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6-*O*-α-L-ARABINOPYRANOSYL-β-D-GLUCOPYRANOSIDES AS AROMA PRECURSORS FROM PASSION FRUIT

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Key Word Index—*Passiflora edulis*; Passifloraceae; passion fruit; linalyl 6-*O*-α-L-arabinopyranosyl-β-D-glucopyranoside; benzyl 6-*O*-α-L-arabinopyranosyl-β-D-glucopyranoside; 3-methyl-but-2-en-1-yl 6-*O*-α-L-arabinopyranosyl-β-D-glucopyranoside; aroma precursor.

Abstract—The 6-*O*-α-L-Arabinopyranosyl-β-D-glucopyranosides of linalool, benzyl alcohol and 3-methyl-but-2-en-1-ol were isolated from passion fruit (*Passiflora edulis*) by adsorption chromatography on XAD-2 resin, then further extracted on the same resin after partial enzymic hydrolysis and semi-preparative chromatography on RP-18 phase by HPLC. Their structures were identified by ^{1}H NMR spectroscopy and mass spectral analysis and by methylation analysis of the carbohydrate moieties.

INTRODUCTION

A considerable number of glycosidically bound volatile compounds, considered as potential aroma precursors, have been reported in various fruits and vegetables [1, 2]. The presence of aroma compounds such as glycosylated conjugates in purple passion fruit (*Passiflora edulis* Sims) have been ascertained by the release of volatile compounds [3–5] during enzymic and acid hydrolysis of a crude extract obtained after adsorption on XAD-2 resin [1].

The structure of passion fruit bound volatile components is not yet known, as only preliminary results have so far been obtained. The presence of prunasin has, however, been reported [6], and information regarding molecular weights and carbohydrate sequences have been obtained using NCI mass spectrometry of glycoside trifluoroacetylated derivatives [5].

In the present paper, we report on the isolation of the 6-α-L-arabinopyranosyl-β-D-glucopyranosides of linalool, previously reported in other plants [7, 8], benzyl alcohol and 3-methyl-but-2-en-1-ol from passion fruit juice and the consequent identifications.

RESULTS AND DISCUSSION

The passion fruit glycosidic fraction was isolated by the procedure of Günata *et al.* [1] using XAD-2 resin and methanol elution, and the total glycosidic isolate was submitted to enzymic hydrolysis with Hemicellulase REG 2. Certain glycosides were found to be resistant to hydrolysis, as observed by TLC and GC after trifluoroacetylation. These results, in agreement with previous reported data [5, 9], suggest that the enzymic preparation used did not contain the glycosidases involved in the first step of hydrolysis of these compounds [10].

A second chromatography on an Amberlite XAD-2 column was performed to remove the aglycones and sugars released during enzymic hydrolysis. The methanol fraction containing the 'resistant' glycosides was fractionated using semi-preparative reverse phase HPLC. Isolation and purification of these compounds were monitored by GC after trifluoroacetylation of selected fractions, enabling three unknown glycosides to be detected in fractions 30, 32–34, and 56, 57 3-Methyl-but-2-en-1-ol, benzyl alcohol and linalool, released respectively from these fractions by the use of sweet almond glucosidase preparation (emulsin), were identified by GC–mass spectrometry. It has been previously reported that whereas arabinopyranosidase activity is present in sweet almond glucosidase, it is not present in hemicellulase REG 2 [5].

Useful information concerning the sugar and aglycone moieties was obtained following trifluoroacetylation. Indeed, in the EI mass spectrum, three compounds exhibited the same characteristic fragment ions at *m/z* 193 and 421 (tri-trifluoroacetylated pentosyl fragment and subsequent loss of two trifluoroacetoxy groups OTFA), and at *m/z* 319 (di-trifluoroacetylated hexosyl fragment)

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indicative of the presence of a pentosyl hexoside moiety [11]. This disaccharidic moiety was confirmed by NCI mass spectrometry; the presence of ions at *m/z* 887 and 437 were specific to this trifluoroacetylated sugar moiety [12]. Furthermore, M_rs of 956 (**3b**), 978 (**2b**) and 1024 (**1b**) were obtained for the derivatized glycosides, corresponding to aglycones with M_rs of 86, 108 and 154, respectively, were obtained by NCI mass spectrometry.

GC and GC–mass spectral analysis of partially methylated alditol acetates obtained from the three purified glycosides revealed the formation of 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methylhexitol with a 1,5-di-*O*-acetyl-2,3,4,-tri-*O*-methylpentitol in a molar ratio 1:1. Final identification by co-chromatography with authentic reference compounds suggested the presence of a terminal arabinopyranoside unit 1,6-linked to a glucopyranoside unit in the three isolated glycosides. This hypothesis was confirmed by NMR. As reported for natural products [7, 8], it has been postulated that glucopyranose and arabinopyranose in these glycosides have, respectively, D and L configurations.

The ^{1}H NMR spectrum of **1a** (Table 1) indicates the presence of three methyl groups [δ 1.36, 1.60 and 1.65 (each 3H)], four olefinic protons [δ 5.17, 5.26, 5.27, 5.87 (each 1H)] and two anomeric protons [δ 4.50 (1H, *d*, J = 8.0 Hz) and 4.35 (1H, *d*, J = 7.5 Hz)]. These ^{1}H NMR signals are in good agreement with those reported by Watanabe *et al.* [8] for (*R*)-linalyl 6-*O*-α-L-arabinopyranosyl-β-D-glucopyranoside (**1a***) isolated from *Gardenia jasminoïdes* flowers. The chirality of linalool released from **1a** was determined to be (*S*) by chiral-GC (Cyclodex-B). The structure of **1a** was thus determined to be (*S*)-linalyl 6-*O*-α-L-arabinopyranosyl-β-D-glucopyranoside, this compound also having been identified in raspberry fruit [7].

The ^{1}H NMR spectrum of **2a** revealed the existence of aromatic protons [δ 7.43 (5H)] and two aliphatic protons [δ 4.91 (1H) and 4.73 (1H)] corresponding to the benzyl aglycone moiety, and two anomeric protons [δ 4.51 (1H) and 4.38 (1H)]. The ^{1}H and ^{13}C NMR spectra indicated the presence of one β-glucopyranosyl unit [H-1′: δ 4.51 (*d*, J = 8.1 Hz), C-1′: δ 102.1] and one α-arabinopyranosyl unit [H-1″: δ 4.38 (*d*, J = 7.5 Hz), C-1″: δ 104.4] (Tables 1 and 2). Thus, compound **2a** was benzyl 6-*O*-α-L-arabinopyranosyl-β-D-glucopyranoside. The ^{13}C NMR data recorded for the sugar moiety in **2a** were coincident with those provided by Mizutani *et al.* [13] for α-L-arabinopyranosides, confirming our hypothesis that

Table 1. ^{1}H NMR data for compounds **1a**, **2a** and **3a**

H	**1a*** (D_2O, 400 MHz)	**1a** (D_2O, 500 MHz)	**2a** (D_2O, 500 MHz)	**3a** (D_2O, 500 MHz)
Aglycone				
1a	5.29 *br d* (17.6)	5.26 *d* (17.5)		4.32 *dd* (7.1, 12.4)
1b	5.28 *br d* (11.0)	5.27 *d* (11.0)	ca	4.23 *dd* (7.0, 12.4)
2	6.01 *dd* (17.6, 11.0)	5.85 *dd* (17.5; 11.0)		5.35 *m*
3				
4	1.69–1.66	1.63–1.58	7.43	1.75 *s*
5	2.08–2.02 *m*	1.93–2.02 *m*		1.68 *s*
6	5.22 *br t* (7.0)	5.17 *br t* (7.1)	*m*	
7a			4.91 *d* (11.9)	
7b			4.73 *d* (11.9)	
8	1.70 *s*	1.65 *s*		
9	1.63 *s*	1.60 *s*		
10	1.36 *s*	1.36 *s*		
Glucose				
1′	4.53 *d* (8.1)	4.50 *d* (8.0)	4.51 *d* (8.1)	4.44 *d* (8)
2′	3.23 *dd* (8.8, 8.1)	3.19 *m*	3.28 *br t* (8.8, 8.1)	3.23 *br t* (8.8)
3′	3.5–3.45 *m*	3.45–3.38 *m*	3.48–3.40 *m*	3.40–3.48 *m*
4′	3.5–3.45 *m*	3.45–3.38 *m*	3.48–3.40 *m*	3.40–3.48 *m*
5′	3.5–3.45 *m*	3.5 *m*	3.57 *m*	3.51–3.59 *m*
6′a	4.09 *br d* (11.4)	4.3 *br d* (11.0)	4.13 *dd* (11.6, 1.8)	4.12 *br d* (11.6)
6′b	3.81 *dd* (11.4, 4)	3.75 *dd* (11.0, 5.4)	3.82 *dd* (11.6, 5.6)	3.80 *dd* (11.6, 5.4)
Arabinose				
1″	4.39 *d* (7.3)	4.35 *d* (7.5)	4.38 *d* (7.5)	4.36 *d* (7.5)
2″	3.60 *dd* (9.5, 7.3)	3.55 *dd* (9, 7.5)	3.6–3.54 *m*	3.51–3.67 *m*
3″	3.68 *dd* (9.5, 3.3)	3.64 *dd* (9, 2.7)	3.62 *dd* (9.3, 3.1)	3.64 *dd* (9.1, 3.2)
4″	3.95 *m*	3.91 *m*	3.90 *m*	3.86–3.93 *m*
5″a	3.93 *dd* (12.8, 2.6)	3.88 *br d* (13.0)	3.89 *dd* (12.0, 2.4)	3.86–3.93 *m*
5″b	3.66 *br d* (12.8)	3.62 *br d* (13.0)	3.61 *br d* (12.0)	3.63 *br d* (12.0)

1a* = (*R*)-linalyl 6-*O*-α-L-arabinopyranosyl-β-D-glucopyranoside [8].

Table 2. ^{13}C NMR data for compound **2a** (50 MHz, D_2O)

C	δ (ppm)
1	137.5
2/6	129.5
3/5	129.4
4	129.2
7	72.3
1′	102.1
2′	73.8
3′	75.8
4′	70.2
5′	76.5
6′	69.1
1″	104.4
2″	71.5
3″	73.0
4″	69.0
5″	66.9

arabinopyranose has L configuration in these isolated glycosides.

The ^1H NMR spectrum of **3a** revealed the existence of two methyl groups [δ 1.68 and 1.75 (each 3H, *s*)], two aliphatic protons [δ 4.32 (1H) and 4.23 (1H)] and one olefinic proton [δ 5.35 (1H, *m*)] corresponding to the 3-methyl-but-2-en-1-yl aglycone moiety. Comparison of the signals for the sugar moiety with those obtained for glycosides **1a** and **2a** made it possible to attribute to **3a** the structure 3-methyl-but-2-en-1-yl 6-*O*-α-L-arabinopyranosyl-β-D-glucopyranoside (Table 1).

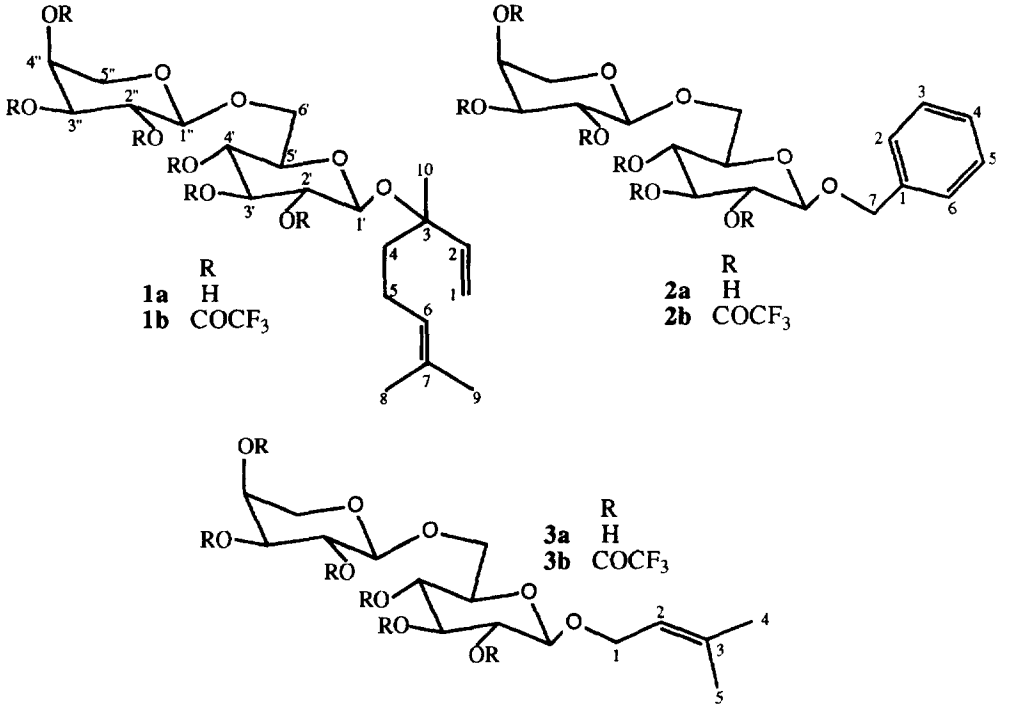
To our knowledge, **2a** and **3a** have not been previously reported as natural products. Linalool, benzyl alcohol and 3-methyl-but-2-en-1-ol have been reported as aroma compounds in passion fruit [14], so these identified glycosides could be considered to be aroma precursors of this fruit.

EXPERIMENTAL

General. NMR: 500 MHz (^1H) and 50 MHz (^{13}C) in D_2O soln using TMS as ext. standard; TLC: silica gel using EtOAc–*iso*-PrOH–H_2O (12:7:2) and detection with *N*-(1-naphthyl)-ethylenediamine dihydrochloride [15]; GC and GC-MS: DB-5MS fused silica capillary column (30 m × 0.32 mm i.d.; df = 0.25 μm) [9]. The column was programmed from 125° to 220° at 3° min^{-1} and then increased at 2° min^{-1} up to 280°. FID temp. was 250°. Injection was on-column. The injector was programmed from 90° to 280° at 60° min^{-1}, then held at this temp. for 55 min. Carrier gas was H_2 for GC-FID with a flow rate of 1.4 ml min^{-1} and He for GC-MS with a flow rate of 1.1 ml min^{-1}. EIMS was performed at 70 eV and NCIMS at 200 eV with CH_4 as reagent gas at 80 Pa [12].

Plant material. Mature purple passion fruits (*P. edulis* Sims) from Zimbabwe were purchased in France (Rungis) and were worked up immediately after arrival.

Isolation and fractionation glycosides. Passion fruits (4 kg) were cut, and the seeds removed by filtration through gauze. The juice and pulp were centrifuged (30 min, 10 000 g) at 4°, and the supernatant chromatographed on an Amberlite XAD-2 column (220 × 2.5 cm i.d.) [1]. After washing the column contents with H_2O, the adsorbed materials were eluted with azeotropic



pentane–CH_2Cl_2 (2:1) and MeOH. The MeOH elutate was concd *in vacuo* to dryness and redissolved in 40 ml 0.2 M citrate–phosphate buffer (pH 5). A partial enzymic hydrolysis of the glycosidic extract was carried out at 40° for 16 hr using 125 mg of a commercial prepn of hemicellulase REG-2 (Gist Brocades) [5]. After this time, a TLC spot (R_f 0.45) characteristic of disaccharide compounds was detected. Released sugars and aglycones were removed by CC on Amberlite XAD-2 as previously described. Unhydrolysed glycosides were thus purified on XAD-2 as for the crude glycosidic extract. The MeOH fr. was concd and subjected to semi-prep. HPLC (Kromasil BP18, i.d. 7.5 mm; eluent MeCN–H_2O with a gradient of 15–40% MeCN for 20 min, then isocratic at 40% MeCN; flow rate 1.5 ml min^{-1}; UV) to give 60 frs (each 15 ml) with 10 injections.

Enzymic hydrolysis. Almond glucosidase (emulsin) (6 mg) was added to 1/80 part of the 2nd MeOH fr. in 0.6 ml 0.2 M citrate–phosphate buffer (pH 5). Incubation was carried out at 40° for 16 hr. The released aglycones were extracted with azeotrope pentane–CH_2Cl_2 (2:1) and were subjected to conventional work-up to give an aroma concentrate. Identifications were based on RR_t and confirmed by GC-MS using a DB-5MS column [split injection (1:20); injector at 250°, over 40° increased to 200° at 2° min^{-1}; He at 1.8 ml min^{-1}].

Derivatization. Aliquots of frs selected by TLC were trifluoroacetylated in 20 μl pyridine with 20 μl *N*-methyl bis-trifluoroacetamide by heating at 60° for 20 min [11]. The derivatized compounds were analysed by GC and GC-MS in EI and NCI modes as mentioned above.

Methyl alditol acetates analysis. Frs 30, 33, and 56, 57 were methylated using the procedure of ref. [16] as described in ref. [17]. After hydrolysis with 2 M TFA, the partially methylated sugars were converted into partially methylated alditol acetates and analysed on OV-1 and OV-225 capillary columns [18]. Identifications were based on RR_t using authentic reference compounds and confirmed by GC-MS using a DB-1 column [split injection (1:20); injector at 250°, oven hold for 10 min at 145°, then programmed to 210° at 2° min^{-1}; He at 1.4 ml min^{-1}].

Chirality analysis. The chirality of linalool released from **1a** was determined by GC using a chiral column of Cyclodex-B (30 m × 0.25 mm i.d.): temp. 60° (10 min)–230°, 3° min^{-1}; H_2, 1.2 ml min^{-1}. The RR_ts of linalool (4-nonanol as ref. compound) were 1.031 and 1.026 for (*S*)- and (*R*)-linalool, respectively.

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