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# 3-OXO-α-IONOL, VOMIFOLIOL AND ROSEOSIDE IN *VITIS VINIFERA* FRUIT

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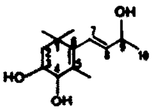
**Key Word Index**—*Vitis vinifera*; Vitaceae; grapes; 3-oxo-α-ionol; vomifoliol; dehydrovomifoliol; roseoside; hydrolytic studies.

**Abstract**—3-Oxo-α-ionol, vomifoliol and dehydrovomifoliol were identified for the first time in fruit from *Vitis vinifera*. The last named compound was mainly present free in the juice while the others existed predominantly as conjugates. In the case of vomifoliol, the conjugation was with glucose, i.e. as roseoside. Hydrolytic studies on 3-oxo-α-ionol and vomifoliol gave a range of compounds which have been recognized as fruit and plant products.

## INTRODUCTION

Many volatile flavorants of *Vitis vinifera* juices and wines are formed by acid hydrolysis from non-volatile precursors in the fruit. Precursors of a diverse range of compounds, including monoterpenes [1, 2], phenolics [3], benzyl alcohol, 2-phenylethanol [4] and $C_{13}$ nor-isoprenoids [5], have been found. Studies on precursors of monoterpenes and aromatic alcohols in Muscat and Riesling juices demonstrated that these compounds comprised a complex mixture of acid-labile glycosides [6]. In work on the $C_{13}$ nor-isoprenoids, triols **1** and **2** have been identified and shown to be precursors of a number of volatiles in grape juices and wines [5].

The present communication reports for the first time for grapes, the identification of 3-oxo-α-ionol **3**, vomifoliol **4a** and (tentatively) dehydrovomifoliol **5**. Roseoside **4b**, a glucoside derived from vomifoliol, has also been isolated from Riesling juice and characterized. The roles of **3** and **4a** as precursors of volatile flavorants are examined.



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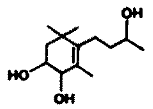

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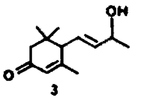

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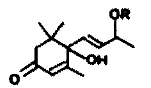

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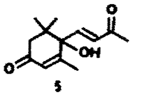

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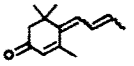

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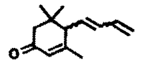

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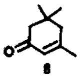

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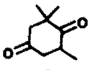

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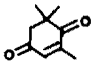

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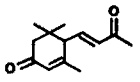

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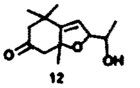

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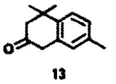

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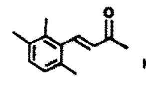

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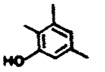

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## RESULTS AND DISCUSSION

Isolates from juice of the *Vitis vinifera* varieties, Muscat of Alexandria, Muscat à petit grains blanc, Riesling, Gewürztraminer, Shiraz and Chardonnay, were obtained from $C_{18}$ reversed phase adsorbent by methanol elution, as previously described [7]. Treatment of these isolates with the crude commercial pectinase, Rohapect C [7], yielded **3** and **4a** in amounts up to 1 mg/l. from each variety examined. The approximate proportions of **3** to **4a** released in these experiments were 5:1 (Muscat of Alexandria), 2:1 (Muscat à petit grains blanc), 1:2 (Riesling), 1:6 (Shiraz), 4:1 (Gewürztraminer) and 1:4 (Chardonnay). This indicated that considerable variation was possible in the relative amounts of each compound in fruit from the species *Vitis vinifera*. Moreover, it suggested that **3** and **4a** are ubiquitous in *V. vinifera* fruits, since varieties examined here included red and white, as well as those which are highly flavoured and more neutral.

As free compounds, **3** and **4a** were found only in trace amounts in the juices examined. Dehydrovomifoliol **5**, which, for lack of a reference sample, was so designated from mass spectral data [8], also co-occurred with **3** and **4a**. However, diketone **5** was present mainly free in the juice. In an effort to determine the nature of the conjugation involved with these compounds, the bound form of vomifoliol was isolated from Riesling juice. Spectral studies established this to be a diastereoisomeric mixture of the roseosides **4b**.

Roseoside **4b** was first isolated and characterized in *Vinca rosea* [9] and has been subsequently identified in *Betula alba*, *Cydonia oblonga*, *Chaenomeles japonica* [10] and *Nicotiana* sp. [11]. In this last species, **4b** has been found to co-occur with glucosides of several $C_{13}$ nor-isoprenoids, including that of **3** [12]. It now seems likely that this co-occurrence of glycosides is the case in *Vitis* sp. as well, although the bound form of **3** has not as yet been isolated in a pure state from grape juice.

There has been considerable speculation as to the role of $C_{13}$ compounds in plants. Many of the compounds found so far are skeletally related to known carotenoids [13]. However, vomifoliol **4a** also bears a structural similarity to the well-known plant hormone abscisic acid (ABA). Jamaican workers have shown that **4a** was as effective as ABA in causing stomatal closure in epidermal strips from *Eichhornia crassipes*, but, unlike ABA, it exhibited no growth effects [14, 15]. It has been suggested that **3** might also possess biological activity [14], but there have been no reports so far to confirm this.

Our interest in **3** and **4a** was not related to biological activity, but stemmed from their possible role as precursors of volatile flavorants in grape juices, wines and brandies. Model hydrolyses were carried out on the authentic materials to investigate such a hypothesis.

Ketoalcohol **3** was found to be only slightly susceptible to acid catalysed reaction at natural juice pH (*ca* 3), but decomposed readily when heated at pH 1 in aqueous solution. Megastigmatrienones **6** and **7** were major products, along with an unknown possessing the following EI mass spectrum at 70 eV; $m/z$ (rel. int.): 190 (53), 175 (69), 148 (25), 147 (50), 133 (75), 106 (100), 105 (35), 91 (80), 77 (23), 41 (40). Although water elimination was clearly the favoured process during acid treatment of **3** at pH 1, small quantities of isophorone **8**, as well as diones **9** and **10**, were also formed by degradation involving loss of the side chain. Aasen *et al.* [16] found the megastigmatrienones **6** and **7** as tobacco constituents and prepared them by acid treatment of **3**. They did not, however, report any additional products of the reaction.

Vomifoliol **4a** was resistant at pH 3, but rearranged extensively with heat at pH 1 to produce a series of ketones—3-oxo-α-ionone **11**, the four diastereoisomers of dihydro-oxoactinidol **12**, the naphthalenone derivative **13** and the trimethylphenylbutenone **14**. Three $C_9$ compounds were also formed as a result of loss of the $C_4$ side chain, i.e. trimethylphenol **15** and ketones **8** and **10**. It is noteworthy that the dihydro-oxoactinidols **12** were also observed in trace amounts as natural products amongst the enzyme-liberated compounds from the Gewürztraminer juice.

Many of the compounds formed here by acid hydrolysis from **3** and **4a** were first reported as grape products when an isolate of Muscat of Alexandria juice was heated at pH 1 [17]. Subsequently, juices or isolates from several varieties have been heated in our laboratory and most of the volatile $C_{13}$ and $C_9$ compounds obtained hydrolytically from **3** and **4a** have been observed after such treatment. It thus seems reasonable that glucosides of **3** and **4a** are the precursors of these volatiles. The product distributions obtained are of course dependent upon the conditions of treatment and may not reflect differences in composition of the precursors in the fruit. For example, Davis *et al.* [18] have shown that ketone **11** will cyclize to naphthalenone derivative **13** under acidic conditions.

## EXPERIMENTAL

*Isolation and analysis of free and bound compounds from grape juice.* Solvent extraction of free volatiles and the methods used for isolation and enzyme liberation of bound compounds were essentially the same as those described in ref. [7]. Analysis by GC-MS [17] was carried out using a quartz silica BP10 column (SGE, Melbourne), held at 100° for 1 min and then programmed at 4°/min to 260°.

*Identification of volatiles.* Authentic samples of **3** [19], a mixture of **6** and **7** [16], and **9** [20] were prepared by lit. methods, **8** was commercially obtained and **4a** [21] and **12** [22] were donated. Spectral data for the above compounds, including **8** and **9** [23], agreed closely with those given in the lit. cited. EIMS of **4a**, $m/z$ (rel. int.) 168 (3), 150 (5), 135 (5), 125 (10), 124 (100), 121 (8), 111 (6), 79 (13), 55 (12), 45 (18), 43 (50), 41 (13).

Positive identifications of volatiles from juice isolates were made by co-chromatography with authentic materials to give symmetrical peak enhancement and identical MS with those of the ref. compounds. Components **5** [8], **10** [24], **11** [19], **13** [18], **14** [25] and **15** [23] were identified on the basis of published MS data alone.

*Isolation and identification of roseoside* **4b**. A $C_{18}$ reversed phase isolate from Riesling juice (12 l.) was treated with PVPP and subjected to droplet counter current analysis (DCCC) [3]. Roseoside **4b** was isolated from a DCCC fraction as its tetra-*O*-acetyl derivative (10.9 mg) after flash chromatography on silica gel [26]. The isolated diastereoisomeric compound ($R_f$ 0.28 on silica gel 60 with $Et_2O$ as solvent) had the following spectral properties. IR (KBr disc): 1660 $cm^{-1}$, 3500 $cm^{-1}$. $^{13}C$ NMR (22.49 MHz; $CDCl_3$; M equals megastigmane; G equals glucose): $\delta$18.9 ($CH_3$-M5); 22.0, 22.9 and 24.2 (M10 and $CH_3$-M1; not specifically assigned), 41.2 (M1), 49.6 (M2), 61.8 and 62.1 (G6), 68.5 (G4); 71.5 and 71.9 (G2 and G5); 72.9 (G3), 74.8 (M9); 79.0 (M6); 98.4 and 100.0 (G1); 127.0 (M4); 130.6, 131.8, 132.4, 133.4 (M7 and M8); 162.2 (M5); 20.6 (acetate $CH_3$); 169.3–170.6 (acetate CO). A signal for M3 not observed. $^1H$ NMR (90 MHz, $CDCl_3$): $\delta$0.97 and 1.06 (6H, 2*s*, $CH_3$-M1), 1.20 and 1.27 (3H, 2*d*, $CH_3$-M9), 1.7 (1H, *b*, OH) 1.86 and 1.89 (3H, 2*d*, $J$ = 1.5 and 1.2 Hz, respectively; $CH_3$-M5), 1.98–2.05 (12H, acetate methyls 2.2–2.4 (2H, *m*, $H_2$-M2), 3.6 (1H, *b*, H-G5), 4.2 (2H, *m*, H-G6), 4.4–4.6 (2H, *m*, H-M9, H-G1), 4.8–5.3 (3H, *m*, H-G2, G3 and G4), 5.6–6.0 (3H, *m*, H-M4, M7 and M8), *cf.* ref. [9]. EIMS (probe, 70 eV): $m/z$ 554 $[M]^+$ (0.04), 539 (0.04), 331 (11), 207 (21), 206 (15), 169 (41), 151 (22), 150 (48), 139 (25), 135 (17), 127 (20), 123 (27), 122 (30), 115 (21), 109 (46), 107 (17), 98 (19), 97 (25), 95 (30), 85 (29), 83 (16), 82 (18), 81 (31), 79 (22), 69 (35), 67 (24), 55 (36), 43 (100).

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