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A CYANOGENIC GLYCOSIDE FROM *PASSIFLORA EDULIS* FRUITS

DAVID CHASSAGNE and JEAN CROUZET*

Laboratoire de Génie Biologique et Sciences des Aliments, Unité de Microbiologie et Biochimie Industrielles, Associée à l'INRA, Université de Montpellier II, 34095 Montpellier Cedex 05, France

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Abstract—Cyanogenic β-rutinoside ((R)-mandelonitrile α-*L*-rhamnopyranosyl-β-D-glucopyranoside) was isolated from fruits of *Passiflora edulis*. Its structure was elucidated mainly by enzymatic hydrolysis using a specific enzyme, GC-mass spectrometric analysis of trifluoroacetyl derivatives, and NMR spectroscopy.

INTRODUCTION

The presence of cyanogen in purple passion fruit (*Passiflora edulis*) is well-established [1, 2]. It was first characterised as prunasin [2], and more recently, in addition to this compound, sambunigrin and amygdalin were identified in the glycosidal extracts of this fruit and other edible *Passiflora* species [3]. During the course of our study on cyanogenic glycosides, two mandelonitrile β-rutinosides were also detected in *P. edulis*, (40.4 and 10.4 mg kg^{-1}, respectively, in juice). The aim of this work was to isolate and characterise the glycoside present in purple passion fruits using chromatographic and spectroscopic techniques.

RESULTS AND DISCUSSION

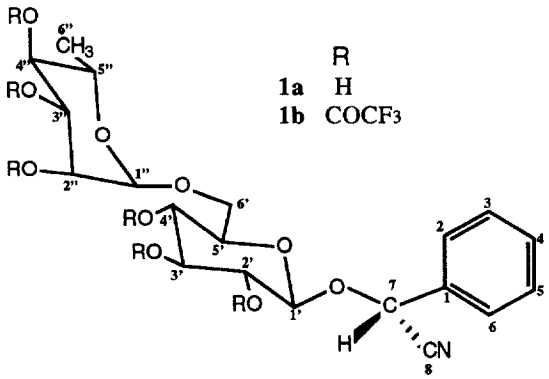
The glycosidic fraction from fruit juice was submitted to enzymatic hydrolysis with almond glucosidase. The use of this preparation, known to be free of rhamnosidase activity required for the first step of the sequential enzymatic of rutinoside hydrolysis [4], allowed the concentration of the rutinoside derivatives. The efficiency of this treatment was checked by GC-mass spectrometry after trifluoroacetylation. Liquid chromatography on XAD-2 resin and silica gel was performed to remove the aglycones and sugars released during enzymatic hydrolysis and to pre-fractionate the non-hydrolysed glycosides. Final purification of **1a**, previously tentatively identified as a mandelonitrile rutinoside [3], was achieved by semi-preparative reverse-phase HPLC, with monitoring by TLC and GC after trifluoroacetylation. TLC of **1a** showed a yellow spot at R_f 0.56 after detection with *N*-(-1-naphthyl)-ethylenediamine dihydrochloride, below prunasin at R_f 0.80, which was used as reference substance. Positive FAB mass spectrometry of **1a** gave a pseudo-molecular ion at *m/z* 442 $[M+1]^+$ together with fragments at *m/z* 309 and 147, indicating a diglycoside made up of a hexose and a deoxy-hexose. Similar information could be deduced from the NCI mass spectrum of the trifluoroacetyl derivative (**1b**), obtained by GC-MS, which showed the $[M]^+$ at *m/z* 1017 and a major ion at *m/z* 901, characteristic of a hexa-trifluoroacetylated deoxyhexosyl hexoxyl fragment [4]. GC-EI mass spectrometry [5] of **1b** gave ions at *m/z* 435, 207, 292 and 193, indicative of this saccharide moiety, and other fragments at *m/z* 133, 116 and 89, presumably arising from the aglycone. The latter fragments were identical to those obtained with prunasin tetra-trifluoroacetate, identified earlier by Chassagne *et al.* [3], suggesting the presence of the same aglycone residue, 2-phenylacetonitrile.

The ^1H NMR spectrum (Table 1) of **1a** in D_2O showed signals between 7.4 and 7.51 ppm corresponding to a monosubstituted aromatic ring system, a singlet at 5.76 ppm indicating an isolated methine proton, and except for H-1′, some signals characteristic of a 6-rhamnosyl glucosyl residue [6, 7]. The ^{13}C and 2D NMR data corroborated the occurrence of mandelonitrile rhamnosyl glucoside. The chemical shift of the anomeric carbon C-1′ at 101.9 and the coupling constant ^3J (H-1′/H-2′) of 7.8 Hz indicated a β-linkage between the glucose unit and the aglycone. Concerning the terminal *L*-rhamnose, the chemical shift of C-1″ and a small coupling constant between H-1″ and H-2″ indicated an α-linkage with the glucose unit. The chemical shift of C-7 at 69.7 ppm was reasonably similar to that given by Nahrstedt

* Author to whom correspondence should be addressed.

Table 1.

Position	^{13}C NMR	^{1}H NMR
1	132.7	—
2, 6	129.7	
3, 5	128.2	7.40–7.51 (5H, *m*)
4	130.8	
7	69.7	5.76 (*s*)
8	118.7	—
1′	101.9	4.52 (*d*, 7.8)
2′	73.0	3.24 (*dd*, 7.8, 9.4)
3′	75.3	3.08
4′	69.7	−3.36
5′	75.7	3.44 (*m*)
6′	66.7	a3.55 (*dd*, 5.7, 11.3)
		b3.87 (*dd*, 1.4, 11.3)
1″	100.9	4.7 (*d*, 1.4)
2″	70.3	3.89 (*dd*, 1.4, 3.4)
3″	70.5	3.71 (*dd*, 3.4, 9.7)
4″	72.4	3.08–3.36
5″	69.0	3.62 (*dd*, 6.3, 9.7)
6″	17.0	1.19 (*d*, 6.3)



[8] for (*R*)-prunasin (69.6 ppm in D_2O), suggesting that this carbon has an (*R*)-configuration. Partial hydrolysis of **1a**, performed using α-*L*-rhamnopyranosidase isolated from an *Aspergillus niger* pectinase preparation [9] is in favour of the presence of an α-*L*-rhamnopyranoside unit. Comparison of the GC retention times of the trifluoroacetyl derivatives of the hydrolysate with those of authentic compounds enabled identification of (*R*)-prunasin. This second result confirms the presence of the glucose unit and an (*R*)-absolute configuration for C-7. Therefore, we can conclude that **1a** is (*R*)-mandelonitrile α-*L*-rhamnopyranoyl-β-D-glucopyranoside. To our knowledge this is the first report of the occurrence of this cyanogenic glycoside in *P. edulis*.

EXPERIMENTAL

General

NMR: 400 MHz (^{1}H) and 100.6 MHz (^{13}C) in D_2O soln using TMS as ext. standard. TLC: silica gel using EtOAc–*iso*-PrOH–H_2O (12:7:2) with *N*-(1-naphthyl)-ethylendiamine dihydrochloride detection [10]. GC and GC-MS: WCOT DB-5MS fused silica capillary column (30 m × 0.25 mm i.d.; $df = 0.25$ μm) [5]. Column temp. programmed from 125° to 220° at 3° min^{-1} then increased at 2° min^{-1} up to 280° and maintained for 15 min. FID and injector temp were 250° and 280°, respectively. A split ratio of 10:1 was used. Carrier gas for GC-FID was H_2 at a flow rate of 1.8 ml min^{-1} and for GC-MS He at a flow rate of 1.4 ml min^{-1}. EIMS was performed at 70 eV and NCIMS at 200 eV with CH_4 at 80 Pa as reagent gas [4]. FAB mass spectrometry was used in the positive mode with a matrix of glycerol-thioglycerol.

Plant material

Mature purple passion fruits (*P. edulis* Sims) from Zimbabwe were purchased in France (Rungis).

Isolation and fractionation of glycosides

Fruits (6 kg) were cut and the seeds removed by pressing through a gauze. Juice and pulp were centrifuged (30 min 10,000 *g*) at 4°. The clear juice obtained was transferred onto a column (25 × 3 cm i.d.) of Amberlite XAD-2 [11]. After washing the column with 600 ml H_2O, the adsorbed materials were eluted with 600 ml pentane–CH_2Cl_2 (2:1) and 600 ml MeOH. The MeOH eluate was evapd to dryness under vacuum and the residue re-dissolved in 18 ml 0.2 M citrate-phosphate buffer (pH 5) containing 5 g l^{-1} almond glucosidase (emulsin). Partial enzymatic hydrolysis of the glycosidal extract was carried out at 40°C for 16 h. To purify the non-hydrolysed glycosides, the released sugars and aglycones were removed by chromatography on an Amerblite XAD-2 column. The new MeOH eluate was evapd to dryness and the residue taken up in the mobile phase and chromatographed on a silica gel column (26 × 2 cm i.d.) with EtOAc–MeOH (9:2), collecting 15 ml frs. The frs were monitored by TLC (R_f 0.56) and GC (*RI* 2251) under the conditions given above, and the cyanogenic material purified by semi-prep. HPLC (Kromasil BP18, i.d. 7.5 mm; mobile phase MeCN-H_2O with a gradient 15–40% MeCN for 20 min, then isocratic at 40% MeCN; flow rate 1.5 ml min^{-1}; UV) to give 72 frs (each 6 ml) with 10 injections. The new compound was present in frs 30–32.

Derivatization

Aliquots of frs selected by TLC were trifluoroacetylated in 20 μl pyridine with 20 μl of MBTFA (*N*-methyl *bis*-trifluoroacetamide) by heating at 60° for 20 min [6]. The derivatives were analysed by GC and GC-MS in EI and NCI modes, as described above.

Determination of C-7 configuration

The diastereoisomeric (*R*)- and (*S*)-mandelonitrile glucosides were shown [3] to be well-resolved under the GC conditions described above. Thus, the assignment of the (*R*)-configuration of C-7 was established by comparing authentic (*R*)-prunasin with the corresponding compound released by partial enzymatic hydrolysis of **1a**. The enzyme used was an α-*L*-rhamnopyranosidase isolated from an *Aspergillus niger* pectinase prepn [9]. The *RR's* of trifluoroacetylated derivatives of (*S*)-sambunigrin and (*R*)-prunasin (phenyl β-D-glucopyranoside as ref.) were 1.552 and 1.621, respectively.

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