New Monoterpene Ethyl Ethers in Grape Wines and Brandies

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The monoterpene ethyl ethers (*E*)-7-ethoxy-3,7-dimethylocta-1,5-dien-3-ol and the two diastereoisomers of 5-ethoxy-3,7-dimethylocta-1,6-dien-3-ol have been identified in grape wines and brandies. Formation of these components has been rationalized as occurring by ethanolysis of (*E*)-3,7-dimethylocta-1,5-diene-3,7-diol a major monoterpenoid constituent of many grapes and wines.

In the course of studies into the monoterpene composition of wines and spirits, three unknown components with mass spectra similar to those of hydroxylated linalool derivatives (Williams et al., 1980a; Rapp and Knipser, 1979) have been routinely observed. These compounds, which show retention indices (Van den Dool and Kratz, 1963) of 880, 955, and 1057 when gas chromatographed on an SP1000 column, have now been identified as ethyl ethers formed by acid-catalyzed reaction of ethanol with (*E*)-3,7-dimethylocta-1,5-diene-3,7-diol (1) or 3,7-dimethylocta-1,7-diene-3,6-diol (4) (see Figure 1).

EXPERIMENTAL SECTION

Capillary Gas Chromatography (GC) and Gas Chromatography–Mass Spectrometry (GC–MS). Analytical GC and GC–MS were carried out as described previously (Williams et al., 1982; Rapp et al., 1983).

Nuclear Magnetic Resonance (NMR) and Infrared (IR) Spectra. NMR spectra were recorded on a Jeol FX90Q spectrometer or a Bruker WP80 instrument using Fourier transform, with $CDCl_3$ as solvent and Me_4Si as the reference standard.

The IR spectrum of compound (3) was recorded as a liquid film with a Perkin Elmer 1320 infrared spectrophotometer.

Model Reactions. Two separate 10% ethanol solutions (250 mL) acidified with L-tartaric acid (2 g) and containing octan-3-ol (0.2 mg) as internal standard and either diene diol 1 (1 mg) or diene diol 4 (1 mg) were prepared. Each solution was then continuously extracted for 20 h with Freon F11/CH_2Cl_2 (9:1 by vol, 60 mL). The extracts were concentrated to about 100 μL and the concentrates analyzed by GC and GC–MS.

An aqueous solution (250 mL) containing L-tartaric acid (2 g), diene diol 1 (1 mg) and octan-3-ol was similarly extracted and analyzed.

Preparation and Isolation of Hydroxy Ethers 2a, 2b, and 3 (see Figure 1). Diene diol 1 (190 mg) was dissolved in EtOH (95% v/v, 10 mL) and 1.8 M H_2SO_4 (0.2 mL) added. The progress of the reaction at room temperature was monitored by thin-layer chromatography on silica gel 60, with 5% MeOH/CH_2Cl_2 as solvent. After 36 h, the solution was poured onto ice (30 g) and extracted with cold Freon F11 (3 × 30 mL). The organic phase was washed with cold water (1 × 20 mL), dried with $MgSO_4$, concentrated on the rotary evaporator, and analyzed by GC and GC–MS. Sole products observed were hotrienol (1% of the product distribution), as well as hydroxy ethers **3**, **2a**, and **2b**, (62%, 25%, and 12%, respectively).

Hydroxy ether **3** (106 mg isolated yield) was separated from **2a** and **2b** which remained unresolved after flash chromatography with CH_2Cl_2 as eluent. Rechromatography of the diastereoisomeric pair, with Et_2O/pentane (1/10) as solvent, afforded **2a** and **2b** individually.

^1H NMR spectral data (90 MHz, $CDCl_3$, Me_4Si) for the isolated ethers were as follows. Compound **3**: δ 1.08 (t, 3 H, J = 7.1 Hz, CH_3CH_2O), 1.21 (s, 9 H, CH_3COH and $(CH_3)_2C$), 1.74 (bs, 1 H, OH), 2.23 (m, 2 H, HC=CHCH_2), 3.28 (q, 2 H, J = 7.1 Hz, CH_3CH_2O), 5.09 (m, 2 H, H_2C=), 5.48 (m, 2 H, CH_2CH=CH), 5.88 (dd, 1 H, HC=CH_2)

Compound **2a**: δ 1.12 (t, 3 H, J = 7.1 Hz, CH_3CH_2O), 1.20 (s, 3 H, CH_3COH), 1.60 and 1.69 (2 d, 6 H, J = 1.2 Hz, $(CH_3)_2C$=), 1.75 (m, 2 H, CH_2CHOEt), 3.32 (m, 2 H, CH_3CH_2O), 4.22 (ddd, 1 H, $HCOEt$), 4.72 (bs, 1 H, OH), 5.20 (m, 3 H, HC=CH_2 and HC=C$(Me)_2$), 5.92 (dd, 1 H, HC=CH_2).

Compound **2b**: δ 1.16 (t, 3 H, J = 7.1 Hz, CH_3CH_2O), 1.32 (s, 3 H, CH_3COH), 1.66 and 1.71 (2 d, 6 H, J = 1.5 Hz, $(CH_3)_2C$=), 1.74 (m, 2 H, CH_2CHOEt), 3.41 (m, 2 H, CH_3CH_2O), 4.34 (s, 1 H, OH), 4.36 (m, 1 H, $HCOEt$), 5.10 (m, 3 H, HC=CH_2 and HC=C$(Me)_2$), 5.89 (dd, 1 H, HC=CH_2).

RESULTS AND DISCUSSION

The two unknown wine or spirit components of retention index 880 and 955 had the following EIMS at 70 eV. (a) Compound with retention index 880: m/z (relative intensity) 165 (2), 137 (5), 126 (8), 114 (9), 113 (86), 85 (100), 83 (19), 82 (30), 81 (18), 71 (40), 67 (30), 55 (34), 43 (69), 41 (36). (b) Compound with retention index 955: m/z (relative intensity) 165 (2), 137 (6), 126 (3), 114 (7), 113 (82), 85 (100), 83 (16), 82 (28), 81 (15), 71 (36), 67 (26), 55 (30), 43 (58), 41 (43).

Owing to the similarity of these mass spectra, the two compounds appeared to be isomeric. Structures **2a** and **2b** were proposed for these unknowns on the basis of the postulated mass spectral fragmentations shown in Figure 2.

The remaining unknown wine or spirit compound of retention index 1057 had the following EIMS at 70 eV: m/z (relative intensity) 137 (0.4), 125 (0.7), 113 (9), 85 (11), 83 (17), 82 (100), 71 (76), 67 (30), 55 (12), 43 (69), 41 (14). This is a spectrum similar to those of hotrienol (Bayonove et al., 1976) and 3,7-dimethylocta-1,5-diene-3,7-diol (1) (Rapp and Knipser, 1979), but included additional small, but significant ions such as m/z 113 and m/z 85. Structure **3** was considered likely for this compound.

Reaction of ethanol with monoterpenes under the acidic conditions existing during the percolation of spices with aqueous alcohol or in wines during storage or distillation has been investigated by several authors (Taskinen and Nykänen, 1976; De Smedt and Liddle, 1976; Strauss and Williams, 1983). In addition, recent studies on the *Vitis*

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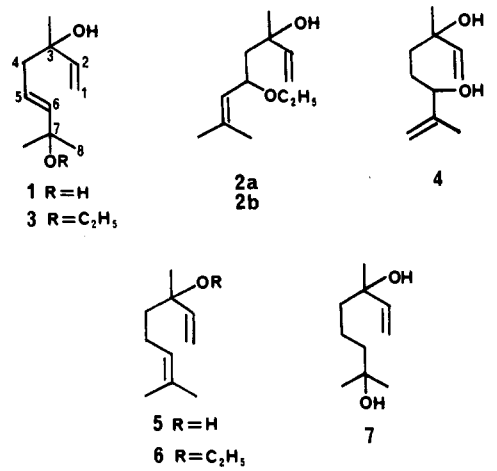

Figure 1. Some Monoterpenes referred to in this work.

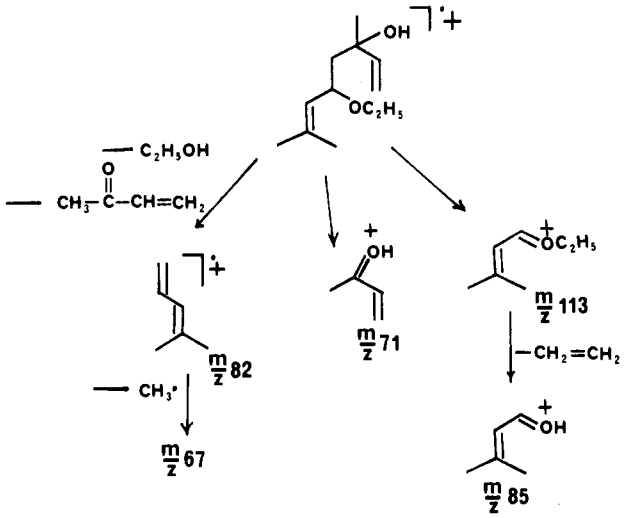

Figure 2. Postulated mass spectral fragmentation of **2a** and **2b**.

vinifera variety Muscat of Alexandria have shown that diol 1 can be present in juice at concentrations exceeding the combined total of all other monoterpenes (Wilson et al., 1984). Diol **4** has also been found to be a significant component of juices and wines, although present in lesser amounts than diol **1**. These factors suggested that the proposed ethers **2a**, **2b**, and **3** could have arisen by a solvolytic reaction of diols **1** or **4** with dilute aqueous ethanol.

Experiments were thus carried out in which diols **1** and **4** were allowed to stand at room temperature in model wine solutions. Diol **1** readily decomposed in the ethanolic solution to form the three unknown wine or spirit compounds along with hotrienol and 2-vinyl-2-methyltetrahydrofuran-5-one. The lactone has previously been identified in grape extracts (Schreier et al., 1976). The control experiment in which diol **1** was treated under aqueous conditions in the absence of ethanol yielded hotrienol and 2-vinyl-2-methyltetrahydrofuran-5-one only, thus adding weight to the suggestion that the unknowns were ethyl ethers.

When diol **4** was allowed to stand in acidified ethanolic solution it too formed the unknown ethyl ethers along with the anhydrofuran ring linalool oxides (Williams et al., 1980b) and 2-vinyl-2-methyltetrahydrofuran-5-one. The extent of reaction in this case was less than that obtained with diol **1**.

A preparative reaction of diol **1** with acidified ethanol gave volatile products of which the three unknowns comprised 99% and this allowed isolation and characterization of the individual compounds.

Table I. ^{13}C NMR Spectra Data

C	chemical shift of each compd[a]					
	1	**4**[c]	**6**	**2a**	**2b**	**3**
1	112.1	112.1	113.8	112.0	111.0	111.0
2	145.1	145.4	143.7	144.8	145.8	144.8
3	72.9	73.0	76.9	73.3	72.4	72.5
CH_3-C3	27.5	27.9 and 28.3	22.2	29.6	26.9	27.3
4	45.1	37.9 and 38.4	39.5	45.9	46.0	45.4
5	121.9	29.5 and 29.3	22.2	74.8	73.8	124.1
6	142.7	75.8	124.6	125.7	126.0	140.5
7	70.7	147.8	131.2	135.1	135.5	74.5
CH_3-C7[b]	29.9	18.0	17.4	18.1	18.3	26.5
8	29.9	111.0	25.5	25.7	25.7	26.5
CH_3CH_2O			15.8	15.4	15.4	16.1
CH_3CH_2O			57.2	63.1	63.1	57.7

[a] For compound numbers see Figure 1. For chemical shift data for other monoterpenoids including **5** and **7** see Wehrli and Nishida (1979). [b] Cisoid where applicable. [c] Diastereoisomeric.

The ^1H NMR spectra of the isolated materials showed these to be diol monoethyl ethers with structures consistent with those proposed for the wine and spirit ethers. However the spectra gave little indication as to which of the two oxygens on each terpene was etherified. Evidence for this was obtained by the use of ^{13}C NMR spectroscopy.

In Table I, ^{13}C NMR spectral data for some monoterpenes containing a 1,2-double bond and a hydroxyl group at position 3 (for numbering system see Figure 1) of the monoterpene skeleton have been collected. For all such listed compounds the C3 carbon gave a signal at about δ 73. When the hydroxyl group on position 3 was replaced by an ethoxyl function, as in the case of linalyl ethyl ether **6**, the signal for C3 was shifted downfield by about 4 ppm. In the ^{13}C spectra of the three isolated ethers signals were observed at δ 72.4, 73.3, and 72.5, respectively, indicating the presence of a hydroxyl and not an ethoxyl function on C3 in each compound. Furthermore, the downfield shift for C7 in ether **3** compared with the signal for the corresponding carbon in diol **1** confirmed that the ethoxyl group lay on C7 in this ether.

In the ^1H NMR spectrum of ether **3**, the hydrogens on carbons 5 and 6 were nearly equivalent. This spectrum was remarkably similar to that of diol **1**, in which the stereochemistry of the 5,6-double bond is trans. The only significant difference between the proton spectra of ether **3** and diol **1** was the additional absorption of the ethoxyl group in place of the hydroxyl proton. So the stereochemistry of the 5,6-double bond of ether (**3**) was assigned as trans also, an assignment supported by the presence of a moderately strong band at 980 cm^{-1} in the IR spectrum of ether **3**. This absorption has been noted as a significant feature in the IR spectra of compounds possessing analogous trans double bonds such as diol **1** (Kjøsen and Liaaen-Jensen, 1973) and the sesquiterpene 3,11-dihydroxy-3,7,11-trimethyldodeca-1,6,9-triene (Stoessl et al., 1975).

In the ^1H NMR spectra of synthetic ethers **2a** and **2b**, the individual methylene protons of the ethoxyl groups were nonequivalent and gave complex signals. This implied that rotation of the ethoxyl group was restricted and suggested that there was hydrogen bonding between the hydroxyl proton on position 3 and the ethoxyl oxygen, in each isomer. Although rigorous assignment of the relative stereochemistry at positions 3 and 5 in isomeric ethers **2a** and **2b** was beyond the scope of the present work, this problem was investigated by using the available spectral data and with the aid of models.

From models, the methyl group attached to C3 of the 3*R**,5*R** diastereoisomer was proximal to C6. The protons

of the C3 methyl group of this isomer should then have been shielded by the π electrons of the 6,7-double bond, while the methyl carbon should have been deshielded owing to the δ-substituent effect from C6 (Stothers et al., 1976; Grover et al., 1973; Mann et al., 1978). The respective ^1H NMR and ^{13}C NMR spectra of isomer **2a** showed an upfield shift for these methyl protons and a downfield shift for the methyl carbon relative to the positions of these signals in corresponding spectra of isomer **2b**. Therefore the diastereoisomeric ether **2a** was tentatively assigned 3*R**,5*R** configuration about its asymmetric centres and isomer **2b** assigned the 3*R**,5*S** configuration.

Finally, an extract of a commercial wine made from Muscat of Alexandria grapes was analyzed by GC and GC–MS. This extract contained the three unknowns. Cochromatography of the isolated and characterized monoterpene ethyl ethers **2a**, **2b**, and **3** with this extract confirmed the identity of the unknowns in the wine.

The ethers **2a**, **2b**, and **3** have been found to occur at higher concentrations in muscat wines than in wines prepared from less aromatic grape varieties. They could play a significant role in recent studies into the characterization of cultivars from the variety Riesling (Guentert, 1984; Rapp and Guentert, 1985). It has been demonstrated here that these ethers owe their origin in wines and spirits to the reactivity of diol **1** and to a lesser extent diol **4** in acidic ethanolic solution. An additional product was formed from diol **4** in acidified ethanol and this showed the following EIMS: *m/z* (relative intensity) 137 (3), 112 (4), 100 (7), 99 (100), 84 (5), 82 (8), 81 (7), 71 (90), 69 (10), 68 (9), 67 (19), 55 (17), 43 (42). This compound had a retention time longer than that of ether **3** and was tentatively identified from its MS as 3-ethoxy-3,7-dimethylocta-1,7-dien-6-ol.

It thus appears that ethyl ether formation is a significant reaction pathway in wines and spirits and could warrant further study.

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Registry No. 1, 51276-34-7; (*R**,*R**)-**2a**, 95694-07-8; (*R**,*S**)-**2b**, 95694-08-9; **3**, 95694-06-7; **4**, 51276-33-6; ethanol, 64-17-5.

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The Nature of the Protein Constituent of Commercial Lemon Juice Cloud

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The cloud protein content of two commercial lemon juice concentrates was 29.8% of the original cloud. The protein can be completely solubilized in 10 M urea–6% citric acid, pH 2.5. The insolubility of the cloud protein could be attributed to at least three causes: heat denaturation, inherent insolubility, or a complex of protein with another constituent of the fruit. We present evidence that heat-denatured proteins do not contribute to the cloud, but that inherently insoluble and/or complexed protein are responsible for this constituent.

INTRODUCTION

A fine suspension of particulate material, known as cloud, contributes substantially to quality factors such as color, flavor, and texture of citrus juices. Most research on citrus cloud has dealt with the causes of its instability in orange juice (Baker and Bruemmer, 1970), a problem which is due mainly to soluble factors in the juice and which can usually be prevented by appropriate heat treatment during processing. Cloud instability may also be encountered when citrus-flavored beverages are formulated, and in such cases the problem resides in the nature of the cloud particles.

A better knowledge of the basic components and of the

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