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NEW LINALOOL DERIVATIVES IN MUSCAT OF ALEXANDRIA GRAPES AND WINES

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Key Word Index—*Vitis vinifera*; Muscat of Alexandria; grapes; wine; acyclic monoterpenoids; 3,7-dimethyloct-1-en-3,6,7-triol; 3,7-dimethyloct-1-en-3,7-diol; 3,7-dimethylocta-1,7-dien-3,6-diol; 3,7-dimethylocta-1,5-dien-3,7-diol.

Abstract—The $CHCl_3$ extract of Muscat of Alexandria grapes as well as extracts of wines made from these grapes were found to contain 3,7-dimethyloct-1-en-3,6,7-triol, 3,7-dimethyloct-1-en-3,7-diol, 3,7-dimethylocta-1,7-dien-3,6-diol and 3,7-dimethylocta-1,5-dien-3,7-diol.

INTRODUCTION

Several monoterpenes have been identified in muscat grape varieties [1]. We now report the identification of four hydroxylated linalool derivatives in *Vitis vinifera* var. Muscat Gordo Blanco (syn. Muscat of Alexandria) which have not been previously recognised in grapes.

RESULTS AND DISCUSSION

A water-soluble fraction partitioned from a $CHCl_3$ extract of Muscat of Alexandria juice was separated by PLC into a major, high R_f zone and a minor band. Elution of the major zone and analysis by GLC showed it to consist of a mixture of the diendiols **1** and **2** (70 and 15%, respectively) together with the endiol **3** (15%). The identities of these three components were confirmed by GLC, GC–MS and ^1H NMR comparison with authentic reference compounds [2, 3]. Grape diendiol **1** has been assigned the *trans*-stereochemistry as it was identical with a synthetic diendiol (**1**) prepared by photo-oxygenation of linalool. This method of synthesis is known to give the *trans*-isomer (**1**) [4, 5].

The minor band eluted from the PLC was shown to be the triol **4**. This structure was proven by comparison of the TLC, MS and ^1H NMR characteristics with that of a synthetic sample of the triol (**4**). The latter material was obtained by acid hydrolysis of the epoxide ring of (±)-6,7-epoxydihydrolinalyl acetate (**5**) followed by deacetylation.

The ^1H NMR spectrum of the synthetic triol (**4**) showed two signals (quartets) for the vinyl proton at C-2. By contrast this C-2 vinyl proton in the spectrum of the grape triol (**4**) showed predominantly one quartet signal indicating the presence of a single diastereoisomer. This conclusion was confirmed by formation of only one diastereoisomeric acetonide (**7**) after treatment of the natural triol (**4**) with acetone and anhydrous $CuSO_4$; the synthetic triol (**4**) afforded both acetonide diastereoisomers. Thus the two chiral centres in the natural triol (**4**) are related and if the triol is derived from an optically pure linalool then the centre at C-6 is also optically active.

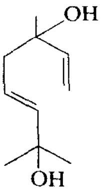
Muscat of Alexandria grapes harvested at different maturities showed varying proportions of compounds **1**–**4**.

The linalool derivatives **1**–**4** were also identified in $CHCl_3$ extracts of table wines prepared from Muscat of Alexandria grapes, thus confirming that at least some quantity of each of these fruit compounds survives fermentation and other winemaking conditions.

It seems probable that an unknown component observed in methylene chloride extracts of Muscat de Frontignan grapes by French workers [1, 6] and labelled 'compound Y' in their publications is the diendiol (**1**). This follows from the similarity in GLC behaviour and MS characteristics published for 'compound Y' [6] and those of the diendiol (**1**).

The diendiols **1** and **2** have been isolated from *Cinnamomum camphora* [7] as has another grape monoterpene, hotrienol (**8**) [8]. Triol **4** has not been reported previously and endiol **3**, although known synthetically [3] has not been found in a natural source.

The dye-sensitized photo-oxygenation reaction has been considered [9] as a biomimetic process involved in the formation of such hydroxylated products as the diendiols **1** and **2** from linalool. However the co-occurrence of diols **1** and **2** together with endiol **3** and triol **4** in Muscat of Alexandria grapes and wines suggests 6,7-epoxydihydrolinalool (**6**) or a derivative thereof as a precursor. This proposal is given support by the reported [10] isolation of a seasonally variable 'salvage enzyme' system from tissues of *Tanacetum vulgare* capable of oxidizing hemi- and monoterpenes by epoxidation and ring-opening to give products analogous to those found here.



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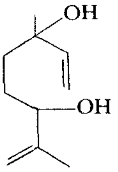

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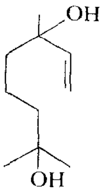

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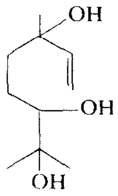

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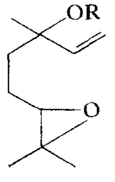

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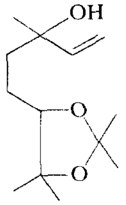
6 R = H



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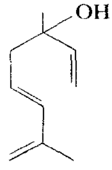

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EXPERIMENTAL

MS were determined at 70 eV by direct insertion on a probe or by GC–MS. ^{1}H NMR spectra of synthetic products were obtained at 60 MHz without Fourier transform and those of isolated natural products at 80 or 90 MHz with Fourier transform in $CDCl_3$ solvent with TMS as int. standard.

Separations by PLC were carried out using Si gel 60 (1 mm) with $CHCl_3$–MeOH (4 : 1) as developing solvent. Anisaldehyde in HOAc–H_2SO_4 (1 : 100 : 2) or *dodeca*-molybdophosphoric acid in EtOH (1 : 20) were used for visualization. Material was recovered from the Si gel with MeOH at room temp.

GLC was carried out using FID under the following conditions. (a) A Carbowax 20 M SCOT column was operated isothermally at 50° for 10 min then temp. programmed to 170° at 1°/min and held at 170° for 20 min. Injector and detector temps were 225°. Carrier gas was N_2 at 2.7 ml/min flow rate with septum purge of 150 ml/ min. (b) An SF96 SCOT column was programmed from 80° to 170° at 0.5°/min. Injector and detector temps were 225°. Carrier gas was He at 5.0 ml/min. GC–MS was carried out on a Carbowax 20 M SCOT column with the He carrier gas at 2.7 ml/min and temp. programme from 60° to 180° at 1°/min. Detection was by total ion monitor. Injector temp. was 225°.

Extraction. Muscat of Alexandria grapes (2 kg fr. wt), harvested from the Waite Agricultural Research Institute vineyards at varying stages of ripeness, were picked from the bunches and blended with NaCl (*ca* 450 g) in a Waring blender. The suspension was filtered through a muslin cloth and the filtrate liquid–liquid extracted with $CHCl_3$ for 48 hr. Solvent was changed after 24 hr. The $CHCl_3$ was evapd to give a gummy residue. This residue was taken up in H_2O (10 ml) and washed with *n*-pentane (6 × 5 ml). The aq. soln was exhaustively extracted with $CHCl_3$ and the solvent evapd and subjected to PLC. Two bands at R_f 0.35–0.45 (fraction A) and R_f 0.45–0.60 (fraction B) were eluted with MeOH.

Compound **4** in fraction A was not amenable to GLC analysis (unless derivatized) and was isolated by further PLC on Si gel 60 with zone concentration section. ^{1}H NMR (90 MHz, $CDCl_3$) and MS and TLC data for the isolated component matched those of the synthetic material.

Compounds **1**, **2** *and* **3** in fraction B were separated by GLC and GC–MS. They all gave symmetrical peak enhancement on co-chromatography under conditions (a), RR_t (geraniol) 1.4, 2.0, 1.6 respectively, and had identical MS and TLC properties with authentic materials. Compounds **1** and **3** were purified by prep. GLC and gave ^{1}H NMR (80 MHz, $CDCl_3$) spectra matching those of the reference materials.

Authentic compounds. Compounds **1** and **2** were prepared by photo-oxygenation of linalool, followed by mild reduction [2] and had identical GLC [conditions (a)] and GC–MS properties to authentic samples donated to the authors. ^{1}H NMR and IR spectral properties of the donated materials corresponded with those in the literature [2, 4].

Compound **3**. Reaction of racemic linalyl acetate with an equimolar quantity of perphthalic acid in dry Et_2O overnight at 0°, afforded diastereoisomeric epoxides **5**: GLC conditions (a) RR_t (geraniol) 0.90 and 0.92, bp 59°/0.3 mm Hg (75% yield). ^{1}H NMR (60 MHz, $CDCl_3$): δ 1.24 and 1.30 (6H, 2*s*, 2Me—C-7), 1.55 (3H, *s*, Me—C-3), 1.60 (4H, *m*, C-4 and C-5), 1.96 (3H, *s*, OAc), 2.66 (1H, *t*, C-6), 5.06 (2H, *m*, C-1), 5.90 (1H, *q*, C-2). MS *m/e*: 41, 43 (base peak), 53, 59, 67, 79.

Epoxide **5** (1.02 g) was reduced with $LiAlH_4$ (0.43 g) in refluxing THF over 20 hr. Work-up and distillation yielded endiol **3**, (0.65 g) bp 82–84°/0.7 mm Hg, mp 48–50°, lit. [3] mp 51–52°. ^{1}H NMR (60 MHz $CDCl_3$): δ 1.20 (6H, *s*, 2Me—C-7), 1.28 (3H, *s*, Me—C-3), 1.45 (4H, *m*, C-4 and C-5), 1.55 (2H, *s*, 2OH), 5.16 (2H, *m*, C-1), 5.92 (1H, *q*, C-2). MS *m/e* (rel. int.): 139 (6), 121 (13), 109 (13), 95 (10), 93 (17), 85 (19), 83 (11), 81 (47), 79 (19), 71 (100), 69 (45), 68 (55), 67 (31), 59 (68), 56 (40), 55 (29), 43 (63), 41 (50).

Compound **4**. To H_2O (40 ml) containing conc H_2SO_4 (0.4 ml) at 0°, was added diastereoisomeric epoxide **5**, (1.0 g) with stirring. After 2 hr, 5% KOH soln (13 ml) was added and the soln warmed on the steam bath for 1 hr. The product was liquid–liquid extracted with $CHCl_3$ for 2 hr and the solvent evapd. Diastereoisomeric triol **4** distilled at bp 126–128°/1 mmHg (0.82 g).

^{1}H NMR (60 MHz, $CDCl_3$): δ1.13 and 1.18 (6H, 2*s*, 2Me—C-7), 1.28 (3H, *s*, Me—C-3), 1.66 (4H, *m*, C-4 and C-5), 2.73 (3H, *br.s*, 3OH), 3.33 (1H, *m*, C-6), 5.13 (2H, *m*, C-1), 5.87 (1H, 2*q*, C-2). MS *m/e* (rel. int.): 155 (4), 143 (3), 142 (1), 137 (2), 111 (7), 94 (22), 93 (10), 85 (10), 81 (16), 72 (44), 71 (55), 69 (11), 68 (67), 67 (19), 59 (100), 57 (11), 55 (17), 43 (67), 41 (28). [Found: C, 63.5; H, 10.4. $C_{10}H_{20}O_3$ requires: C, 63.8; H, 10.7%.]

Acetonide **7**. Synthetic triol **4** (0.08 g) in Me_2CO (4 ml) plus dry $CuSO_4$ (0.17 g) was stirred at room temp. for 17.5 hr. After filtering off the catalyst the Me_2CO was evapd and the product bulb to bulb distilled bp 84–88°/0.7 mmHg. (0.05 g). GLC conditions (a) RR_t (geraniol) 1.0 and 1.1. ^{1}H NMR (60 MHz, $CDCl_3$): δ1.07 and 1.22 (6H, 2*s*, 2Me—C-7), 1.28, 1.31 and 1.40 (9H, 3*s*, Me—C-3, —O—C(Me)$_2$—O—) 1.6 (5H, *br m*, C-4, C-5 and OH), 3.63 (1H, *m*, C-6), 5.10 (2H, *m*, C-1), 5.90 (1H, *q*, C-2). MS *m/e* (rel. int.): 228 (M^+, 0.1), 213 (16), 195 (3), 155 (5), 153 (9), 143 (4), 142 (3), 135 (7), 112 (5), 109 (5), 107 (4), 100 (7), 97 (9), 81 (29), 72 (32), 71 (58), 69 (14), 68 (17), 67 (10), 59 (52), 58 (19), 55 (20), 43 (100), 41 (32). [Found: C, 68.8; H, 10.9. $C_{13}H_{24}O_3$ requires: C, 68.4 H, 10.6%.] Similar reaction on natural triol **4** gave a single acetonide isomer which co-chromatographed with one of the synthetic acetonides under GLC conditions (a) and (b).

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