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Novel Monoterpene Disaccharide Glycosides of *Vitis vinifera* Grapes

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Acuminoside (geranyl 6-*O*-β-D-apiofuranosyl-β-D-glucopyranoside) has been characterized in *Vitis vinifera* vars. Muscat of Frontignan, Muscat of Alexandria, Muscat Ottonel, Muscat of Hambourg, and Gewürztraminer. Moreover, similar disaccharide glycosides of monoterpenols, of 2-phenylethanol, and of benzyl alcohol were tentatively identified on the basis of the GC–EIMS and GC–CIMS of their trimethylsilyl and trifluoroacetyl derivatives.

1. INTRODUCTION

Since Cordonnier and Bayonove (1974) first suggested the occurrence of monoterpene glycosides in *Vitis vinifera* var. Muscat of Alexandria, extensive research has been carried out on their chemical structure. These compounds, although odorless, can be hydrolyzed under certain conditions to yield increases in the concentrations of volatile flavorants with quite low aroma threshold values (Günata et al., 1989). Williams et al. (1982, 1983) first identified them as 6-*O*-α-L-rhamnopyranosyl-β-D-glucopyranosides (β-rutinosides) (I), 6-*O*-α-L-arabinofuranosyl-β-D-glucopyranosides (II), and β-D-glucopyranosides (III) with monoterpenyl aglycons at the linalool oxidation state and benzyl and 2-phenylethyl aglycons (Figure 1); the same group described later such glycosides with other aglycons, monoterpenoids, C13 norisoprenoids, phenols, and other compounds (Strauss et al., 1987a,b, 1988), and presented data substantiating the importance of these tasteless compounds as a reserve of grape flavor (Strauss et al., 1985; Noble et al., 1987, 1988).

To further progress with the knowledge of their structural, chemical, and biochemical properties, we focused on their chemical synthesis (Voirin et al., 1989; Günata et al., 1989a), their enzymatic hydrolysis (Günata et al.,

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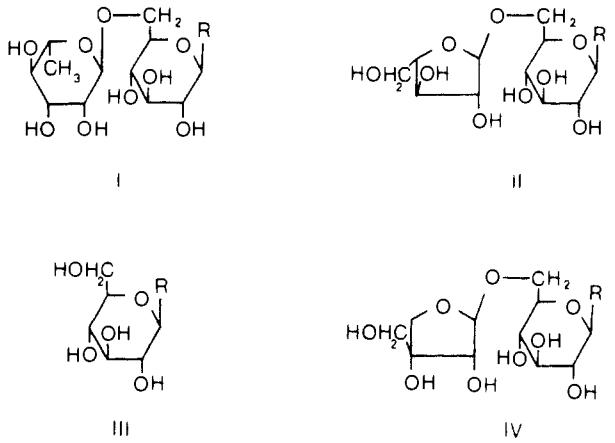

Figure 1. Structure of glycosides with volatile aglycons in grape. R = monoterpenyl, benzyl, 2-phenylethyl group. Compounds I–III were identified in previous studies (see introduction).

1988, 1989, 1990; Cordonnier et al., 1989), and their direct determination using GC (Pierrisnard, 1986; Günata et al., 1989; Bitteur et al., 1989; Voirin, 1990) and HPLC (Günata et al., 1989; Bitteur et al., 1989). Although HPLC allowed direct analysis of monoterpenyl glycosides without derivatization, its main limitation was due to the lack of a routine HPLC–MS coupling (Bitteur et al., 1989). On the contrary, comparative analysis of such synthetic and grape glycosides (*V. vinifera* vars. Muscat of Alexandria, Muscat of Hambourg, Muscat of Frontignan, Muscat Ottonel, and Gewürztraminer) by means of GC–EIMS of their trimethylsilyl (TMS) and trifluoroacetyl (TFA) derivatives proved useful, as it enabled positive or tentative identification (further sustained by CIMS of TMS derivatives) and quantitative determination of the known diglycosides (Pierrisnard, 1986; Voirin, 1990) and also of novel grape disaccharide glycosides, 6-*O*-β-D-apiofuranosyl-β-D-glucopyranosides (IV) (Figure 1) of monoterpenols, 2-phenylethanol, and benzyl alcohol, the structure of which we report here.

2. EXPERIMENTAL PROCEDURES

2.1. Reagents and Reference Samples. Analytical grade solvents were further purified by redistillation before use. Amberlite XAD-2 resin from Rohm and Haas was purified according to the procedure of Günata (1984). TMS and TFA reagent were purchased, respectively, from Touzart et Matignon and Sigma. Phenyl and *p*-nitrophenyl β-D-glucopyranosides were purchased from Sigma. Acuminoside was a gift from Dr. Wentler. Geranyl, neryl, linalyl, benzyl, and 2-phenylethyl 6-*O*-α-L-arabinofuranosyl-β-D-glucopyranosides as well as the corresponding rutinosides were synthesized according to the method of Voirin et al. (1989).

2.2. Glycoside Sampling. Mature, sound grapes (cultivars Muscat of Frontignan, Muscat of Alexandria, Muscat Ottonel, Muscat of Hambourg, and Gewürztraminer) were collected in 1988 at the vineyard of the vine experimental station in Montpellier, France.

Glycoside extracts from grape were obtained according to the method of Günata (1985): a sample of 50 mL of juice, to which were added phenyl and *p*-nitrophenyl β-D-glucopyranosides (1 mg of each one) as internal standards, was passed through a column of Amberlite XAD-2 resin previously equilibrated with water, and the column was washed with water and pentane. Glycosides were then recovered by elution with ethyl acetate, and the eluate was concentrated to dryness.

2.3. Methods of Analysis. *2.3.1. Trimethylsilylation of Glycosides.* A glycoside sample obtained from 10 mL of grape juice as reported above or a mixture of synthetic glycosides (5 μg of each compound) was concentrated to dryness at 60 °C under nitrogen. After the addition of 20 μL of anhydrous pyridine and 20 μL of the TMS reagent [*N*,*O*-bis(trimethylsilyl)-trifluoroacetamide and chlorotrimethylsilane 99-1], the mixture was stirred, stored for 20 min at 60 °C, and then cooled at room temperature.

2.3.2. Trifluoroacetylation of Glycosides. A glycoside sample obtained from 15 mL of grape juice as reported above or a mixture of synthetic glycosides (10 μg of each compound) was treated as above by using 20 μL of the TFA reagent [*N*-methylbis(trifluoroacetamide)] instead of the TMS reagent.

2.3.3. Gas Chromatographic Analysis of TMS Derivatives of Glycosides. The TMS derivatives prepared as reported above were analyzed on an OV1 fused silica capillary column (Delsi Instruments; 50 m × 0.32 mm i.d.; 0.2 μm bonded phase). Injections of about 0.8 μL were on-column: the injector temperature was programmed at 60 °C min^{-1} from 90 to 150 °C and then at 10 °C min^{-1} to 300 °C. The column temperature was programmed at 3 °C min^{-1} from 130 to 300 °C with 2 mL min^{-1} hydrogen carrier gas and fid temperature at 320 °C.

2.3.4. Gas Chromatographic Analysis of TFA Derivatives of Glycosides. The TFA derivatives prepared as reported above were analyzed on a CP-Sil 8 CB fused silica capillary column (Chrompack; 25 m × 0.32 mm i.d.; 1.2 μm bonded phase). Injections of about 0.8 μL were on-column: the injector temperature was programmed at 60 °C min^{-1} from 90 to 150 °C and then at 10 °C min^{-1} to 300 °C. The column temperature was programmed at 3 °C min^{-1} from 125 to 300 °C with 1.3 mL min^{-1} hydrogen carrier gas and fid temperature at 300 °C.

2.3.5. Gas Chromatography Mass Spectrometry of Glycoside Derivatives. Electron impact mass spectra (EIMS) were recorded for the TMS and the TFA derivatives by coupling a Girdel 31 gas chromatograph, equipped with the same fused silica capillary columns described respectively under sections 2.3.3 and 2.3.4 to a Nermag R10-10 mass spectrometer. The transfer line consisted of a platinum capillary tube heated to 260 °C. The source temperature was 200 °C. Mass spectra were scanned at 70 eV in the range m/e 60–1050 at 2.87-s intervals.

Chromatographic conditions were as follows: injections of 2 μL of glycoside derivatives (TMS or TFA prepared as above) were injected with a 10:1 split into an injector held at 320 °C. Helium carrier gas head pressure was 90 kPa for TMS derivatives and 10 kPa for TFA derivatives.

For TMS derivatives, the column was programmed at 3 °C min^{-1} from 130 to 300 °C and for TFA derivatives at 4 °C min^{-1} from 120 to 280 °C.

Chemical ionization mass spectra (CIMS) were recorded only for the TMS derivatives, using the same GC and transfer line conditions as reported for EIMS. The source temperature was 90 °C, and ammonia was used as the reactant gas. Mass spectra were scanned at 70 eV in the range m/e 60–1050 at 2.87-s intervals.

3. RESULTS AND DISCUSSION

Surprisingly, in the range of GC retention time of the TMS and TFA derivatives of the diglycosides in the grape extracts appeared a set of unknown glycosides showing EIMS (Tables I and II) similar to those of the corresponding 6-*O*-α-L-arabinofuranosyl-β-D-glucopyranosides (Voirin, 1990) with the same molecular weight according to the CIMS of their TMS derivatives and present in similar amounts (Tables I and II).

For both kinds of derivatives, EIMS data showed characteristic fragment ions derived from both the aglycon and the sugar moiety (especially from both monosaccharide units) as observed for the corresponding known glycosides (Williams et al., 1982, 1983; Pierrisnard, 1986) and for synthetic compounds (Voirin, 1990). This allowed the determination of the monoterpenyl, benzyl, and 2-phenylethyl nature of the corresponding aglycons (Tables I and II list the characteristic fragment ions of these aglycons) and the terminal pentosylhexoside nature of their sugar moiety. Indeed, their TFA derivatives exhibited the characteristic fragment ions at m/e 193 and 421 (tri-TFA pentosyl fragment and subsequent loss of two

Table I. Mass Spectra of TMS Derivatives of 6-*O*-β-D-Apiofuranosyl-β-D-glucopyranosides[a] Detected in *Vitis vinifera* Grape Extracts

aglycon residue[b]	EIMS, characteristic fragment ions of: sugar moiety	aglycon moiety	sugar and aglycon moieties	mol wt[c] MW_s	mol wt of aglycon[d] MW_a	occurrence in grape[e]: F	A	O	H	G	rrt[f]
benzyl	191 (100); 204 (82); 217 (20) 259 (16); 349 (0.2)	91 (28)	209 (6); 469 (1)	834	108	+	+	++	+	++	1.200
a linalyl oxide	191 (100); 259 (59); 217 (48) 204 (32); 349 (0.6)	153 (57); 71 (20) 81 (12); 69 (8) 135 (5); 93 (4)	271 (2); 531 (1)	896	170	+++	+	+	+	++	1.200
a linalyl oxide	191 (100); 259 (53) 204 (50); 217 (8); 349 (2)	153 (12); 71 (5) 81 (0.7); 69 (3) 135 (3)				+	–	–	–	–	1.223
a linalyl oxide	191 (100); 259 (47) 217 (40); 204 (18); 349 (4)	153 (94); 71 (57) 135 (11); 93 (2)				+	–	–	–	–	1.226
neryl[g]	259 (100); 191 (74) 217 (40); 204 (13); 349 (1)	81 (16); 69 (12) 137 (6); 136 (2) 95 (3); 93 (1)	515 (0.1)	880	154	+++	+	+++	+++	+	1.230
2-phenylethyl	204 (100); 191 (95); 217 (28) 259 (24); 349 (1)	105 (27) 95 (3); 93 (1)	223 (10); 483 (2)	848	122	–	+	++	+	–	1.257
geranyl[h]	259 (100); 191 (68); 204 (42) 217 (39); 363 (15); 273 (5) 349 (2)	69 (23); 81 (12) 137 (3); 136 (1) 95 (2); 93 (1)	515 (0.4); 255 (1)	880	154	+	++	++	++	++	1.268

[a] See Table III for unsubstituted glycosides. [b] Positive identification for geranyl; otherwise, tentative identification. [c] From CIMS with ammonia as reactant gas. [d] Obtained as: $MW_a = MW_s - 727$ (molecular weight of the sugar moiety) + 1. [e] F, A, O, H: respectively, muscat of Frontignan, Alexandria, Ottonel, Hambourg, G: gewürztraminer. Quantities were estimated by comparison of the total ion count for the MS of each peak with that of *p*-nitrophenyl β-D-glucopyranoside internal standard; –, not detected; +, <100 μg/L; ++, 100–400 μg/L; +++, >400 μg/L. [f] Retention time relative to *p*-nitrophenyl β-D-glucopyranoside (GC-EIMS conditions, see Experimental Procedures). [g] This is "unknown peak e" in the gas chromatograms of the TMS derivatives of grape glycosides after enzymic hydrolysis (Günata et al., 1988). [h] Coeluted with geranyl β-rutinoside; this is "unknown peak g" in the gas chromatograms of the TMS derivatives of grape glycosides after enzymic hydrolysis (Günata et al., 1988).

TFA–OH groups) and at *m/e* 319 (di-TFA hexosyl fragment) (König et al., 1973), although the last two had weak intensities. Furthermore, their TMS derivatives showed the characteristic fragment ions at *m/e* 349 and 259 (tri-TMS pentosyl fragment and subsequent loss of one TMS–OH group) and at *m/e* 361 and 331 (tri-TMS hexosyl fragment and subsequent loss of formaldehyde) (De Jongh et al., 1969; Martinelli, 1980). On the other hand, the high abundance of fragment ion at *m/e* 191 with respect to that of ions at *m/e* 204 (a di-TMSO-ethenyl) and 217 (a di-TMSO-propenyl) in the EIMS of the TMS derivatives of the unknown compared to those of the corresponding 6-*O*-α-L-arabinofuranosyl-β-D-glucopyranosides (ion at *m/e* 191 very weak with respect to ions at *m/e* 204 and 217) was of high diagnostic value to distinguish between them. However, this ion, probably TMSO–CH=O$^+$TMS, was not characteristic of a particular sugar, as it is found in the EIMS of almost all glycoside TMS derivatives (De Jongh et al., 1969; Inouye et al., 1976; Martinelli, 1980).

Similar discrimination between the TFA derivatives of the unknown diglycosides and those of the 6-*O*-α-D-arabinofuranosyl-β-D-glucopyranosides was not so obvious. The one slight difference we could find dealt with the ratio of the intensity of the fragment (generally <5%) at *m/e* 279 (a di-TFAO-butenyl) to that of the fragment at *m/e* 265 (a di-TFAO-propenyl) (Table II). This ratio was greater than 1 for the unknown and smaller than 1 for the other compounds. However, due to the weak intensity of these ions, this rule must be applied cautiously, especially in the case of eventual coelution; thus, tentative identifications were also based on comparison of the GC retention times of the two sets of diglycosides and, when available, ascertained by synthetic compounds for the 6-*O*-α-L-arabinofuranosyl-β-D-glucopyranosides (Voirin, 1990).

Moreover, in the range of retention times between those of the monoglucosides and those of the diglycosides TMS derivatives of grape extracts appeared the two known unsubstituted diglycosides, i.e., TMS-rutinose and TMS-6-*O*-α-L-arabinofuranosyl-D-glucopyranose, along with another unsubstituted diglycoside presenting EI and CI mass spectra (Table III) similar to those of TMS-6-*O*-α-L-arabinofuranosyl-D-glucopyranose but with the same major difference in their EIMS as reported above, i.e., high-intensity fragment ion at *m/e* 191. Their EIMS exhibited different characteristic fragments relative to the terminal sugar, i.e., *m/e* 363–273 for rutinosides and *m/e* 349–259 for the two other ones, but the same characteristic fragment ions for the reducing hexose, i.e., prominent ions at *m/e* 495–496 (probably carbonyl and formyl TMS-hexosyl fragments) and small fragment ions at *m/e* 466–377 (loss of formaldehyde then of TMSO from ion at *m/e* 496), which is strongly indicative of a 6-*O*-pentosylglucose for the unknown. In addition, their CIMS with ammonia as reactant gas showed pseudomolecular ion corresponding to 1-amino derivatives, i.e., *m/e* 775 for rutinosides and *m/e* 761 for the two other ones, probably due to the high reactivity of these TMS glycosides toward ammonia in the MS source conditions. It is interesting to note that these unsubstituted diglycosides were absent in the gas chromatograms of the corresponding grape TFA glycoside extracts, in which, conversely, the corresponding diglycosides of neric and geranic acid (1-O ester linkage between aglycon and sugar moieties), absent in the grape TMS glycoside extracts, were tentatively identified. Indeed, these ester diglycosides were probably deglycosylated by chlorotrimethylsilane present in the silylating reagent (BSTFA + 1% TMCS in pyridine), giving rise to the corresponding unsubstituted glycosides, as already reported for other 1-*O*-acyl glycosides (Martinelli, 1980).

Table II. Mass Spectra of TFA Derivatives of 6-*O*-β-D-Apiofuranosyl-β-D-glucopyranosides Detected in *Vitis vinifera* Grape Extracts

aglycon residue[a]	b	EIMS, characteristic fragment ions of sugar moiety	EIMS, characteristic fragment ions of aglycon moiety[c]	occurrence in grape[d] F	A	O	H	G	rrt[e]
benzyl	H	193 (19); 279 (2); 421 (0.8)	91 (100); 107 (24); 92 (9); 108 (6)	+	+	++	+	++	1.736
linalyl	H	193 (15); 279 (2); 421 (2) 265 (0.4)	69 (100); 93 (66); 81 (66); 80 (45) 136 (22); 137 (13); 121 (12); 111 (5); 153 (3)	+	++	-	+	-	1.739
	P	193 (16); 325 (3); 211 (0.7) 421 (0.7)	69 (100); 81 (37); 93 (27); 80 (13) 136 (11); 111 (10); 137 (8); 121 (4); 153 (4)	-	+	-	-	-	1.979
a furan linalyl oxide	H	193 (14); 421 (10); 279 (2) 265 (0.1)	111 (100); 153 (20); 69 (18); 71 (17) 93 (14); 94 (8)	+	-	+	-	-	1.744
a furan linalyl oxide	H	193 (20); 421 (7); 279 (3) 319 (0.5); 265 (0.2)	111 (100); 69 (29); 93 (28); 153 (16) 81 (15); 71 (15)	++	-	+	-	+	1.751
	P	193 (19); 325 (10); 319 (5) 211 (2)	111 (100); 69 (46); 93 (38); 153 (23) 135 (11); 71 (11)	+	-	-	-	-	2.015
a pyran linalyl oxide	H	193 (73); 421 (27); 279 (14) 319 (0.7); 265 (0.4)	94 (100); 68 (34); 81 (28); 93 (19); 95 (18) 111 (16); 71 (16); 153 (11)	+++	-	-	-	++	1.847
	P	325 (61); 193 (55); 211 (9) 319 (0.4)	94 (100); 81 (36); 68 (30); 111 (23) 69 (21); 71 (18); 95 (18); 153 (12)	+	-	-	-	+	2.084
neryl	H	193 (17); 421 (3); 279 (3) 319 (1); 265 (0.3)	69 (100); 81 (26); 93 (14); 123 (10) 137 (8); 95 (8); 136 (6); 154 (5)	++	+	+++	+++	+	1.806
	P	193 (16); 325 (11); 211 (2) 319 (2)	69 (100); 81 (43); 93 (29); 68 (20) 137 (14); 95 (12); 136 (11); 154 (10); 123 (7)	+	+	+	+	+	2.041
2-phenylethyl	H	193 (22); 279 (4); 421 (2) 265 (0.4); 308 (0.4)	105 (100); 104 (61); 106 (20); 91 (20)	-	+	++	+	-	1.845
	P	193 (19); 325 (11); 319 (2) 211 (1)	105 (100); 104 (77); 106 (15); 91 (11)	-	-	+	-	-	2.081
geranyl	H	193 (11); 421 (2); 279 (2) 265 (0.2)	69 (100); 81 (20); 123 (11); 93 (11) 95 (8); 136 (6); 137 (4); 154 (2)	++	+++	++	++	++	1.893
	P	193 (8); 325 (8); 319 (2) 211 (0.8)	69 (100); 81 (19); 123 (14); 93 (14) 68 (8); 136 (8); 95 (6); 137 (4); 154 (2)	+	+	+	+	+	2.127
a 3,7-dimethylocta-2,6-dienoyl[f]	H	193 (13); 279 (2); 265 (0.7)	69 (100); 123 (24); 151 (23); 82 (18) 168 (12)	+	+	+	+	+	2.015
a 3,7-dimethylocta-2,6-dienoyl[f]	H	193 (19); 279 (3); 319 (0.6) 421 (0.6); 265 (0.4)	69 (100); 123 (24); 151 (21); 82 (17) 168 (12)	++	+	+	++	+	2.030
	P	193 (13); 325 (7); 211 (3) 319 (2)	69 (100); 123 (26); 168 (18); 82 (18) 151 (16)	-	-	-	+	-	2.188

[a] Positive identification for geranyl; otherwise, tentative identification. [b] H, hexatrifluoroacetylated disaccharide [2,3,4-tri-*O*-trifluoroacetyl-6-*O*-(2,3,3′-tri-*O*-trifluoroacetyl-β-D-apiofuranosyl)-β-D-glucopyranoside]. P, pentatrifluoroacetylated disaccharide [2,3,4-tri-*O*-trifluoroacetyl-6-*O*-(2,3′-di-*O*-trifluoroacetyl-β-D-apiofuranosyl)-β-D-glucopyranoside]. [c] A small portion of *m/e* 69 can be accounted for by CF_3^+. [d] See Table I for F, A, O, H, G meaning; quantities were estimated by comparison of the total ion count for the MS of each peak with that of phenyl β-D-glucopyranoside internal standard; -, not detected; +, <100 μg/L; ++, 100–400 μg/L; +++, >400 μg/L. [e] Retention time relative to phenyl β-D-glucopyranoside (GC–EIMS conditions, see Experimental Procedures). [f] Glycoside esters of neric and geranic acids.

Table III. Mass Spectra of TMS Derivatives of Unsubstituted Disaccharides Tentatively Identified in *Vitis vinifera* Grape Extracts

disaccharide[a]	EIMS	other significant peaks	mol wt from CIMS[b] (ammonia)	rrt[c]
Rhap-O-Glcp	204 (100); 73 (57); 495 (32); 217 (17) 496 (15); 205 (21); 75 (8); 363 (6)	273 (4); 466 (0.2) 377 (5); 741 (0.04)	757	0.967
Araf-O-Glcp	217 (100); 73 (36); 218 (23); 259 (17) 495 (17); 219 (10); 103 (8); 496 (7)	349 (0.5); 466 (0.7) 377 (2); 727 (0.02)	743	0.956
Apiof-O-Glcp	191 (100); 73 (53); 259 (29); 495 (27) 217 (21); 103 (18); 192 (16); 204 (15)	349 (0.3); 466 (3) 377 (3); 727 (0.1); 496 (10)	743	0.964

[a] Rhap-O-Glcp: 6-*O*-α-L-rhamnopyranosyl-α(β)-D-glucopyranose. Araf-O-Glcp: 6-*O*-α-L-arabinofuranosyl-α(β)-D-glucopyranose. Apiof-O-Glcp: 6-*O*-α-L-apiofuranosyl-α(β)-D-glucopyranose. [b] Corresponding to 1-amino derivatives (see text). [c] Retention time relative to *p*-nitrophenyl β-D-glucopyranoside (GC–EIMS conditions, see Experimental Procedures).

Furthermore, in addition to D-glucose, L-arabinose, and L-rhamnose (Williams, 1982; Günata, 1984), D-apiose [3-*C*-(hydroxymethyl)-D-erythrofuranose] was identified recently (Brillouet et al., 1989) as a component of the sugar moiety of muscat grape glycosides resistant to selective enzymic hydrolyses (see footnotes *g* and *h* of Table I). As it was reported to be in a terminal nonreducing position, a 6-*O*-D-apiosyl-D-glucoside structure for the disaccharide moiety of the unknown diglycosides was highly probable.

Another feature of the gas chromatograms of the trifluoroacetyl derivatives of natural glycosides gave further support to this disaccharide structure. In the range of high retention times, small peaks were found which corresponded to 2,3,4-tri-*O*-trifluoroacetyl-6-*O*-di-(*O*,*O*′-trifluoroacetylpentosyl)-D-glucosides with the same aglycons as those of the major peaks of the unknown diglycosides. Indeed, in addition to the characteristic fragment ions due to the aglycons, the EIMS of these compounds (Table II) exhibited glycosidic fragment ions

at m/e 325, 211, and 193 corresponding respectively to the breakage of the pentosidic linkage and to the subsequent loss of trifluoroacetic acid and water. These partial trifluoroacetylations were consistent with the fact that the hindered tertiairy 3-hydroxy group of apiose is hardly acetylated (Tronchet and Tronchet, 1974; Iwagawa and Hase, 1983).

These deductions were confirmed for the corresponding geranyl diglycoside by comparison of GC–EIMS of TFA derivatives and GC–EI and CIMS of TMS derivatives of the grape precursors with those of acuminoside (geranyl 6-*O*-β-D-apiofuranosyl-β-D-glucopyranoside) which was recently isolated from *Hypoxis acuminata* plants by Bredenkamp et al. (1989). Fortunately, the stereochemistry of the unknown geranyl 6-*O*-D-apiosyl-D-glucoside was the same as that of acuminoside, which behaved in a very similar way. This allowed the positive identification of this component in the five grape cultivars and gave further support to the tentative identification of the other related diglycosides (Tables I and II).

Although in other known plant apioglucosides with clearly established structure apiose has the same β-D-erythrofuranose configuration as acuminoside, its linkage with D-glucose is either 1–6 or 1–2 (Watson and Orenstein, 1975; Grisebach, 1980). Furthermore the corresponding known aglycons are mostly phenolic and seldom of another kind as in acuminoside (geraniol: Bredenkamp et al., 1989), urceolide [(*E*)-8-hydroxy-2,6-dimethyl-2-octenoic acid: Iwagawa and Hase, 1983], and benzyl 6-*O*-β-D-apiofuranosyl-β-D-glucopyranoside (Suzuki et al., 1988); this last compound was also tentatively identified in our grape extracts (Tables I and II).

Positive identification of the other 6-*O*-β-D-apiosyl-β-D-glucosides present in our grape extracts is under investigation, involving both chemical synthesis of these diglycosides and ^{1}H NMR, ^{13}C NMR, GC–EI, and CIMS investigations of grape glycosidic extracts first purified by selective enzymatic hydrolysis of the known glycosides using pure β-D-glucopyranosidase, α-L-rhamnopyranosidase, and α-L-arabinofuranosidase (Günata, 1988, 1989). Further disclosure of their chemical and biochemical properties is needed for the optimal use of these technologically interesting compounds.

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Novel Polysulfides Identified in the Volatile Components from Welsh Onions (*Allium fistulosum* L. var. maichuon) and Scallions (*Allium fistulosum* L. var. caespitosum)

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Volatile components were isolated from Welsh onions (*Allium fistulosum* L. var. maichuon) and scallions (*A. fistulosum* L. var. caespitosum) by steam distillation and by dichloromethane extraction. The components of distilled oils and solvent extracts were analyzed by gas chromatography and gas chromatography–mass spectrometry (EI and CI). Sulfur-containing compounds accounted for 85% and 77% of the total volatiles in the distilled oils of Welsh onions and scallions, respectively. In addition to the sulfur compounds commonly reported in genus *Allium*, 25 novel volatile polysulfides were found in the distilled oils from both varieties of green onions. These compounds can be grouped as (a) alk(en)ylthioalkyl alk(en)yl disulfides, (b) alkyl tetra- or pentathiaalkanes or -alkene(s), and (c) thiaheterocycles. All these compounds are being reported in Welsh onions and scallions for the first time. Thermal reaction may be responsible for the formation of these volatile polysulfides.

INTRODUCTION

Onions, garlic, leeks, chives, and shallots are vegetables of the *Allium* species widely used to flavor foods. In addition to their flavoring application, medicinal properties of garlic and onions have been known for centuries. Recently, some biologically active sulfur compounds have been isolated from garlic and onions (Block et al., 1986; Bayer et al., 1989). The characteristic aromas of the *Allium* species are contributed to by the sulfur-containing volatiles. The composition and formation of volatiles in garlic and onion have been extensively studied and reviewed (Freeman and Whenham, 1975; Whitaker, 1976; Fenwick and Hanley, 1985; Carson, 1987). It is known that the volatile components of the *Allium* genus are produced by enzymic splitting of the nonvolatile precursors, *S*-alk(en)ylcysteine sulfoxides, when the plants are crushed. The alk(en)yl groups are mainly a combination of propyl, 1-propenyl, allyl, and methyl groups, depending on the species.

Green onions, *Allium fistulosum* L. var. maichuon (Welsh onions) and *A. fistulosum* L. var. caespitosum (scallions), are used as vegetables or spices in many countries. They are important ingredients in Chinese cuisine (Ho et al., 1989). Kameoka et al. (1984) reported sulfides and furanones from steam volatile oils of Welsh onions and scallions. The reported sulfur-containing volatiles were only 40–48% in the neutral fraction of the total volatiles. The present study investigated the volatile components of distilled oils and solvent extracts from Welsh onions and scallions, with emphasis on the effect of heat on the formation of thermally generated polysulfides.

MATERIALS AND METHODS

Materials. Green onions were purchased from the local market. Methylene chloride was obtained from Fisher Scientific (Malvern, PA). Silica gel (60–200 mesh) was obtained from Mallinckrodt, Inc. (Paris, KY). A standard of *n*-paraffins (C_5–C_{26}) was purchased from Alltech Associates Inc. (Deerfield, IL).

Preparation of Distilled Oils and Solvent Extracts. Homogenized green onion samples were prepared by blending 1 kg of freshly cut stalks and foliage with 2 L of distilled water.

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