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Bound Terpenoids in the Juice of the Purple Passion Fruit (*Passiflora edulis* Sims)

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Enzymatic hydrolysis of C_{18} reversed-phase isolates from the juice of the purple passion fruit led to the identification of a wide range of terpenoids. In addition to the main component linalool and other monoterpenoids, the following C_{13} norterpenoid aglycons were identified for the first time in passion fruit: 4-hydroxy-β-ionol; 4-oxo-β-ionol; 4-hydroxy-7,8-dihydro-β-ionol; 4-oxo-7,8-dihydro-β-ionol; 3-oxo-α-ionol; isomeric 3-oxoretro-α-ionols; 3-oxo-7,8-dihydro-α-ionol; 3-hydroxy-1,1,6-trimethyl-1,2,3,4-tetrahydronaphthalene; vomifoliol; dehydrovomifoliol.

About 150 volatiles are known as purple passion fruit constituents, with esters and terpenoids being the most abundant classes of flavor components (Whitfield and Last, 1986). Among the terpenoids numerous monoterpenes have been found as degradation products of nonvolatile precursor compounds such as hydroxylated linalool derivatives (polyols) as well as monoterpene glycosides (Engel and Tressl, 1983). Similar pathways have been suggested for the generation of the carotenoid-derived C_{13} norterpenoids, the second group of passion fruit terpenoids. But the natural precursors of these components, including the strongly odoriferous megastigma-4,6,8-trienes and isomeric edulans, key flavor components of the purple variety, remained unknown. The present work reports for the first time the occurrence of glycosidically bound C_{13} norterpenoids in the juice of the purple passion fruit and discusses their possible role as flavor precursors.

EXPERIMENTAL SECTION

Fruits. Fresh ripe purple passion fruit (*Passiflora edulis* Sims) was available from the local market in Adelaide, South Australia.

Isolation of Glycosidic Components. The pulp of 5 kg of passion fruit (approximately 1.2 L) was centrifuged at 20000g for 1 h. The juice (720 g), possessing a pH value of 3.2, was diluted with distilled H_2O (500 mL), and the pH was adjusted to 5.0 by adding an aqueous solution of sodium hydroxide (2 N). After storage at 4 °C overnight, and further centrifugation (1 h, 20000g), the juice was peristaltically pumped down a glass column (470 × 15 mm (i.d.)) containing C_{18} reversed-phase adsorbent. The column was washed with water (200 mL), and the retained components were subsequently eluted with methanol (250 mL). After evaporation of the solvent, the residue was

taken up in H_2O (50 mL). This solution was liquid–liquid extracted (16 h) with Freon F 11 to ensure removal of any volatiles prior to enzymatic hydrolysis.

Enzymatic Hydrolysis. In a typical experiment the extracted residue (10 mL) was diluted with 0.2 M phosphate buffer (pH 5) (30 mL). Almond glucosidase (50 mg; Serva) and *n*-octyl β-D-glucopyranoside (100 μg; internal standard) were added, and the incubation was carried out for 48 h at 37 °C. The liberated aglycons were liquid–liquid extracted (16 h) with dichloromethane and subjected to HRGC-MS analysis.

Capillary Gas Chromatography–Mass Spectrometry (HRGC-MS). HRGC-MS analyses were made with a Finnigan 4021 GC-MS data system. The chromatograph was equipped with a J&W DB-1701 capillary column (30 m × 0.25 mm (i.d.), df = 0.25 μm); helium was used as carrier gas (2.5 mL/min). The temperature program was 1 min isothermal at 60 °C and then from 60 to 250 °C at 4 °C/min.

C_{13} **Norterpenoid Reference Compounds.** Authentic samples of compounds 1–5, 9, and 10 were available from previous studies (Winterhalter and Schreier, 1988a,b). Compounds 6A/B and 7 were donated samples. Compound 8 was prepared by the method of Davis et al. (1976) and showed a ^{1}H NMR spectrum that was in close agreement with the published data. For MS data see Table I.

RESULTS AND DISCUSSION

Isolates of freshly prepared passion fruit juice were obtained from C_{18} reversed-phase adsorbent by methanol elution (Williams et al., 1982). After removal of the free volatiles by freon extraction, the residue was incubated with a commercially available β-glucosidase (from sweet almond; emulsin) and the liberated aglycons were extracted with dichloromethane.

Monoterpenoids. As shown in Figure 1 the main monoterpenoid liberated by emulsin was linalool. Approxi-

Terpenoids in Passion Fruit Juice

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Table I. Mass Spectral Data of the C_{13} Norterpenoid Aglycons Enzymatically Liberated from a Glycosidic Extract from Purple Passion Fruit

peak no. (Figure 1)	compound	EIMS at 70 eV: m/z (%)
12	unknown **A**	43 (66), 55 (8), 65 (5), 77 (11), 91 (15), 109 (10), 119 (5), 121 (8), 135 (19), 149 (7), 159 (3), 177 (100), 192 (4), 210 (2)
13	unknown **B**	43 (32), 55 (14), 69 (11), 81 (60), 95 (30), 109 (14), 123 (10), 133 (13), 151 (100), 163 (1), 177 (12), 192 (10), 210 (7)
14	3-hydroxy-1,1,6-trimethyl-1,2,3,4-tetrahydronaphthalene (8)	41 (20), 77 (20), 91 (27), 105 (16), 115 (24), 119 (18), 128 (17), 129 (16), 131 (14), 142 (28), 147 (11), 157 (100), 175 (32), 190 (18, M$^+$)
15	4-hydroxy-β-ionol (1)	43 (100), 55 (30), 69 (20), 77 (21), 93 (35), 107 (21), 111 (48), 121 (43), 135 (13), 149 (14), 159 (14), 163 (6), 177 (22), 192 (20, M$^+$ – H$_2$O)
16	4-hydroxy-7,8-dihydro-β-ionol (3)	43 (53), 55 (25), 67 (13), 81 (16), 95 (28), 96 (22), 109 (43), 119 (16), 123 (19), 138 (100), 161 (3), 176 (0.5), 179 (1), 194 (2, M$^+$ – H$_2$O)
17	3-oxo-α-ionol (5)	43 (18), 53 (4), 67 (3), 79 (7), 91 (8), 108 (100), 123 (3), 135 (5), 152 (12), 175 (0.2), 208 (0.5, M$^+$)
18	4-oxo-β-ionol (2)	43 (68), 55 (28), 69 (13), 77 (19), 91 (31), 107 (34), 123 (27), 137 (41), 147 (6), 165 (100), 175 (3), 193 (20), 208 (4, M$^+$)
19	unknown **C**	41 (46), 55 (10), 65 (9), 71 (11), 79 (100), 93 (63), 99 (26), 105 (13), 119 (20), 133 (5), 149 (4), 177 (6), 192 (24)
20	unknown **D**	41 (42), 53 (10), 65 (10), 71 (11), 79 (100), 93 (54), 99 (24), 105 (14), 119 (21), 133 (8), 149 (4), 177 (4), 192 (24)
21	4-oxo-7,8-dihydro-β-ionol (4)	43 (100), 55 (61), 67 (37), 79 (39), 95 (42), 109 (73), 121 (40), 137 (82), 152 (66), 165 (33), 195 (26), 210 (9, M$^+$)
22	3-oxoretro-α-ionol (isomer 1) (6A)	45 (64), 55 (10), 65 (6), 77 (16), 91 (19), 105 (14), 121 (23), 135 (10), 149 (100), 164 (53), 175 (0.3), 193 (1), 208 (1, M$^+$)
23	3-oxo-7,8-dihydro-α-ionol (7)	41 (100), 55 (28), 69 (49), 79 (32), 84 (28), 93 (69), 108 (92), 123 (36), 135 (82), 150 (24), 177 (20), 192 (3), 195 (2), 210 (17, M$^+$)
24	3-oxoretro-α-ionol (isomer 2) (6B)	45 (72), 55 (8), 65 (5), 77 (15), 91 (15), 105 (13), 121 (25), 136 (6), 149 (100), 164 (47), 175 (0.3), 193 (0.2), 208 (1, M$^+$)
25	vomifoliol (9)	43 (24), 55 (7), 69 (4), 79 (10), 95 (5), 107 (3), 111 (7), 124 (100), 135 (8), 150 (6), 168 (5), 206 (1, M$^+$ – H$_2$O)
26	dehydrovomifoliol (10)	43 (46), 55 (9), 69 (8), 95 (12), 124 (100), 149 (6), 166 (10), 204 (0.1, M$^+$ – H$_2$O)
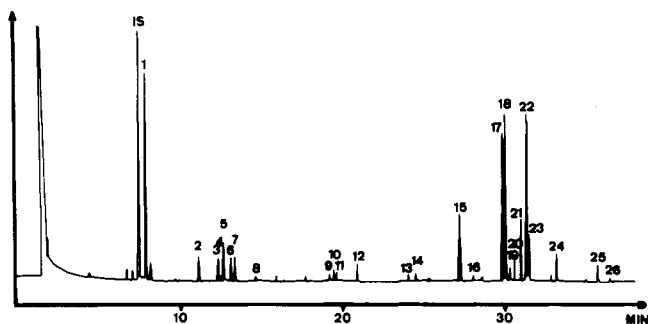
Figure 1. HRGC separation of passion fruit aglycons on a J&W (30 m × 0.25 mm (i.d.)) DB-1701 WCOT capillary column (df = 0.25 μm; IS internal standard, 1-octanol): 1, linalool; 2, α-terpineol; 3, theaspirane (isomer 1); 4, nerol; 5, citronellol; 6, theaspirane (isomer 2); 7, geraniol; 8, 2,6-dimethyloct-7-ene-2,6-diol; 9, (Z)-2,6-dimethylocta-2,7-diene-1,6-diol; 10, 3,7-dimethyl-1,7-octanediol; 11, (E)-2,6-dimethyl-2,7-diene-1,6-diol; 12–26, C_{13} norterpenoid aglycons; cf. Table I.

mately 250 μg/kg of juice was released by β-glucosidase activity during 48-h incubation. Additionally, the monoterpene alcohols α-terpineol, citronellol, and geraniol were identified in smaller quantities, and nerol was only present in trace amounts. These results confirmed earlier findings of Engel and Tressl (1983), who detected linalool, α-terpineol, geraniol, and nerol in a ratio of 91:4:5:1 after enzymatic cleavage of a glycosidic passion fruit extract.

In the present work, several hydroxylated monoterpenols, i.e., 2,6-dimethyloct-7-ene-2,6-diol, (Z)- and (E)-2,6-dimethylocta-2,7-diene-1,6-diol, and (tentatively) 3,7-dimethyl-1,7-octanediol, were newly identified as aglycons from passion fruit. However, these bound components occurred only at low levels, i.e., below 20 μg/kg of juice. The last mentioned diol was identified on the basis of published MS data alone (Rapp et al., 1983).

C_{13} **Norterpenoids.** Surprisingly, the majority of the aglycons observed were C_{13} norterpenoids, with the main products occurring in amounts up to 200 μg/kg of juice.

A total of 15 C_{13} norterpenoid aglycons were detected for the first time in purple passion fruit. Among these, the structures of 11 components were elucidated by comparison of their linear retention data and mass spectra with those of authentic reference compounds. The MS data of the C_{13} aglycons are listed in Table I; their structures are outlined in Figure 2.

Two groups of norterpenoid aglycons predominated in the purple passion fruit juice: (i) a series of β-ionol derivatives with a further oxygen function in the 4-position (1–4); (ii) a series of α-ionol derivatives, oxygenated in the 3-position (5–7).

The 4-oxygenated β-ionol derivatives 1–4 have been found in nature only rarely. 4-Hydroxy-β-ionol (1) and 4-oxo-β-ionol (2) have been previously reported in *Osmanthus absolute* (Kaiser and Lamparsky, 1978) and quince fruit (Winterhalter and Schreier, 1988b). In addition, diol 1 has been identified in tobacco (Weeks and Seltmann, 1986). Mild acid treatment of 1 led to the formation of a wide range of volatiles (Winterhalter and Schreier, 1989), including the isomeric megastigma-5,8-dien-4-ones, which have been already described as flavor components in the yellow passion fruit variety (Demole et al., 1979). Free 4-hydroxy-7,8-dihydro-β-ionol (3) has been recently identified in quince fruit as the natural precursor of the isomeric theaspiranes (Winterhalter and Schreier, 1988a). Compound 3 is known to be very labile. The relatively easy cyclization of 3 to the theaspiranes is likely to explain the occurrence of these latter compounds among the liberated aglycons as products formed from diol 3 under the experimental conditions. Diol 3 and the last representative in this group, 4-oxo-7,8-dihydro-β-ionol (4), were observed in this study as bound compounds for the first time. However, free keto alcohol 4 has been already identified in *Osmanthus absolute* (Kaiser and Lamparsky, 1978) and guava fruit (Idstein and Schreier, 1985).

Among the 3-oxo derivatives 5–7, compound 5 is well-known as the synthetic precursor of the isomeric
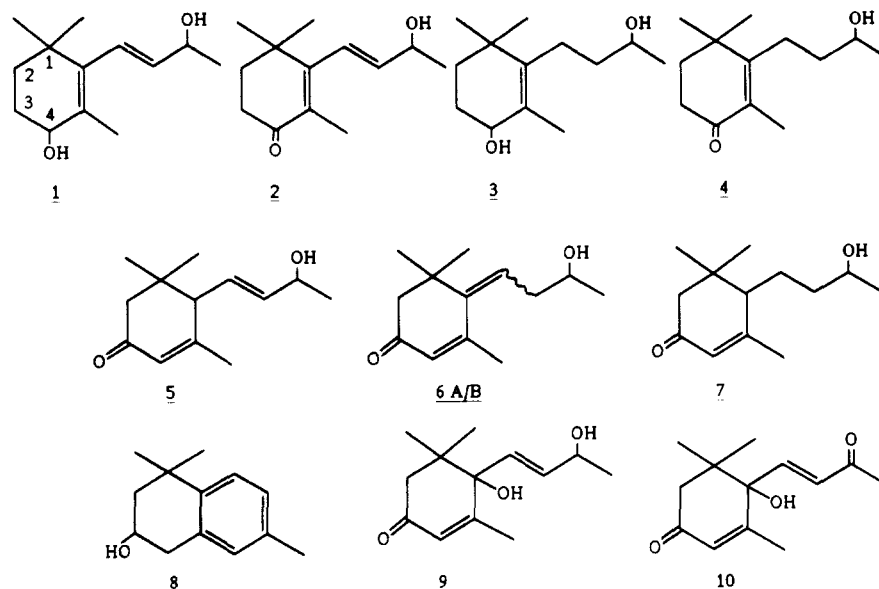
Figure 2. Structures of C_{13} norterpenoid aglycons identified after emulsin hydrolysis of purple passion fruit glycosides.

megastigma-4,6,8-trien-3-ones, key flavor components in burley tobacco (Aasen et al., 1972). However, recent model studies showed that **5** was only slightly susceptible to acid-catalyzed reactions at natural pH conditions (Strauss et al., 1987). This is consistent with the fact that the isomeric megastigma-4,6,8-trien-3-ones have not been found as yet among the passion fruit norterpenoids. In addition to **5**, the closely related components **6A/B**, with a rearranged double bond, as well as keto alcohol **7**, with a saturated side chain, were identified. Isomeric 3-oxoretro-α-ionols **6A/B** can be regarded as the most probable precursors of the 7-oxo-7,8-dihydroedulans, passion fruit constituents, which have been described to possess an oriental tobacco-type aroma (Winter et al., 1979). Compounds **6A/B** and **7** have been previously characterized from tobacco (Lloyd et al., 1976; Fujimori et al., 1976), and their glycosidically bound forms have been most recently identified in grapes (Sefton et al., 1989). Compound **7** is also known as blumenol C, indicating its first identification in *Podocarpus blumei* (Galbraith and Horn, 1972).

Precursor of 1,1,6-Trimethyl-1,2-dihydronaphthalene (TDN). There has been considerable speculation about precursors of TDN. This component, which possesses a low flavor threshold (20 ppb), exhibits undesirable flavor properties, which have been described as "hydrocarbon" or "kerosene-like" (Simpson, 1978). The fact that TDN could not be detected in the headspace of fresh passion fruit juice but was present in excessively pasteurized juice (Whitfield and Sugowdz, 1979) clearly indicated its formation from a labile precursor. For passion fruit it has been proposed that TDN is a decomposition product of the isomeric megastigma-4,6,8-trienes (Whitfield and Last, 1986). In other fruits, different C_{13} norterpenoids, including 5,6-epoxy-β-ionon (Stevens et al., 1975) and 4-hydroxy-1,1,6-trimethyl-1,2,3,4-tetrahydronaphthalene (Di Stefano, 1985), have been suggested as TDN precursors. Eventually a C_{13} norterpenoid triol, i.e., megastigma-5,7-diene-3,4,9-triol, which has been isolated from grape juice, gave TDN as a minor component upon heat treatment at pH 3 (Strauss et al., 1986).

None of these above-mentioned precursor compounds could be detected in the present work. Instead, 3-hydroxy-1,1,6-trimethyl-1,2,3,4-tetrahydronaphthalene (**8**) was identified among the liberated aglycons. As alcohol **8** is known to give TDN in acidic media (Davis et al., 1976), the gly-

cosidically bound form of **8** can be regarded as the most likely precursor of TDN in purple passion fruit. This is the first time that component **8** has been identified as a natural product.

Other C_{13} Norterpenoid Compounds. Vomifoliol (**9**), dehydro derivative **10**, and four unknown norterpenoids **A–D** were also observed as aglycons. Compound **9** most likely does not influence the flavor of passion fruit, as it was shown to be resistant to heat treatment at pH 3 (Strauss et al., 1987). Structural elucidations of the unknown components as well as biomimetic studies with the newly identified aglycons are under investigation.

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Headspace Compounds from Flowers of *Nicotiana tabacum* and Related Species

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Volatile compounds from flowers of four lines of tobacco, KY 14, TI 1068, TI 1112, and TI 1406, were entrained in air and trapped on Tenax. Each headspace sample was eluted with hexane and separated by capillary GC, and the components were analyzed by GC–MS. Total yields of volatiles ranged from approximately 600 to 900 ppb, which was 30–100 times greater than those from foliage of the corresponding plants. Caryophyllene was the predominant compound in three lines and a major component in the fourth, TI 1068. Studies of volatiles from five other *Nicotiana* species, *Nicotiana alata*, *Nicotiana rustica*, *Nicotiana suaveolens*, *Nicotiana sylvestris*, and *Nicotiana tomentosiformis*, showed that total volatile yields ranged from 88 ppb for *N. rustica* to 2424 ppb for *N. sylvestris*. There was wide diversity in the composition of compounds from the various species studied. *N. tomentosiformis* and *N. sylvestris*, which are putative male and female progenitors of tobacco, respectively, yielded several compounds also identified as tobacco flower headspace components.

Nicotiana is a large predominantly neotropical genus of approximately 57 known species. This genus contains several ornamentals, but tobacco (*Nicotiana tabacum*) is by far its most important member. Tobacco is one of the world's most extensively studied crop plants, not only because of its economic importance but also because of human health concerns. In addition, the genetics of tobacco and other members of the *Nicotiana* fam-

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