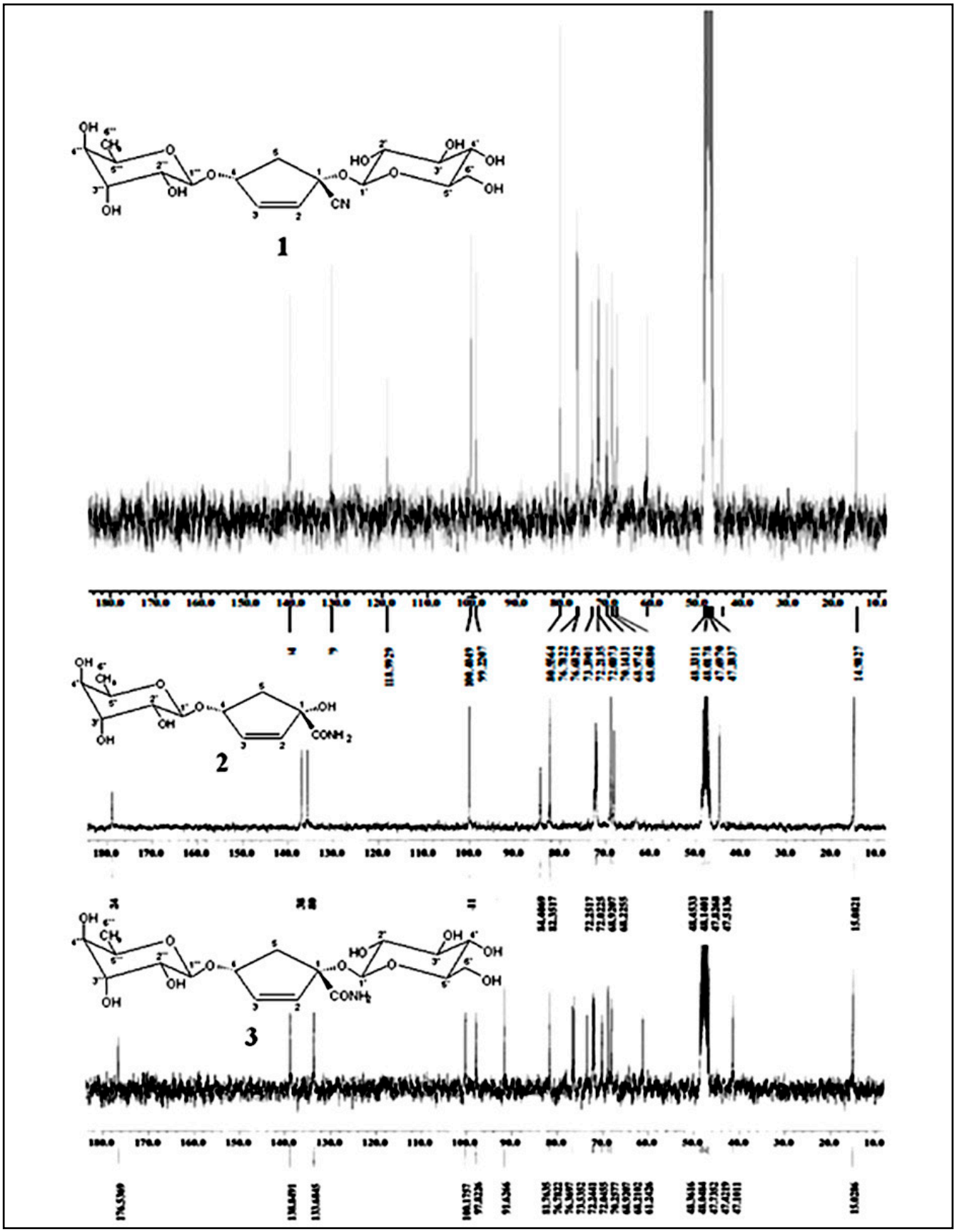

Figure 2. ^{13}C-NMR spectra of compounds **1-3**

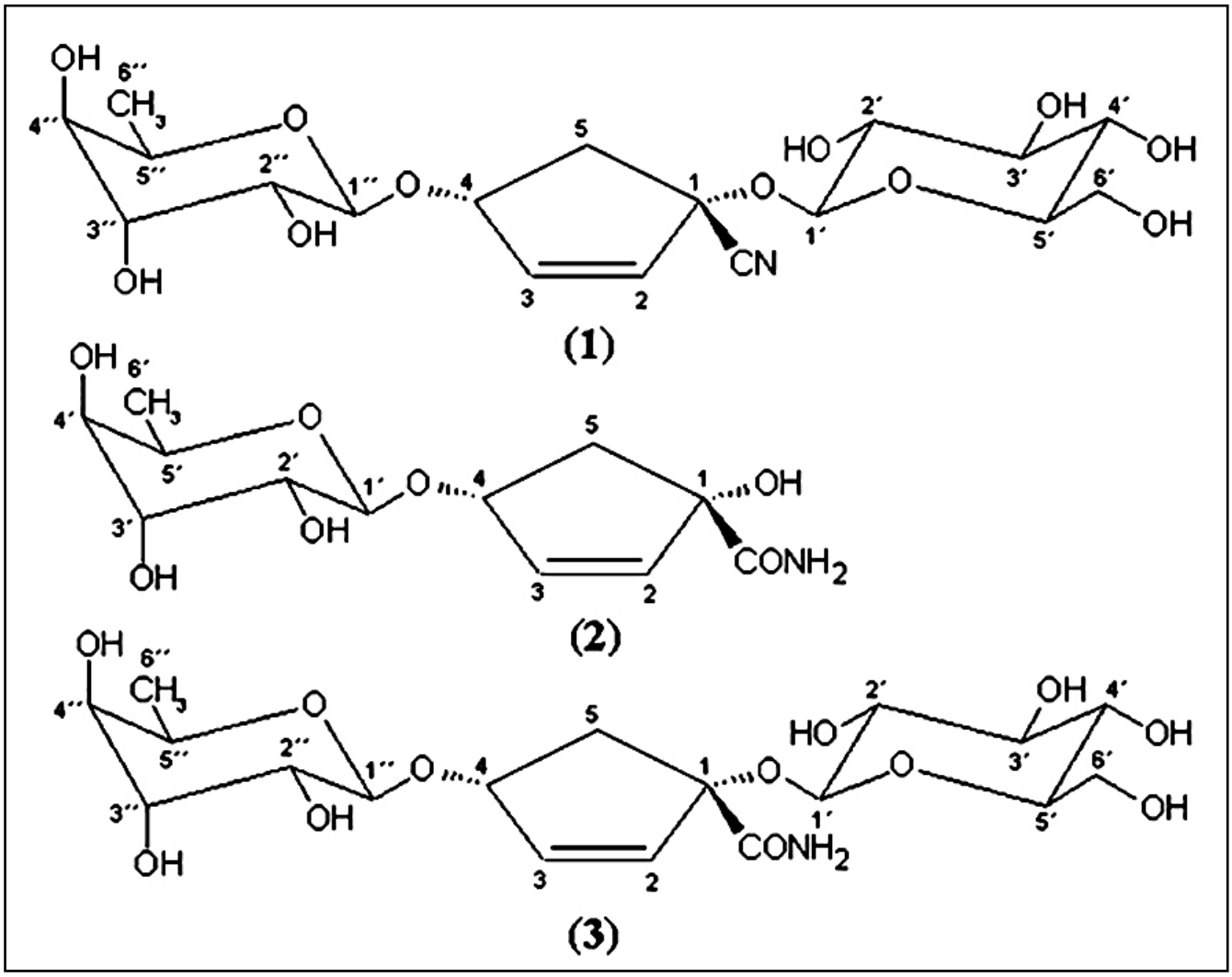

Figure 3. Structures of compounds 1-3

moiety (100.4, 68.0, 71.9, 72.2, 68.9 and 14.9). The previous spectral data (**Tables I** and **II**) were identical to published spectra for passibiflorin (Olafsdottir *et al.*, 1989) confirming that the cyanogen detected in *P. punctata* foliar tissue was the cyclopentanoid cyanogenic glycoside (1*S*,4*R*)-1-(β-D-glucopyranosyloxy)-4-(6-deoxy-β-D-gulopyanosyloxy)-2-cyclopentene- 1-carbonitrile, known as passibiflorin (**1**) (**figure 3**) .

The cyanogenic glycoside passibiflorin was previously found in species belonging to the sections Decaloba, Murucuja and Pseudomurucuja (Kingma and Moerman, 2001). *P. punctata*, as the species *P. biflora*, *P. colinvauxii*, *P. cuneata* and *P. talamancensis*, is a member of the section Decaloba, series Punctatae (Adsersen *et al.*, 1993, Kingma and Moerman, 2001). This is of taxonomic significance as it confirms their relationship as sister species.

After extraction of dry leaves from P. *punctata*, two compounds (**2** and **3**) were isolated with ^{1}H-NMR spectra similar to passibiflorin. However, the proton spectrum of **2** (**figure 1**) did not show the resonance belonging to β-D-glucopyranosyloxy unit. The spin connectivity patterns were readily distinguishable in a COSY spectrum (**figure 4**). Moreover, the ^{13}C-NMR spectrum (**figure 2**), DEPT 135 and the HETCOR revealed the presence

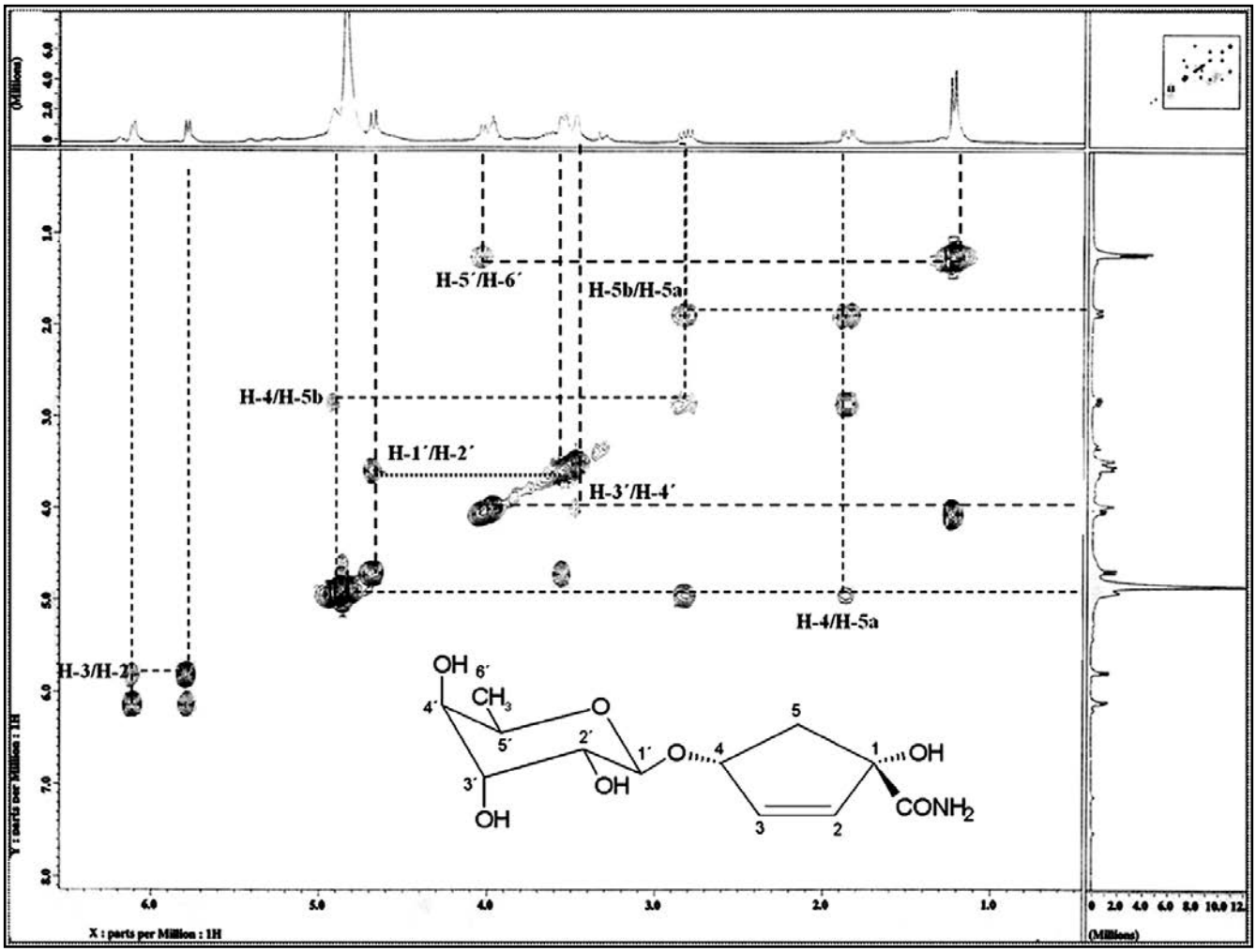

Figure 4. ^{1}H-1H-COSY spectrum of compound **2**

of an amide group at δ 178.8, which was confirmed by IR data (υ_{max} 1673 cm^{-1}). It also displayed long range correlations in the ^{13}C ^{1}H FLOCK (**figure 5**) of the signal at δ 178.8 with the signal at δ 1.82 (H-5), of the signal at δ 136.9 with the signals at δ 5.77 (H-2) and 2.78 (H-5), of the signal at δ 135.6 with the signal at δ 6.10 (H-3), δ 84.4 with the signals at δ 5.77 (H-2) and 1.82 (H-5), of the signal at δ 82.3 with the signals at δ 6.10 (H-3), 5.77 (H-2), 4.67 (H-1´) and 1.82 (H-5), These correlations indicate the cyclopentanoid moiety. The signal 100.0 is correlated with the signals at δ 3.97 (H-3´´) and 3.56 (H-2´´) and the signals at δ 68.9 and 15.0 with the signals at δ 1.21 (H-6´´) and 4.01 (H´5´´), respectively. These signals correspond to the sugar unit. The ESI mass spectrum of **2** revealed an $(M-H)^{+}$ ion peak at m/z 288, suggesting M_r = 289 and solving for $C_{12}H_{14}NO_7$. Based on the preceding data (**Tables I** and **II**) and comparison of the data given in the literature (Jaroszewski *et al.*, 1987 and 2002), the structure of compound **2** was identified as (1*S*,1*R*)-1-hydroxy-4-(6-deoxy-β-D-gulopyranosyloxy)-2-cyclopentene-1-carboxamide (**figure 3**).

The ^{13}C-NMR spectrum of **3** (**figure 2**) also showed the resonance of an amide group at δ 176.5. The absorption band at 1673 cm^{-1} in IR spectrum confirmed the presence of this group. The ESIMS of **3** proved an $(M-H)^{+}$ ion peak at m/z 450, suggesting M_r = 451 and solving for

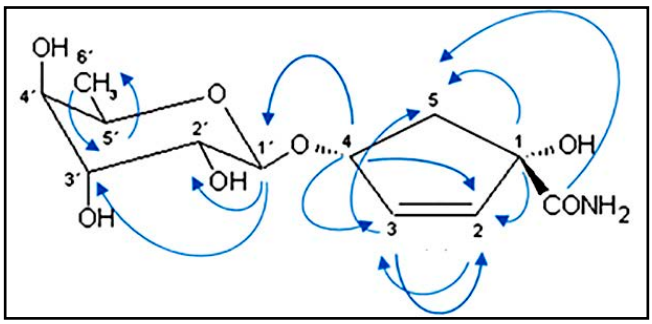

Figure 5. FLOCK correlations of compound **2**

$C_{18}H_{29}NO_{12}$. The structure of **3** was identified as (1*S*,4*R*)-1-(β-D-glucopyranosyloxy)-4-(6-deoxy-β-D-gulopyranosyloxy)-2-cyclopentene-1-carboxamide (**figure 3**) on the basis of comparison of the above data (**Tables I** and **II**) with those given in the literature (Adsersen *et al.*, 1993).

Similar α-hydroxy- and α-glucosyloxyamides have been reported as artifacts formed from the respective cyanogenic glycoside (α-glycosyloxynitriles). They are considered a consequence of enzymatic hydration of the nitrile group to the amide during sample processing, mostly during the drying of fresh plant material (Jaroszewski *et al.*, 1987; Olafsdottir *et al.*, 1989; Adsersen *et al.*, 1993; Nahrstedt and Rockenbach, 1993; Jaroszewski *et al.*, 2002; Hungeling *et al.*, 2009). The primary amides **2** and **3** were not detected in the fresh leaves of *P. punctata*. These are most probably formed during the drying process from passibiflorin (**1**), which was isolated only in fresh leaves. These results agree with those previously mentioned in the literature.

However, Nahrstedt and Rockenbach (1993) who identified prunasin together with prunasin amide in the air-dried leaves of *Olinia ventosa*, argued that the detected amide was not an artificial product, in view of its high amount and the conservation of its stereochemistry. Similarly, Picmanova *et al.* (2015) indicated that artificial hydrolysis of nitriles to amides during processing can be ruled out, as it requires strong acidic or basic conditions that could not have been provided during extraction in 85% methanol. Moreover, all enzymatic activities are inhibited by snap freezing of the plant material in liquid nitrogen and by its subsequent boiling in methanol.

On the other hand, the conversion of nitriles into amides in plants can be explained either by: (a) the in vivo occurrence of a Radziszweski process, driven by the presence of hydrogen peroxide evolved upon oxidative stress such as air-drying, light exposure, senescence, or as a result of the oxidative burst caused by a fungal infection; or by (b) the endogenous turnover of cyanogenic glucosides catalysed by specific turnover enzymes probably bifunctional nitrilase / nitrile hydratase enzymes that can form amides as major products from nitriles. The formation of amides might be followed by the enzymatic hydrolysis of the amide function into a carboxylic acid function, and thereby release ammonia. Such reactions are catalysed by the enzyme amidase (Sendker and Nahrstedt, 2009; 2010; Picmanova *et al.*, 2015).

The antibacterial activity of **2** and **3** was evaluated against five pathogenic organisms, *S. aureus*, *B. cereus*, *E. coli*, *P. aeruginosa* and *C. tropicalis*, using the diffusion method on agar plates. The results obtained are summarized in **Table III** and are expressed as inhibition zone diameter in mm. The antibiotics used were shown to have specific activity against those microorganisms. The amides showed broad spectrum antimicrobial activity, they were active against the Gram-positive and Gram-negative bacteria and yeast. Noteworthy here is activity against *P. aeruginosa*, since it is very resistant to a large number of commercial antibiotics.

No inhibitory activity was observed by the solvent mixture. To our knowledge, this is the first report of the antibacterial activity of α-hydroxy- and α-glucosyloxyamides.

Conclusions

In this study, the cyclopentanoid cyanogenic bis-glycoside, passibiflorin (**1**) was isolated together with the carbohydrate D-antiarose (**4**), α- and β-D-glucose (**5**) and sucrose (**6**) from the fresh leaves of *Passiflora punctata*. However, two primary amides, (1*S*,1*R*)-1-hydroxy-4-(6-deoxy-β-D-gulopyranosyloxy)-2-cyclopentene-1-carboxamide (**2**) and (1*S*,4*R*)-1-(β-D-glucopyranosyl-oxy)-4-(6-deoxy-β-D-gulopyranosyloxy)-2-cyclopentene-1-carboxamide (**3**) were isolated from the plant dry leaves. These amides are possibly formed from the glycoside passibiflorin by enzymatic hydration of the nitrile group to the amide during the drying of fresh plant material. In addition, **2** and **3** showed microbial activity against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa* and antifungal activity against *Candida tropicalis*.

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References

Adsersen A, Brimer L, Olsen C, Jaroszewski J. 1993. Cyanogenesis of *Passiflora colinvauxii*, a species endemic to the Galápagos Islands. Phytochemistry 33: 365–367.

Castro A, Rodríguez M. Reacción de Guignard para detectar compuestos del ácido cianhídrico en sorgo. 2012. Available in: https://inta.gob.ar/documentos/reaccion-de-grignard-para-detectar-compuestos-del-acido-cianidrico-en-sorgo (30 may 2016)

Hungeling M, Lechtenberg M, Fronczek F, Nahrstedt A. 2009. Cyanogenic and non-cyanogenic pyridone glucoside from *Acalypha indica* (Euphorbiaceae). Phytochemistry 70: 270–277.

Janssen A, Scheffer J, Baerheim S. 1987. Antimicrobial activity of essential oils: a 1976-1986 review. Aspects of tests methods. Planta Med 53: 395–398.

Jaroszewski J, Olafsdottir E, Cornett C, Schaumburg K. 1987. Cyanogenesis of *Adenia volkensii* Harms and *Tetrapathaea tetrandra* Cheeseman (Passifloraceae) Revisited: Tetraphyllin B and Volkenin. Optical rotatory power of cyclopentenoid cyanohydrin glucosides. Acta Chem Scand B 41: 410–421.

Jaroszewski J, Olafsdottir E, Wellendorph P, Christensen J, Franzyk H, Somanadhan B, Budnik B, Jorgensen L, Clausen V. 2002. Cyanohydrin glycosides of *Passiflora*: distribution pattern, a saturated cyclopentane derivative from *P. guatemalensis*, and formation of pseudocyanogenic α-hydroxyamides as isolation artefacts. Phytochemistry 59: 501–511.

Kingma K, Moerman P. *Passiflora*: Exotische verrassingen in de Lage Landen. Terra: Warnsveld; Lannoo: Tielt. 2001. p. 214.

Nahrstedt A, Rockenbach J. 1993. Occurrence of the cyanogenic glucoside prunasin and its corresponding mandelic acid amide glucoside in *Olinia* species (Oliniaceae). Phytochemistry 34: 433–436.

Neil E. NMR spectroscopy explained simplified theory, applications and examples for organic chemistry and structural biology. John Wiley & Sons: New Jersey. 2007.

Olafsdottir E, Cornett C, Jaroszewski J. 1989. Cyclopentanoid cyanohydrins glycosides with unusual sugar residues. Acta Chem Scand B 43: 51–55.

Picmanova M, Neilson E, Motawia M, Olsen C, Agerbirk N, Gray Ch, Flitsch S, Meier S, Silvestro D, Jorgensen K, Sanchez-Perez R, Moller B, Bjarnholt N. 2015. A recycling

pathway for cyanogenic glycosides evidenced by the comparative metabolic profiling in three cyanogenic plant species. Biochem J 469: 375–389.

Roslund R, Tähtinen P, Niemitz M, Sjöholm R. 2008. Complete assignments of the ^{1}H and ^{13}C chemical shifts and J H,H coupling constants in NMR spectra of D-glucopyranose and all D-glucopyranosyl-D-glucopiranosides. Carbohydr Res 343: 101–112.

Sambrook J, Fritsch E, Maniatis T. Molecular cloning: A laboratory manual, Vol. 3, Appendix A, 2nd. ed. Cold Spring Harbor Laboratory Press: New York. 1989. pp. A.1.

Sendker J, Nahrstedt A. 2009. Generation of primary amide glucosides from cyanogenic glucosides. Phytochemistry 70(3): 388–393.

Sendker J, Nahrstedt A. 2010. Synthesis and characterisation of α-Glycosyloxyamides derived from cyanogenic glycosides. Phytochem Anal 21(6): 575–581.

Van den Eynden V, Cueva E, Cabrera O. Plantas silvestres comestibles del sur del Ecuador. Abya Yala: Quito. 1999. p. 64.

Wagner H, Bladt S, Zgainski E. Plant drug analysis. A thin layer chromatography atlas. Springer: Berlin. 1984. p. 299.

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