Studies on the Hydrolysis of Two Megastigma-3,6,9-triols Rationalizing the Origins of Some Volatile C_{13} Norisoprenoids of *Vitis vinifera* Grapes

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Heating diastereoisomeric mixtures of synthetic megastigm-4-ene-3,6,9-triol or megastigma-4,7-diene-3,6,9-triol in aqueous acid at pH 3–3.5 gave a number of volatile C_{13} norisoprenoid products as well as thermodynamically more stable rearranged triols. All of the compounds formed in these reactions have been found in grape products. The first of the synthetic norisoprenoid triols gave predominantly isomeric vitispiranes, 8-hydroxytheaspiranes and 3,4,6,8a-tetrahydro-2,5,5,8a-tetramethyl-2*H*-1-benzopyran-4a-(5*H*)-ols as volatiles together with megastigm-5-ene-3,4,9-triols. Minor volatiles identified were an edulan, 4-(2,3,6-trimethylphenyl)butan-2-ol, and 4-(2,4,6-trimethylphenyl)butan-2-ol. The second synthetic substrate gave, as major volatile dehydration products, the *trans*-actinidols and 2-(3-hydroxybut-1-enyl)-2,6,6-trimethylcyclohex-3-enone, together with lesser amounts of the *cis*-actinidols, dehydroionone, 1,1,6-trimethyl-1,2-dihydronaphthalene, and isomers of megastigma-4,6,8-trien-3-one. Megastigma-5,7-diene-3,4,9-triols were also formed in this reaction. Separate reactions of the two rearranged grape-derived C_{13} triols with aqueous acid gave the same volatile products as those given by the synthetic substrates.

Many highly potent norisoprenoid aroma compounds have been found in fruits and vegetables and most notably in the leaf products tea and tobacco (Ohloff, 1978). The formation of these compounds has been attributed to the degradation of higher molecular weight terpenoids such as carotenoids in the plant tissues (Enzell et al., 1977).

Several volatile C_{13} norisoprenoid compounds have also been identified in grapes, wines, and distilled grape spirits (Marais, 1983; Nykänen and Suomalainen, 1983). The extent of occurrence of these grape constituents with 13 carbons was made more apparent when C_{18} reversed-phase isolates from grape juice were hydrolyzed with acid. Under these conditions isolates from muscat grapes gave damascenone, vitispirane, 1,1,6-trimethyl-1,2-dihydronaphthalene, 4-(2,3,6-trimethylphenyl)but-3-en-2-one, 4-(2,3,6-trimethylphenyl)butan-2-one, 4-(2,3,6-trimethylphenyl)butan-2-ol, isomeric megastigma-4,7,9-trien-3-ones, and megastigma-4,6,8-trien-3-ones, along with several other unidentified C_{13} norisoprenoids (Williams et al., 1982a).

Subsequently it was demonstrated that a part of these reversed-phase grape isolates was made up of glycosidic derivatives of monoterpene flavorants of the fruit (Williams et al., 1982b). However, the nature of substances giving the C_{13} norisoprenoid volatiles is unknown as are the pathways by which these volatiles arise as grape and wine aroma compounds.

Recently the actinidols (**12**, **13**) (Figure 2) were identified as further norisoprenoids occurring naturally in grapes (Dimitriadis et al., 1985). In the course of this work the actinidols (**12** and **13**) were prepared by aqueous acid-catalyzed rearrangement of triols **11** or **15**.

It has been established that triols **15** and their dihydro analogues **7** (Figures 1 and 2) are present in grapes, bound most probably as glycosides. However, triols **1** and **11** have not as yet been observed in these fruits.

The hydrolytic chemistry of triols **1**, **7**, **11**, and **15** has been studied here to account for the origins of many of the volatile norisoprenoids seen in grapes and wine.

EXPERIMENTAL SECTION

Analytical Techniques. Analytical TLC and GLC, low-resolution MS, GC-MS, and NMR were carried out as previously described (Dimitriadis et al., 1985). In addition, GC–MS on the C_{13} triols and their acetate derivatives was carried out on a wide-bore fused silica column 25 m in length, with a 0.5-μm film thickness bonded phase of methylsilicone (supplied as a BPl column from SGE, Melbourne). The column was operated isothermally at 140 °C for 10 min and then programmed to 280 °C at 2 °C/min and held at the upper temperature.

Preparation of Megastigm-4-ene-3,6,9-triols (1). Dehydroionone (**17**) was prepared by the method of Surmatis and Thommen (1967). Regiospecific reduction of the side-chain double bond in **17** afforded megastigma-3,5-dien-9-one in 92% yield (Wolf and Zink, 1973). This ketone was then reduced with $NaBH_4$ in MeOH to megastigma-3,5-dien-9-ol, which was obtained as a colorless oil in 89% yield. Megastigma-3,5-dien-9-ol showed the following spectral properties: ^{1}H NMR (90 MHz, $CDCl_3$) δ 0.97 [6 H, s, $(CH_3)_2C$], 1.21 (3 H, d, J = 6 Hz, CH_3COH), 1.52 (3 H, m, OH, $CH_2CH_2CHOHCH_3$), 1.70 (3 H, s, $CH_3C{=}C$), 2.01 (2 H, d, J = 3 Hz, $Me_2CCH_2C{=}C$), 2.13 (2 H, m, $CH_2CH_2CHOHCH_3$), 3.80 (1 H, sextet, J = 6 Hz, $HCOH$), 5.63 (2 H, m, $HC{=}CH$); EI MS (70 eV) m/z (relative intensity) 194 (9), 121 (42), 120 (19), 119 (100), 107 (7), 105 (16), 93 (8), 91 (17), 79 (7), 77 (9), 55 (11), 45 (11), 43 (10), 41 (14).

Megastigma-3,5-dien-9-ol (1.45 g) was dissolved in Et_2O (150 mL) with *m*-chloroperoxybenzoic acid (1.53 g). After 2 h at room temperature, 5% aqueous $K_2S_2O_5$ solution (110 mL) was added and the mixture stirred for 30 min. Extraction of the aqueous phase with $CHCl_3$ (4 × 100 mL) gave triols **1**, R_f 0.56 ($MeOH/CH_2Cl_2$, 1/4, as solvent), as a colorless oil (900 mg). ^{1}H NMR of triols **1** (90 MHz, $CDCl_3$) δ 0.90 and 0.94 [6 H, 2 s, $(CH_3)_2C$], 1.13 (3 H, d, J = 6 Hz, CH_3COH), 1.70 (3 H, br s, $CH_3C{=}C$), 1.60 (6 H, m, 3 CH_2), 2.93 (3 H, br s, 3·OH), 3.64 (1 H, m, CH_3CHOH), 4.13 (1 H, m, $C{=}CCHOH$), 5.43 (1 H, br s, $HC{=}C$); EI MS of **1** (70 eV) m/z (relative intensity) 155 (43), 154 (4), 137 (17), 136 (7), 109 (51), 98 (16), 95 (37), 70 (10), 69 (13), 55 (16), 43 (100), 41 (33).

Rearrangement of Megastigm-4-ene-3,6,9-triols (1). Triols **1** (450 mg) were dissolved in saturated potassium hydrogen tartrate solution, pH 3.57 (100 mL), and heated on a boiling water bath for 20 min. After cooling in ice, the mixture was extracted with Freon F11 (2 × 150 mL), and the pooled extracts were dried with $MgSO_4$, and the solvent was distilled off at bath temperature 35 °C through a small column packed with Fenske's helices to yield an

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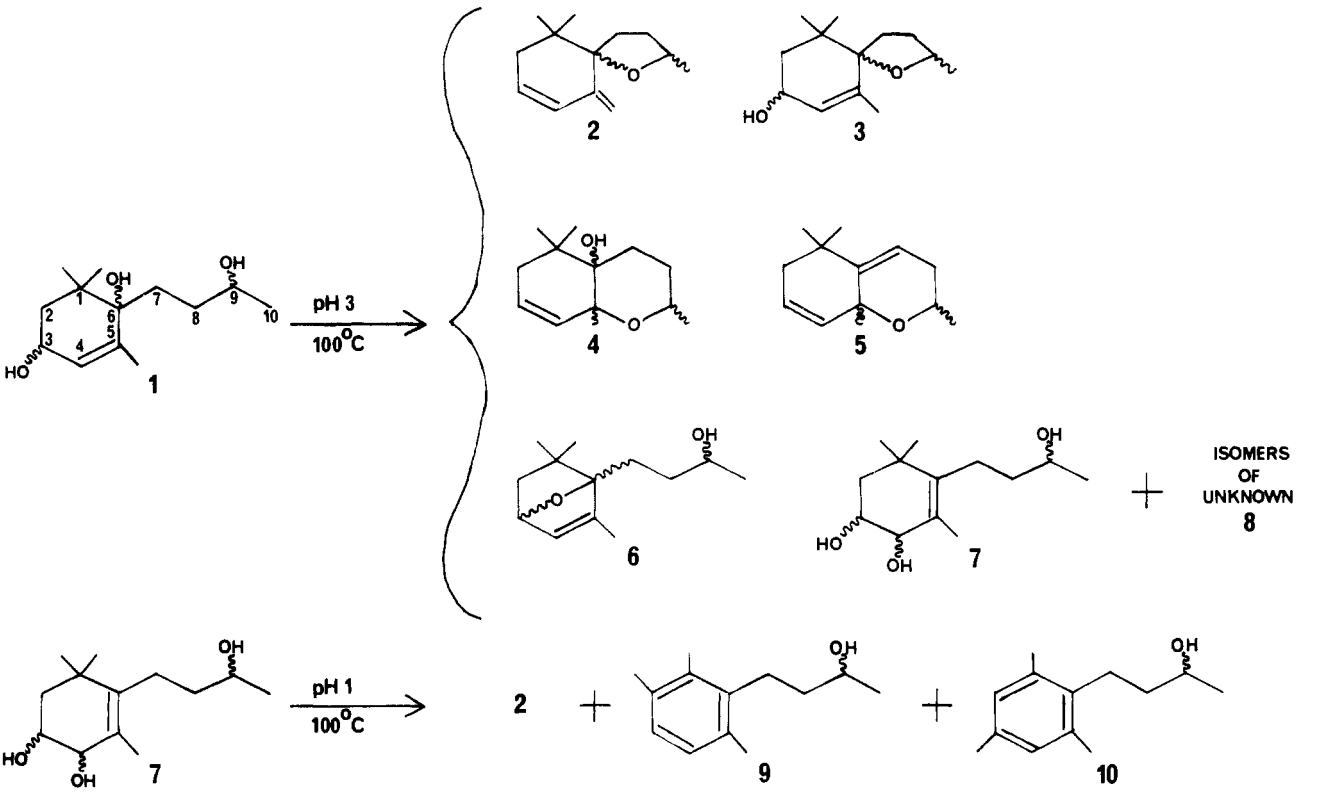

Figure 1. Reactions in aqueous acid of megastigm-4-ene-3,6,9-triols and megastigm-5-ene-3,4,9-triols.

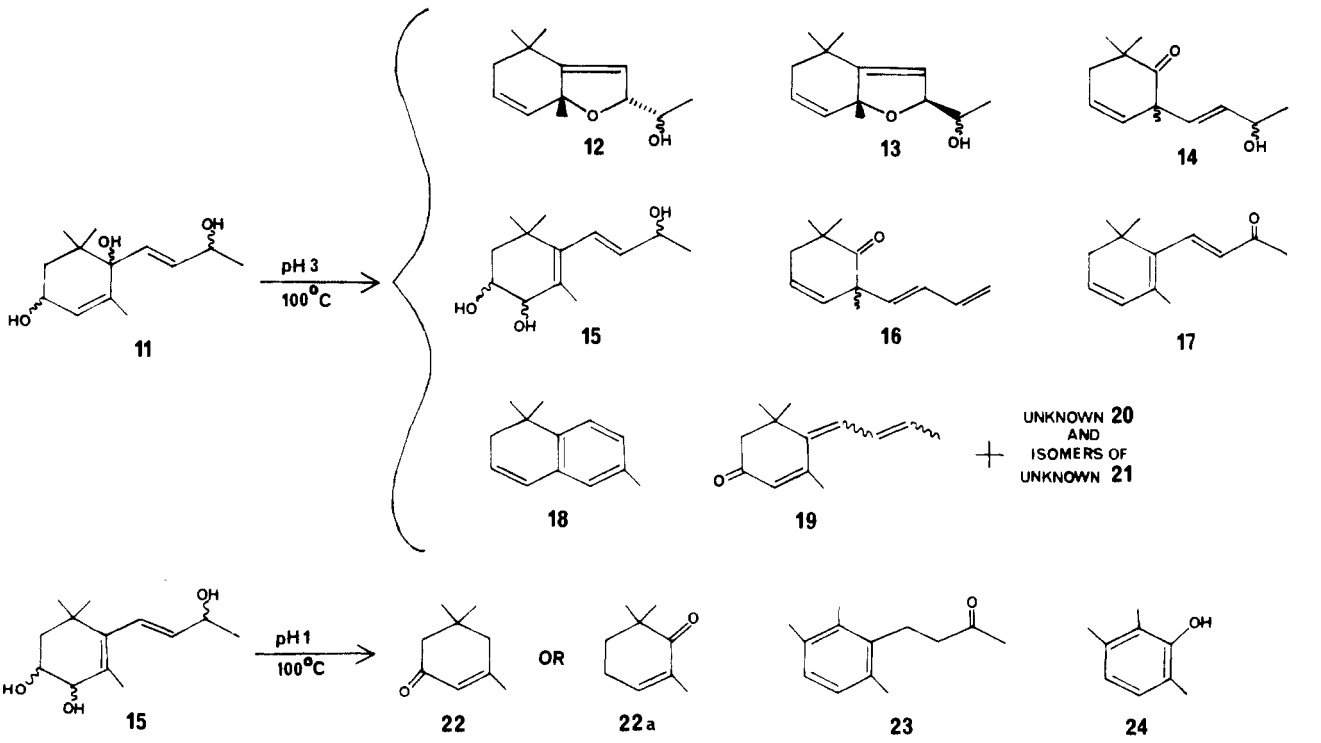

Figure 2. Reactions in aqueous acid by megastigma-4,7-diene-3,6,9-triols and megastigma-5,7-diene-3,4,9-triols.

oil (170 mg). The aqueous phase was further extracterd with $CHCl_3$ (3 × 100 mL), and this extract, after drying and concentration, afforded triols **7** (142 mg), R_f 0.55 ($MeOH/CH_2Cl_2$, 1/4, as solvent), as a colorless oil.

^1H NMR of triols **7** (90 MHz, $CDCl_3$) δ 1.02 and 1.04 [6 H, 2 s, $(CH_3)_2C$], 1.19 (3 H, d, J = 6 Hz, CH_3CHOH), 1.60 (5 H, m, 2 CH_2CHOH, 1 OH), 1.68 and 1.77 (3 H, 2 s, $CH_3C{=}C$), 1.99 (4 H, m, $CH_2C{=}C$, 2 OH), 3.65 (3 H, m, 3 HCOH); EI MS of triols **7** (70 eV) m/z (relative intensity) 210 (1), 195 (1), 177 (2), 166 (2), 154 (54), 137 (13), 135 (15), 126 (12), 125 (13), 121 (13), 111 (24), 109 (32), 107 (19), 95 (18), 93 (19), 91 (16), 81 (21), 79 (16), 69 (22), 67 (24), 57 (15), 55 (52), 53 (15), 45 (31), 43 (100), 41 (67).

Triols **7** were further characterized as their diastereoisomeric triacetate derivatives, R_f 0.58 (Et_2O solvent), after acetylation with Ac_2O/pyridine.

^1H NMR of triacetates of triols **7** (90 MHz, $CDCl_3$) δ 1.06 1.10, 1.13 [6 H, 3 br s, $(CH_3)_2C$], 1.25 (3 H, d, J = 6 Hz, CH_3CHOAc), 1.55 and 1.64 (3 H, 2 s, $CH_3C{=}C$), 1.68 (4 H, m, 2 CH_2CHOAc), 1.99, 2.02, 2.05, 2.07, 2.10 (11 H, 5

Table I. Volatile Products from Aqueous Acid Treatment of C_{13} Norisoprenoid Triols

compd[a]	ret index[b]	evidence for assgnt	ref for assgnt	ref as grape prod.	rel proportn[d] of volatiles given by substr at pH 3–3.5 1	pH 3–3.5 7	pH 3–3.5 11	pH 3–3.5 15	pH 1–1.5 7	pH 1–1.5 15
vitispirane (2) (isomer 1)	840	A, B	*e*	*e, f*	++	++			++++	
vitispirane (2) (isomer 2)	842	A, B	*e*	*e, f*	++	++			++++	
edulan (5)	936	C	*g*	*h*	+	+				
hydroxy ether 6 (isomer 1)	1175	D		*i*	+					
hydroxydihydroedulan 4 (isomer 1)	1185	E	*j, k*	*i, l*	++++	+++			+	
hydroxy ether 6 (isomer 2)	1186	D		*i*	++	+			++	
hydroxydihydroedulan 4 (isomer 2)	1244	E	*j, k*		+++	++				
8-hydroxytheaspirane (3) (isomer 1)	1378	A, B	*m*	*i, l*	++	++				
8-hydroxytheaspirane (3) (isomer 2)	1389	A, B	*m*	*i*	+++	+++				
8-hydroxytheaspirane (3) (isomer 3)	1397	A, B	*m*	*i*	++	++				
hydroxy ether 6 (isomer 3)	1409	D			+	+				
hydroxy ether 6 (isomer 4)	1416	D		*i*	++	+			+	
8-hydroxytheaspirane (3) (isomer 4)	1424	C	*m*	*i*	++	+++				
unknown 8 (isomer 1)	1463				++	++				
unknown 8 (isomer 2)	1474				++	++				
unknown 8 (isomer 3)	1536				++	++				
unknown 8 (isomer 4)	1547				++	++				
4-(2,3,6-trimethylphenyl)butan-2-ol (9)	1500	C	*n*	*f*	+	+			++++	
4-(2,4,6-trimethylphenyl)butan-2-ol (10)	1540	D		*i*	+	+			+++	
trienone 16	1006	D		*l*			++	+		++
1,1,6-trimethyl-1,2-dihydronaphthalene (18)	1060	C, F		*f, o*			++	+		++
unknown 20	1127	D		*l*			++	+		
trans-actinidol (12) (isomer 1)	1200	A, F	*p*	*l, f, p*			+++	++++		
trans-actinidol (12) (isomer 2)	1211	A, F	*p*	*l, f, p*			++++	++++		
dehydroionone (17)	1250	A, E	*q*	*l*			++	++		
cis-actinidol (13) (isomer 1)	1257	A, F	*p*	*l*			+	++		
unknown 21 (isomer 1)	1326			*l*			++	++		
cis-actinidol (13) (isomer 2)	1334	A, F	*p*	*l*			+	++		
megastigma-4,6,8-trien-3-one (19) (isomer 2)	1388	C	*r*	*f*			+	++		
hydroxydienones 14	1414	A, E		*l*			++++	++++		
megastigma-4,6,8-trien-3-one (19) (isomer 3)	1436	C	*r*	*f*			+	+		
unknown 21 (isomer 2)	1440							++		
megastigma-4,6,8-trien-3-one (19) (isomer 4)	1455	C	*r*	*f*				++		
isophorone (22) or 2,6,6-trimethylcyclohex-2-enone (22a)	693	C	*s*	*f*						++++
4-(2,3,6-trimethylphenyl)butan-2-one (23)	1445	C	*t*	*f*						++
2,3,6-trimethylphenol (24)	1290	C	*s*							+

[a] For compound numbers, see Figures 1 and 2. [b] Van Den Dool and Kratz, 1963. [c] A, the mass spectrum of the component was identical with that of the reference compound when recorded under similar conditions; B, the peak was enhanced by the reference compound when cochromatgraphed on the SP1000 or the BP1 column; C, the mass spectrum was consistent with that of published data; D, structure tentatively assigned on the basis of mass spectral interpretation; E, established in this work (see the Experimental Section); F, established previously in this laboratory. [d] Peak sizes were ranked as follows: +, <1% of the total volatile product; ++, 1–10% of the total volatile product; +++, 10–20% of the total volatile product; ++++, >20% of the total volatile product. [e] Simpson et al., 1977. [f] Williams et al., 1982a. [g] Whitfield and Stanley, 1977. [h] Ter Heide et al., 1978. [i] Observed in this laboratory in acid hydrolysates of C_{18} reversed-phase isolates of Muscat of Alexandria or Rhine Riesling grapes. [j] Etoh et al., 1980. [k] Etoh, 1984. [l] Observed in this laboratory in steam distillates of acidified muscat grape juice. [m] Kaiser et al., 1978. [n] Kaiser and Lamparsky, 1978. [o] Williams and Strauss, 1978. [p] Dimitriadis et al., 1985. [q] Surmatis and Thommen, 1967. [r] Aasen et al., 1972. [s] Heller and Milne, 1978. [t] Thomas et al., 1969.

s plus m, 3 CH_3CO plus $CH_2C{=}C$), 5.01 (2 H, m, 2 CH_2CHOAc), 5.43 (1 H, m, C=CCHOAc); EI MS of triacetates of **7** (70 eV) *m/z* (relative intensity) 294 (2), 252 (6), 234 (6), 210 (4), 192 (24), 177 (23), 159 (11), 150 (33), 137 (44), 135 (34), 121 (12), 119 (16), 55 (16), 43 (100). The concentrated Freon extract (170 mg) from the aqueous acid-catalyzed rearrangement of triols **1** was analyzed by GC and GC–MS. Products observed, along with relative proportions based on GC peak heights, evidence for assignment, and mass spectral data are shown in Tables I and II.

Diastereoisomers 3,4,6,8a-tetrahydro-2,5,5,8a-tetramethyl-2*H*-1-benzopyran-4a(5*H*)-ol (hydroxydihydroedulans **4**) were isolated from the reaction mixture by flash chromatography (Still et al., 1978). The ^1H NMR spectrum of the isomer with GC retention index 1244 (Table I) was identical with that of the hydroxydihydroedulan **4** previously assigned as 2β, 4aα, 8aα (Etoh et al., 1980; Etoh, 1984). However, the ^1H NMR spectrum of the hydroxydihydroedulan **4** isomer with GC retention index 1185 (Table I) differed from that of the 2α, 4aα, 8aα isomer (Etoh, 1984) and showed the following signals (90 MHz, $CDCl_3$): δ 1.00 and 1.05 [6 H, 2 s, $(CH_3)_2C$], 1.10 (3 H, d, J = 6.3 Hz, CH_3CHO), 1.35 (3 H, s, CH_3CO), 1.6 (7 H, m, 3 CH_2, 1 OH), 3.50 (1 H, sextet, CH_3CHO), 5.55 (2 H, m, $HC{=}CH$). There was an impurity in the sample that was altered in proportion but not completely eliminated by repeated chromatography on silica gel 60. This gave signals at δ 1.23 and 1.53.

Preparation of Megastigma-4,7-diene-3,6,9-triols (11). Dehydroionone (**17**) (2.0 g) was added to a solution of 80% *m*-chloroperoxybenzoic acid (2.5 g) in Et_2O (50 mL). The solution was allowed to stand at room temperature for 2 h, and 3% aqueous $Na_2S_2O_5$ (50 mL) was then added. After 2.5 h of stirring at room temperature the mixture was basified with powdered $NaHCO_3$ and the Et_2O phase separated. The aqueous phase was extracted with CH_2Cl_2 (3 × 20 mL), the organic phases were pooled, dried with $MgSO_4$, and concentrated in vacuo, and the residue was subjected to flash chromatography in an Et_2O/CH_2Cl_2 gradient to afford diastereoisomeric 3,6-dihydroxymegastigma-4,7-dien-9-one as a colorless oil (1.5

Table II. Mass Spectral Data for Volatile Products Given on Aqueous Acid Treatment of Triols 1, 7, 11, and 15

compd	signif ions (intens %)
hydroxy ether **6** (isomer 1)	43 (100), 82 (16), 95 (36), 97 (15), 139 (40), 154 (11)
hydroxy ether **6** (isomer 2)	41 (25), 43 (100), 55 (12), 57 (12), 82 (13), 95 (37), 139 (40), 154 (59), 155 (5)
hydroxy ether **6** (isomer 3)	41 (21), 43 (100), 57 (12), 95 (31), 139 (32), 154 (29)
hydroxy ether **6** (isomer 4)	41 (17), 43 (100), 57 (13), 82 (9), 95 (35), 139 (43), 154 (17), 155 (1)
hydroxydihydroedulan **4** (isomers 1 and 2)	41 (29), 43 (56), 55 (16), 67 (23), 69 (18), 70 (33), 71 (13), 84 (28), 85 (27), 111 (17), 126 (100), 127 (13), 195 (3), 210 (0.4)
unknown **8** (isomer 1)	43 (100), 55 (15), 91 (10), 93 (23), 95 (13), 121 (8), 136 (21), 159 (2), 163 (2), 177 (5), 192 (18)
unknown **8** (isomer 2)	43 (100), 55 (17), 93 (21), 95 (13), 121 (10), 136 (18), 150 (4), 159 (3), 177 (5), 192 (13)
unknown **8** (isomer 3)	43 (100), 55 (22), 77 (17), 91 (18), 93 (40), 95 (28), 107 (13), 119 (20), 121 (25), 136 (25), 159 (2), 177 (6), 192 (3)
unknown **8** (isomer 4)	43 (100), 55 (22), 77 (15), 91 (18), 93 (38), 95 (28), 107 (12), 119 (25), 121 (28), 136 (28), 149 (7), 177 (7), 192 (5)
4-(2,4,6-trimethylphenyl)-butan-2-ol (**10**)	77 (17), 91 (30), 105 (24), 115 (21), 117 (31), 119 (95), 120 (20), 132 (52), 133 (100), 134 (97), 145 (22), 159 (49), 192 (43), 193 (5)
trienone **16**	41 (58), 55 (27), 77 (33), 79 (21), 91 (66), 92 (25), 105 (91), 119 (58), 120 (100), 147 (6), 162 (4)
unknown **20**	77 (5), 115 (8), 128 (7), 129 (6), 141 (30), 142 (85), 143 (7), 157 (100), 158 (5), 172 (35)
dehydroionone (**17**)	43 (100), 77 (6), 79 (7), 91 (11), 105 (8), 115 (7), 131 (11), 147 (9), 175 (32), 190 (8)
unknown **21** (isomer 1)	43 (100), 57 (60), 77 (28), 91 (40), 105 (28), 107 (25), 119 (22), 131 (25), 137 (15), 149 (60), 164 (58), 175 (8), 193 (95), 194 (10), 208 (2)
hydroxy dienones **14**	43 (100), 55 (15), 67 (12), 69 (10), 77 (15), 79 (18), 81 (20), 91 (15), 93 (22), 95 (75), 107 (35), 108 (25), 122 (18), 138 (20), 147 (2), 164 (3), 180 (1), 190 (0.5), 208 (0.5)
unknown **21** (isomer 2)	43 (85), 55 (22), 57 (20), 77 (22), 91 (30), 105 (25), 107 (26), 119 (16), 131 (25), 149 (50), 164 (18), 175 (5), 193 (100), 194 (10), 208 (2)

g). The isomers showed the following spectral properties: ^{1}H NMR (90 MHz, $CDCl_3$) δ 0.91 and 1.03 [6 H, 2 s, $(CH_3)_2C$], 1.62 (3 H, br s, $CH_3C{=}C$), 1.6–2.1 (2 H, m, Me_2CCH_2), 2.29 (3 H, s, CH_3CO), 2.65 (1 H, s, OH), 3.37 (1 H, br, OH), 4.28 (1 H, br, *H*COH), 5.62 (1 H, br s, *H*C=CMe), 6.34 (1 H, d, J = 16 Hz, C=CHCO) 6.89 (1 H, d, J = 16 Hz, *H*C=CHCO). EI MS probe (70 eV) m/z (relative intensity) 206 (4), 191 (3), 150 (10), 135 (10), 125 (16), 108 (37), 97 (12), 69 (13), 55 (19), 43 (100), 41 (42).

3,6-Dihydroxymegastigma-4,7-dien-9-ones (170 mg) were dissolved in MeOH (10 mL), and $CeCl_3{\cdot}7H_2O$ (100 mg) and $NaBH_4$ (20 mg) were added (Luche, 1978). After 10 min, H_2O (10 mL) was added and the solution saturated with NaCl and extracted with CH_2Cl_2 (7 × 10 mL). The pooled extracts were dried with $MgSO_4$ and concentrated to yield triols **11** (165 mg). ^{1}H NMR (90 MHz, $CDCl_3$) δ 0.89 and 0.98 [6 H, 2 s, $(CH_3)_2C$], 1.27 (3 H, d, J = 6.6 Hz, CH_3CHOH), 1.63 (8 H, br s plus m, $CH_3C{=}C$, 3 OH, $-CH_2-$), 4.25 (2 H, m, C=CC*H*OH), 5.4–6.0 (3 H, m, HC=C); EI MS probe (70 eV) m/z (relative intensity) 170 (13), 152 (48), 137 (29), 123 (23), 121 (35), 109 (65), 108 (25), 107 (38), 95 (56), 93 (24), 91 (25), 81 (23), 79 (24), 71 (26), 69 (33), 55 (45), 43 (100), 41 (80).

Rearrangement of Megastigma-4,7-diene-3,6,9-triols (11) at pH 3. Triols **11** (165 mg) were dissolved in tartaric acid/potassium bitartrate buffer at pH 3 (20 mL), and the resultant mixture was heated on a boiling water bath for 15 min. The reaction mixture was cooled and extracted with CH_2Cl_2 (2 × 10 mL) and $CHCl_3$ (12 × 10 mL), and the pooled extracts were concentrated and subjected to flash chromatography on silica gel with a CH_2Cl_2/MeOH gradient. Rearranged triols **15** (45 mg) were the only isolated products not amendable to gas chromatography on the SP1000 column. The relative proportions of volatile products given by the hydrolysis, along with evidence for the assignments, and mass spectral data, are shown in Tables I and II.

Megastigma-5,7-diene-3,4,9-triols (**15**) had the following spectral properties: ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.03 and 1.06 [6 H, 2 s, $(CH_3)_2C$], 1.32 (3 H, d, J = 7 Hz, CH_3CHOH), 1.5–2.0 (2 H, m, $-CH_2-$), 1.85 (3 H, s, $CH_3C{=}C$), 2.12 (3 H, br, 3 OH), 3.7–3.9 [2 H, m, $-CHOHCHOHC(Me){=}C$], 4.39 (1 H, quintet, J = 7 Hz, CH_3CHOH), 5.54 (1 H, dd, J = 7 Hz and 17 Hz, C=CHCHOHMe), 6.04 (1 H, br d, J = 17 Hz, *H*C=CHCHOHMe); EI MS (70 eV) m/z (relative intensity) 208 (10), 190 (2), 179 (2), 162 (5), 149 (8), 137 (15), 135 (23), 123 (20), 121 (32), 109 (25), 107 (26), 95 (15), 91 (15), 55 (23), 43 (100), 41 (35).

2-(3-Hydroxybut-1-enyl)-2,6,6-trimethylcyclohex-3-enone (hydroxydienones **14**) had the following NMR spectrum (90 MHz, $CDCl_3$): δ 1.12 [6 H, s, $(CH_3)_2C$], 1.22 (3 H, 2 d partly obscured, CH_3CHOH), 1.24 (3 H, s, CH_3 tertiary C), 1.54 (1 H, b, OH), 2.24 (2 H, d, J = 3 Hz, $-CH_2-$), 4.27 (1 H, m, CH_3CHOH), 5.73 (4 H, m, HC=C).

RESULTS AND DISCUSSION

The various hydrolytic reactions studied in this work are shown in Figures 1 and 2. Evidence for the structures of the compounds is summarized in Table I. Additionally, Table I gives the occurrence in grapes, as well as other grape-derived sources, of the various products obtained from aqueous acid treatment of triols **1**, **7**, **11**, and **15**. Figure 1 shows the reaction of diastereoisomeric megastigm-4-ene-3,6,9-triols (**1**) in tartrate buffer at pH 3.5 and 100 °C. Products from this hydrolysis were obtained in 75% yield based on starting triols, and, of these, 43% were the rearranged triols **7**. Less polar rearrangement products were isolated as a Freon-soluble oil and this was analyzed by GC. Major volatiles were the spiro ethers, vitispiranes (**2**), and 8-hydroxytheaspiranes (**3**) along with the diastereoisomeric hydroxydihydroedulans **4**. Only small amounts of edulan (**5**), the tentatively assigned hydroxy ethers **6**, isomeric unknowns **8** and aromatic alcohols **9** and **10** were observed.

Separate reaction of rearranged triols **7** at pH 3.5 gave the same volatiles and in similar proportions to those given by **1**. Also, reaction of 8-hydroxytheaspiranes (**3**) at pH 3.5 gave vitispiranes (**2**) as the sole products. The thermodynamic stability of the vitispirane skeleton was further demonstrated by the results of a hydrolytic study on triols **7** at pH 1. This reaction gave vitispiranes (**2**) and the aromatic alcohols **9** and **10** as major volatile products.

On heating at pH 3 diastereoisomeric megastigma-4,7-diene-3,6,9-triols (**11**) gave a range of volatile dehydration products as well as thermodynamically more stable isomers **15** analogous to those given by the reduced compounds **1** (see Figure 2). The volatiles obtained from triols **11** at pH

3 were dominated by the *trans*-actinidols (12) (Dimitriadis et al., 1985) and the hydroxy dienones 14. Only small quantities of other compounds were produced, among which were identified *cis*-actinidols (13), trienone 16, dehydroionone (17), 1,1,6-trimethyl-1,2-dihydronaphthalene (18), and three isomers of megastigma-4,6,8-trien-3-one (19). Additionally, a compound isomeric with the hydrocarbon 18 was observed as unknown 20. One isomer of a pair, i.e. unknowns 21, was also recognized in the mixture.

Independent reaction of megastigma-5,7-diene-3,4,9-triols (15) at pH 3 gave volatile products similar to those formed by the synthetic substrate 11.

Heating triols 15 at pH 1 led to degradation of the megastigmane skeleton and gave isophorone or 2,6,6-trimethylcyclohex-2-enone (22 or 22a) as the major volatile product.

Spiro Compounds and Edulans from 1 and 7. The formation of spiro ethers 2 and 3 in the mild aqueous acid treatment of megastigma-4-ene-3,6,9-triol (1) or megastigm-5-ene-3,4,9-triol (7) is of significance in view of the lack of success reported for the synthesis of vitispiranes by acid-catalyzed reactions, under a variety of conditions, of alcohols analogous to triols 1 and 7 (Schulte-Elte et al., 1978; Etoh et al., 1980). Possibly the aqueous conditions employed in the present study were important to the formation of vitispiranes (2) and 8-hydroxytheaspiranes (3) from 1 or 7, and since these are the conditions existing in wines and grape juice, the occurrence of vitispiranes can be rationalized, at least in part, by this circumstance. However, the presence in grape juices, wines, or spirits of only trace amounts of other major hydrolysis products of 7, i.e. hydroxydihydroedulans 4 and 8-hydroxytheaspiranes (3), suggests that other pathways must exist for the occurrence of vitispiranes (2). Recent studies (Strauss et al., 1984) have indicated that hydrolysis of a glycoside of 8-hydroxytheaspiranes (3) is the major route to vitispiranes (2) in grape products.

Actinidols (12) and 2-(3-Hydroxybut-1-enyl)-2,6,6-trimethylcyclohex-3-enone (14) from 11 and 15. With regard to products formed at pH 3 from the more highly oxidized triols 11 and 15, the *trans*-actinidols (12) have been previously discussed as grape constituents (Dimitriadis et al., 1985). The other major products, hydroxy dienones 14 together with minor compounds 13 and 16–19, have also been observed in grape juice steam distillates and hydrolysates of reversed-phase isolated material from juice (see Table I). In most cases where *trans*-actinidols (12) and hydroxy dienones 14 have been observed together in grape products, the *trans*-actinidols (12) have been present in significantly greater proportion than the latter compounds 14. However, the ratio of 12 to 14 is reversed when they are formed by aqueous acid treatment of triols 11 or 15 or by mild acid hydrolysis of the enzyme-released aglycones from the reversed-phase retained glycosides from Chardonnay or Shiraz juices. These data indicate that a glycosidic derivative of triol 15, or related species, occurs naturally in the fruit and is responsible for the hydrolytic origin of *trans*-actinidols (12) in grapes.

CONCLUSION

This study rationalizes the origin of several C_{13} norisoprenoid volatiles observed in grape products. In particular the vitispiranes (2), compounds long known as wine volatiles (Simpson et al., 1977), and the *trans*-actinidols (12) (Dimitriadis et al., 1985), which are more recently recognized constituents, are accounted for by pathways explored here. A number of other compounds, originating from natural grape triols 7 or 15 are trace compounds of wines, juices, and spirits and had not previously been reported.

Nevertheless, many norisoprenoid products formed on hydrolysis of grape glycoside fractions (Williams et al., 1982a) cannot be explained by the reactions studied here. Importantly, α-ionone, which was reported early as a wine product (Drawert and Rapp, 1968), together with the potent flavorants β-ionone and damascenone, which are both grape and wine volatiles (Schreier, 1979), do not have their origins in triols 7 and 15. The precursors of these highly fragrant flavor compounds are the subject of active research.

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