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Analytical methods for monoterpene glycosides in grape and wine

I. XAD-2 extraction and gas chromatographic–mass spectrometric determination of synthetic glycosides☆

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ABSTRACT

Synthetic monoterpene and aromatic β-D-glucopyranosides, β-rutinosides and 6-O-(α-L-arabinofuranosyl)-β-D-glucopyranosides and their corresponding alcohols, diluted in a synthetic solution imitating wine, were isolated and separated by selective retention on Amberlite XAD-2. The corresponding recoveries and the conditions for the direct determination of these glycosides by gas chromatography and gas chromatography–mass spectrometry after derivatization were determined. Trifluoroacetylation gave the best results but trimethylsilylation provided complementary results. The separation of some diastereoisomeric monoterpene glycosides was also examined.

INTRODUCTION

Several methods for the extraction and determination of free and glycosidically bound volatile components of grape and wine have been described [1]. Although techniques for the extraction and isolation of these compounds and for the determination of free and enzymatically released volatiles have proved satisfactory [2,3], the bound forms have proved difficult to determine directly, using either gas chromatography (GC) or high-performance liquid chromatography (HPLC) [2,4,5]. Accordingly, no quantitative data are yet available for individual components of these precursors. Recently we synthesized many of the main glycosides of grape volatiles [6]; this has allowed further progress in their analysis. In this paper we report the GC–mass spectrometric (GC–MS) analysis of these representative glycosides. This was carried out to establish satisfactory conditions for their separation and identification, to facilitate detection of new glycosides through identification of characteristic fragmentation patterns and to determine individual gly-

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cosides and their aglycones extracted by a method we developed earlier [7].

EXPERIMENTAL

Reagents and reference samples

Analytical-reagent 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 *et al.* [7]. The trimethylsilylating (TMS) reagent [N,O-bis(trimethylsilyl)trifluoroacetamide–chlorotrimethylsilane, (99:1)] and the trifluoracetylating (TFA) reagent [N-methylbis(trifluoroacetamide)] were purchased from Touzart et Matignon and Sigma, respectively. Geraniol, nerol, (*RS*)-linalool, (*RS*)-α-terpineol, benzyl alcohol, 2-phenylethanol, (*RS*)-4-nonanol and (*RS*)-2-octanol were purchased from Fluka. Phenyl and 4-nitrophenyl β-D-glucopyranosides were obtained from Sigma and 4-nitrophenyl β-rutinoside from Sarsynthese. (*S*)-2,6-dimethyl-3,7-octadien-2,6-diol and (3*RS*,6*S*)-2,6-dimethyl-1,7-octadien-3,6-diol were synthesized according to Matsuura and Butsagan [8] by photooxidation of (*S*)-linalool [containing 15% of (*R*)-linalool] obtained from coriander oil. Syntheses of the glycosides used have been described in detail elsewhere [6].

Trimethylsilylation of glycosides

According to the method described by Sweeley *et al.* [9], an ethanolic mixture of the synthetic glycosides mentioned in Fig. 1 (about 5 μg of each compound) was concentrated to dryness in a screw-capped vial at 60°C under nitrogen. Aft dition of 20 μl of anhydrous pyridine and 2(the TMS reagent, the vial was tightly closed, s stored for 20 min at 60°C, then allowed to c room temperature.

Trifluoroacetylation of glycosides

According to the method described by St and Schewe [10], a mixture of the synthetic sides (about 10 μg of each compound) was t as above but using 20 μl of the TFA reagent i of the TMS reagent.

Methylation of 4-nitrophenyl-β-rutinoside

According to the method described by Hak [11], 330 μl of a 2 *M* solution of sodium metl phinylmethide in dimethyl sulphoxide (DMS(added to a solution of 0.11 mmol of 4-nitro β-rutinoside in 1 ml of DMSO under nitroge mixture was stirred at room temperature for 1 and, after cooling to 0°C, 3.2 mmol of methyl were added and the mixture was stirred for a 2 h at room temperature. Water (1 ml) was and the aqueous solution was extracted with 1 dichloromethane. The organic layer was (Na_2SO_4) and concentrated. The crude resid subjected to column chromatography (silica 63–200 μm) with diethyl ether to give 0.033 m permethylated 4-nitrophenyl β-rutinoside (syrup; R_F 0.35 (diethyl ether, Kieselgel 6(NMR, δ (ppm) 1.22 (d, 3H, $J_{5',6'}$ 6.2 Hz, H-6' (s, 3H, OCH_3), 3.50 (s, 3H, OCH_3), 3.55 (

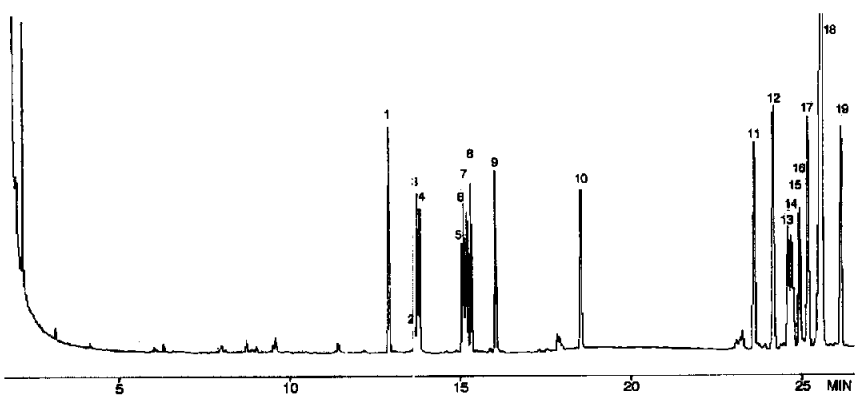

Fig. 1. GC separation (Delsi 50 m × 0.32 mm I.D. fused-silica WCOT OV-1 capillary column, film thickness 0.2 μm) derivatives of glycosides. For conditions, see Experimental. Peak numbers correspond to those in Tables I–III.

$2OCH_3$), 3.65 (s, 3H, OCH3), 3.67 (s, 3H, OCH_3), 4.79 (d, 1H, $J_{1',2'}$ 1.7 Hz, H-1'), 4.93 (d, 1H, $J_{1,2}$ 7.3 Hz, H-1), 7.09 (m, 2H, arom), 8.21 (m, 2H, arom); see below for GC.

According to the method described by Arnarp *et al.* [12], 12 mmol of 2,6-di-*tert.*-butylpyridine (Aldrich) and 6 mmol of methyl trifluoromethanesulphonate were added to a solution of 0.2 mmol of 4-nitrophenyl β-rutinoside in 9 ml of anhydrous chloroform. The mixture was stirred at room temperature for 20 h, then concentrated *in vacuo* at 40°C. The crude residue was subjected to column chromatography (silica gel 60, 63–200 μm) with diethyl ether to give 0.093 mmol of permethylated 4-nitrophenyl β-rutinoside (47%) as described above.

Gas chromatographic analysis of TMS derivatives of glycosides

TMS derivatives were analysed using an OV-1 fused-silica capillary column (Delsi Instruments) (50 m × 0.32 mm I.D.; 0.2-μm bonded phase). Injections of about 0.5 μl were made 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 310°C. The column temperature was programmed at 10°C min^{-1} from 125 to 220°C and then at 4°C min^{-1} to 310°C, with hydrogen as the carrier gas at 2 ml min^{-1} and a flame ionization detector temperature of 320°C.

Gas chromatographic analysis of TMS derivatives of linalyl diglycosides

TMS derivatives of linalyl diglycosides were analysed on a DB-17 fused-silica capillary column (J & W) (15 m × 0.32 mm I.D.; 0.25-μm bonded phase). Injections of about 0.5 μl were made on-column; the injector temperature was programmed at 60°C min^{-1} from 90 to 280°C. The column temperature was programmed at 3°C min^{-1} from 125 to 280°C with helium as the carrier gas at 1.8 ml min^{-1}. The GC instrument was coupled to a Finnigan MAT ITD 700 (see conditions below).

Gas chromatographic analysis of TFA derivatives of glycosides

TFA derivatives were analysed 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 0.5 μl were made 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 4°C min^{-1} from 125 to 280°C, with hydrogen as the carrier gas at 1.3 ml min^{-1} and a flame ionization detector temperature of 300°C.

Gas chromatographic analysis of permethylated 4-nitrophenyl-β-rutinoside

This compound was analysed using the same CP-Sil 8 CB column as above under the same conditions except that the injector temperature was programmed at 60°C min^{-1} from 165 to 300°C and the column temperature at 2°C min^{-1} from 165 to 280°C. One peak was detected at an elution temperature of 268°C.

Gas chromatographic analysis of alcohols

The alcohols were analysed on a CP WAX 52 CB fused-silica capillary column (Chrompack) (25 m × 0.32 mm I.D.; 1.2-μm bonded phase). Injections of about 4 μl were made on-column; the injector temperature was programmed at 180°C min^{-1} from 10 to 250°C. The column temperature was programmed at 2°C min^{-1} from 60°C (3 min isothermal) to 220°C (10 min isothermal) with hydrogen as the carrier gas at 2.5 ml min^{-1} and a flame ionization detector temperature at 250°C.

Gas chromatography–mass spectrometry of glycoside derivatives (TMS or TFA)

Electron impact mass spectrometry (EI-MS) was applied to the TMS and TFA derivatives by coupling a Girdel 31 gas chromatograph equipped with the fused-silica capillary columns described above to a Nermag R 10-10 mass spectrometer. The transfer line consisted of a platinum capillary tube heated at 260°C. The source temperature was 200°C. Mass spectra were scanned at 70 eV in the range m/z 60–1050 at 2.87-s intervals.

The chromatographic conditions were as follows: 2 μl of glycoside derivatives were injected with a 10:1 splitting ratio into an injector held at 320°C. The 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.

TABLE I

MASS SPECTRA OF TMS DERIVATIVES OF β-D-GLUCOPYRANOSIDES

Aglycone residue	Peak No. in Fig. 1	Relative retention time[a]	EI-MS: characteristic fragment ions[b] of			Mol. wt.[c] (MW_s)	Mol. wt.[d] of aglycone (MW_a)
			Sugar moiety	Aglycone moiety	Sugar and aglycone moieties		
Phenyl	1	0.698	361(74), 217(28), 271(10), 243(9.5), 204(5.9), 319(5.2), 191(5.1), 331(3.4), 233(2.4), 305(1.6), 451(0.5)	166(3.3), 151(1.7), 77(1.4), 208(0.7)	195(1.2)	544	94
(*R*)-Linalyl	2	0.739	331(52), 217(41), 233(27), 263(23), 204(19), 361(9.7), 191(6.2), 243(3), 271(1.9), 305(1.9), 319(1.9)	69(46), 81(20), 93(7.3), 80(6.2), 137(4.6)		604	154
(*S*)-Linalyl	3	0.742	217(42), 331(35), 233(28), 204(22), 263(20), 361(8.4), 191(6.1), 305(2.1), 271(1.2), 319(0.8)	69(50), 81(25), 93(11), 80(6.6), 137(6.2)		604	154
Benzyl	4	0.746	204(100), 217(25), 233(4.4), 191(3.8), 331(2.2), 305(1.9), 243(1.8), 263(1.3), 361(0.9), 319(0.8), 271(0.7), 451(0.03)	91(56), 107(0.4)	209(24)	558	108
(*S*)-α-Terpinyl	5	0.815	217(100), 204(32), 331(27), 361(20), 233(19), 263(13), 191(6.7), 243(3.5), 271(2.3), 305(1.8), 319(1.5), 451(0.5)	81(37), 136(15), 69(12), 137(9.9), 95(8.5)		604	154
(*R*)-α-Terpinyl	6	0.818	217(100), 204(34.5), 331(30), 361(24), 233(20), 263(16), 191(10.5), 243(7), 271(6), 305(5)	81(37), 136(20), 69(14), 137(6), 121(3)		604	154
2-Phenylethyl	6	0.818	204(100), 217(17), 191(3.5), 305(1.1), 243(0.7), 233(0.5), 331(0.5), 319(0.4), 361(0.4), 271(0.2)	105(34), 91(0.5)	223(23)	572	122

Neryl	7	0.823	217(74), 204(39), 331(35), 233(31), 263(19), 361(14), 191(7.7), 243(3.7), 305(2.4), 271(2.2), 319(1.5), 451(0.08)	69(49), 81(37), 137(13), 95(8.5), 136(5.5)		604	154
Unknown 1[e]	7	0.821	331(100), 233(47), 217(44), 263(44), 191(36), 204(35), 219(4.3), 243(3.9), 305(3.4), 271(3)	135(30), 225(24), 93(22), 107(21), 143(13), 81(5.1), 241(1.7)		692	170
Unknown 2[e]	7	0.823	331(100), 191(37), 263(36), 217(32), 233(32), 204(25), 305(3.4), 319(2.3), 243(2.2), 271(1.8)	107(30), 93(28), 135(26), 225(24), 143(17), 81(8.3), 241(1.4)		692	170
Diol-3,6[f] (3*RS*,6*S*)	8	0.827	331(92), 217(45), 263(45), 204(42), 233(41), 191(40), 361(25), 243(4.7), 305(4.3), 271(2.3), 319(1.4)	135(30), 225(28), 93(26), 107(24), 143(17), 81(5.5), 241(2.6)		692	170
(*S*)-Citronellyl	8	0.827	204(100), 217(15), 361(10), 191(3.9), 243(1.9), 305(1.8), 271(1.6), 319(1.2), 233(1.1), 331(0.9), 263(0.7), 451(0.6)	69(16), 83(5.7), 95(1.8), 139(1.3), 97(1.3), 109(0.8) 155(0.6), 140(0.5)	257(8.1)	606	156
Geranyl	9	0.866	331(65), 217(59), 233(27), 204(35), 263(29), 361(13), 191(8.1), 243(4), 305(3.5), 271(2.1), 319(1.8), 451(1.7)	69(70), 81(21), 137(6.7), 95(5.2), 93(3.5), 136(2.2)		604	154
4-Nitrophenyl	10	1	361(70), 217(26), 204(16), 271(8.5), 243(7.3), 191(5.8), 319(4.3), 331(2.8), 305(1.4), 233(0.4), 451(0.2)	196(1.8), 76(0.5)	240(2.8)	589	139

[a] Retention time relative to 4-nitrophenyl β-D-glucopyranoside (for TMS and GC conditions, see Experimental).
[b] *m/z*, with relative intensity (%) in parentheses.
[c] From CI-MS with ammonia as reactant gas.
[d] Obtained as $MW_a = MW_s - 451$ (molecular weight of the sugar moiety) + 1, except for the diol-3,6: $MW_a = MW_s - 451 - 73$ (TMS) + 2.
[e] Unknowns 1 and 2 were impurities of β-D-glucopyranoside of diol-3,6 (3*RS*,6*S*) and could be glucosides of diol-3,6 (3*RS*,6*R*) as their synthesis started from (*S*)-linalool containing 15% (*R*)-linalool.
[f] Diol-3,6 = 2,6-dimethyl-3-hydroxy-1,7-octadien-6-yl.

TABLE II

MASS SPECTRA OF TMS DERIVATIVES OF 6-O-(α-L-RHAMNOPYRANOSYL)-β-D-GLUCOPYRANOSIDES

Aglycone residue	Peak No. in Fig. 1.	Relative retention time[a]	EI-MS: characteristic fragment ions[b] of			Mol.wt.[c] (MW_s)	Mol.wt.[d] (MW_a)
			Sugar moiety	Aglycone moiety	Sugar and aglycone moieties		
Benzyl	12	1.304	204(100), 217(14.5), 191(3.1), 363(2.7), 319(1.9), 273(1.7), 305(1.6), 243(1), 245(0.9), 333(0.8), 361(0.4)	91(22)	209(7.6), 469(1.6)	848	108
Neryl	17	1.359	204(100), 217(53), 363(34), 273(12), 191(9), 319(8.4), 245(3.7), 305(3.4), 243(3), 333(1.5), 361(1.3), 271(1.2)	81(26), 69(24), 137(8.8), 95(5.9), 136(4.1)	515(0.3)	894	154
(*S*)-α-Terpinyl	18	1.377	204(100), 217(75), 363(55), 273(16), 319(9.6), 191(8.5), 245(5.3), 243(4.1), 305(3.8), 244(2.7), 361(1.7), 271(1.4), 333(1.4)	81(25), 136(15), 137(6.9), 69(6.5), 95(5.7)	515(0.4)	894	154
(*R*)-α-Terpinyl	18	1.381	204(100), 217(59), 363(31), 273(11), 191(8.5), 319(6.3), 245(4.1), 243(3.2), 244(2), 361(1.1)	81(32), 136(19.5), 69(14), 137(9), 95(6.4)		894	154
2-Phenylethyl	18	1.381	204(100), 217(71), 243(6), 191(4.8), 363(2.9), 305(2.5), 319(2.2), 244(1.9), 245(1.7), 273(1.6), 361(1), 333(1), 271(0.8), 331(0.4)	105(24), 91(4.5), 77(0.5)	223(8.2), 483(1.6)	862	122
Geranyl	19	1.412	204(100), 217(43), 363(41), 273(14), 319(9.3), 191(9.1), 245(3.9), 305(3.5), 243(2.7), 333(1.6), 244(1.3), 361(1.2), 271(1.2)	69(42), 81(17), 137(4.5), 95(3.4), 136(1.9)	515(0.7)	894	154
(*R*)-Linalyl[e]		1.149	204(42), 217(28), 273(28), 363(18), 191(11), 319(11), 245(4.4), 305(3.3)	69(30), 81(23), 155(4.4)			
(*S*)-Linalyl[e]		1.159	204(50), 273(38), 217(38), 363(23), 191(13), 319(13), 245(5.5), 305(1.1)	69(42), 81(33), 155(6.6)			

[a-d] See footnotes in Table I.

[e] Recorded by coupling a DB-17 fused-silica capillary column with a Finnigan MAT ITD 700 (See Experimental).

TABLE III

MASS SPECTRA OF TMS DERIVATIVES OF 6-O-(α-L-ARABINOFURANOSYL)-β-D-GLUCOPYRANOSIDES

Aglycone residue	Peak No. in Fig. 1.	Relative retention time[a]	EI-MS: characteristic fragment ions[b] of			Mol.wt.[c] (MW_s)	Mol.wt.[d] (MW_a)
			Sugar moiety	Aglycone moiety	Sugar and aglycone moieties		
Benzyl	11	1.273	217(100), 204(97), 259(14), 230(5.8), 231(3.2), 191(3.8), 243(3.6), 305(1.5), 349(1.4), 320(1.2), 319(1), 361(0.5)	91(35), 77(0.1)	209(5.8), 469(0.5)	834	108
Neryl	13	1.327	217(100), 259(62), 204(11), 243(7.8), 349(7), 230(4.4), 231(3.2), 191(3), 305(1.5), 361(1.2), 319(0.8), 320(0.7), 271(0.7)	81(12), 69(11), 137(3.9), 95(2.4), 136(1.7)	515(0.05)	880	154
(*S*)-α-Terpinyl	14	1.332	217(100), 259(40), 204(9), 243(6.1), 349(5.5), 230(3.9), 231(2.3), 191(1.6), 361(1.1), 305(1), 271(0.6)	136(20), 81(12), 137(6.2), 95(5.2), 69(3.7)		880	154
(*R*)-α-Terpinyl	15	1.335	217(100), 259(31), 204(10), 243(6), 349(3.7), 230(3.4), 231(2.3), 191(2.5), 305(1.3)	81(20.5), 136(10.5), 69(6), 95(4), 137(4), 121(1.6)		880	154
2-Phenylethyl	16	1.346	204(100), 217(92), 259(11), 230(5.9), 231(2.8), 191(2.7), 243(2.5), 349(1.1), 320(1), 305(0.9), 319(0.7), 361(0.3)	105(26), 77(0.4)	223(8.5), 483(0.5)	848	122
Geranyl	18	1.381	217(100), 259(67), 204(62), 243(8.7), 349(8.1), 230(4.3), 191(4.3), 231(3.8), 305(2.2), 319(1.6), 361(1.1), 320(1.1), 271(0.8)	69(18), 81(5.5), 137(2), 95(1.4), 136(0.9)	515(0.06)	880	154
(*R*)-Linalyl[e]		1.127	259(100), 217(68), 243(10), 191(1.1)	69(36), 81(21), 93(3.3)			
(*S*)-Linalyl[e]		1.136	259(100), 217(56), 243(8.9), 191(4.2), 231(1.8)	69(30), 81(20), 93(2.7)			

[a-e]See footnotes in Table II.

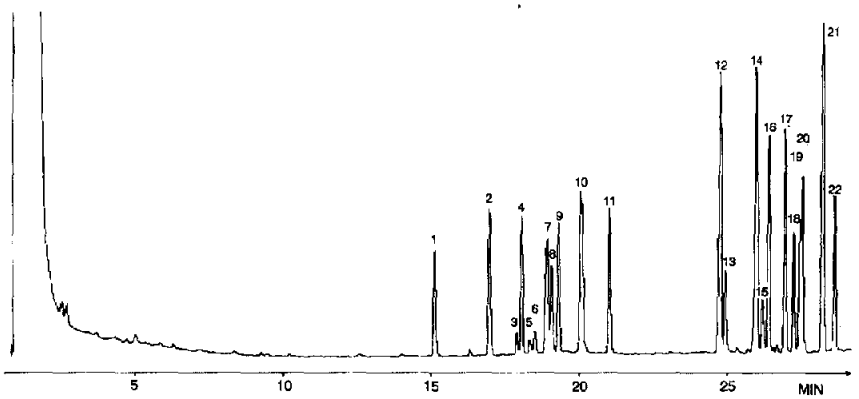

Fig. 2. GC separation (Chrompack 25 m × 0.32 mm I.D. fused-silica WCOT CP-Sil-8CB capillary column, film thickness 1.2 μm) of TFA derivatives of glycosides. For conditions, see Experimental. Peak numbers correspond to those in Tables IV–VI.

Chemical ionization mass spectrometry (CI-MS) was applied using the GC and transfer line conditions as for EI-MS. 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/z* 60–1050 at 2.87-s intervals.

MS monitoring of TMS derivatives of linalyl diglycosides was achieved by coupling the DB-17 fused-silica capillary column (see conditions above) to a Finnigan MAT ITD 700 mass spectrometer. The transfer line, heated at 280°C, consisted of an open-split GC–ITD interface at atmospheric pressure and, as a flow restrictor, a DB-5 fused-silica capillary column (1.2 m × 0.18 mm I.D.; bonded phase). The source temperature was 220°C. Mass spectra were scanned between 50 and 80 eV in the range *m/z* 61–650 at 2-s intervals.

Isolation of alcohols and glycosides from synthetic solutions of wine

To avoid co-elution in the GC analysis of the TFA derivatives of the glycosides, three different synthetic solutions were prepared. Each contained six or seven non-co-eluting glycosides from those reported in Table VIII (phenyl β-D-glucopyranoside as internal standard) and the corresponding terpenic and aromatic alcohols reported in Table VII [(*R,S*)-4-nonanol as internal standard] diluted in 50 ml of a synthetic solution to imitate wine. The amounts used for each component are given in Tables VII and VIII; the synthetic solution consisted of 20 g of tartaric acid, 15 g of malic acid, 0.5 g of acetic acid, 0.125 g of magnesium sulphate, 0.5 g of potassium sulphate, 0.6 kg of ethanol, diluted to 5 l with purified water and adjusted to pH 3.23 with 1 *M* NaOH. Each solution was diluted with 100 ml of purified water, then extracted on Amberlite XAD-2 resin according to the procedure previously described [7]. However, elution of the alcohols (free fraction) was carried out with either 50 ml of pentane or 50 ml of pentane–dichloromethane (2:1) and that of the glycosides (bound fractions) with either 50 ml of ethyl acetate or 50 ml of methanol.

The free fractions were dried with anhydrous sodium sulphate, filtered and concentrated to a final volume of about 1 ml, then 60.4 μg of (*R,S*)-2-octanol were added as external standards. These fractions were analysed by GC as described above. After analysis, the presence of glycosides was checked by GC after trifluoroacetylation according to the procedure described above.

A 10-μg amount of geranyl β-D-glucopyranoside was added as external standard to 0.5 ml of the bound fractions. These fractions were then concentrated to dryness at 60°C under nitrogen then analysed by GC after trifluoroacetylation according to the procedure described above.

Each experiment was performed in triplicate to test the reproducibility of the method.

RESULTS AND DISCUSSION

The various glycosides used (Table I) were available from syntheses reported previously [1,6,13] and have already been described as aroma precursors in grapes [13,14]. Phenyl β-D-glucopyranoside, 4-ni-

trophenyl β-D-glucopyranoside and 4-nitrophenyl β-rutinoside were also commercially available and could be used as chromatographic standards. These glycosides needed to be derivatized prior to their GC analysis in order to increase their volatility and thermal stability.

Simply and versatile methods have been described for the separation and determination of carbohydrates and related polyhydroxy compounds by GC: methylation, acetylation, silylation and trifluoroacetylation were the most frequently used for their derivatization.

Methylation was reported by Khoda *et al.* [15] and Konoshima and Sawada [16] for derivatization of terpene glycosides and by Schwab and Schreier [17] for derivatization of glycosidic conjugates of aliphatic alcohols. However, this required relatively long reaction times and drastic basic conditions. Further, an attempt to methylate 4-nitrophenyl β-rutinoside, either under standard conditions [11] or under mild conditions using methyl trifluoromethanesulphonate and 2,6-di-*tert.*-butylpyridine [12], gave unsatisfactory results as it yielded only 30% and 47% of the permethylated derivative.

Acetylation has been used extensively as a purification step for many natural glycosidic extracts [1]. It was used by Williams *et al.* [13,14] to identify the main grape glycosides. However, although acetyl derivatives give valuable structural information, these were not suited to analysis by GC owing to their limited volatility, especially in the case of terpenyl diglycosides [2]. Moreover, both acetylation and methylation sometimes resulted in incomplete reaction, giving rise to partially derivatized products [17,18].

We therefore focused on TMS ethers and TFA esters, which appeared to have more favourable properties for GC–MS analysis.

Gas chromatographic behaviour of glycoside TMS ethers

Trimethylsilylation, the most commonly used method for the derivatization of sugars and their derivatives, was first extensively studied by Sweeley *et al.* [9]. Later applications of this method to the investigation of plant glycosides, and investigations of silylating reagents used, were reviewed by Martinelli [18] and Voirin [1]. Among the silylating reagents commercially available, we chose N,O-bis-(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% of trimethylchlorosilane as a catalyst. This reagent, which exhibits a high silylating potential towards a wide variety of functional groups, rapidly produced the TMS derivatives of the synthetic glycosides in this study, without noticeable side reactions and under mild conditions. Their GC analysis requirements, discussed earlier [18], were easily met using capillary columns with bonded apolar stationary phases and cold on-column injection with temperature-programmed vaporization to minimize possible thermal degradation during the injection [19]. Owing to the low volatility and high hydrophobicity of the TMS derivatives of monoterpenyl diglycosides (resulting in high distribution constants in apolar phases), columns with high phase ratio were chosen [1]. Under our best conditions (see Experimental), most of the synthetic compounds tested were separated but many were insufficiently resolved (Fig. 1). Elution temperatures of the monoglucosides were between 234 and 246°C and those of the diglycosides between 276°C and 287°C. However, the (*R,S*)-linalyl diglycosides, in contrast to the corresponding glucosides, were not detected under the given experimental conditions, owing to their decomposition in the column at *ca.* 250°C [1], resulting in a baseline rise before the diglycoside range. Using a shorter and more polar capillary column (DB-17) and adjusting the carrier gas flow-rate and temperature programming to allow an elution temperature lower than 250°C allowed their analysis, but the resolution obtained for the diglycosides was unacceptable [1].

Moreover, Martinelli [18] showed that 1-O-acylglycosides were deglycosylated by some silylating reagents, which therefore appear unsuited to analysis of the glycosides of neric and geranic acids found in grapes [1].

Hence silylation appeared to have limitations when applied to the study of complex mixtures of terpenyl diglycosides, such as those in grapes. This method could be used, however, to obtain information complementary to other methods, although other natural diglycosides might behave as the linalyl diglycosides.

Phenyl β-D-glucopyranoside and 4-nitrophenyl β-D-glucopyranoside, both commercial products, were well separated from the glycosides studied and could be used as internal standards in quantitative studies.

TABLE IV

MASS SPECTRA OF TFA DERIVATIVES OF β-D-GLUCOPYRANOSIDES

Aglycone residue	Peak No. in Fig. 2	Relative retention time[a]	EI-MS: characteristic fragment ions[b] of		CI-MS: characteristic fragment ions[d]
			Sugar moiety	Aglycone moiety[c]	
Phenyl	1	1	319(15), 177(3.7), 205(2.5), 193(1.8), 291(0.5), 265(0.3), 547(0.02)	94(100), 97(8), 95(1.7)	432(6.5), 648(1.4)
Benzyl	2	1.122	193(1.7), 319(0.6), 265(0.2), 177(0.1), 205(0.08)	91(100), 92(17), 107(2.4), 108(2.1)	672(47), 446(17), 654(1.1)
(*R*)-Linalyl	3	1.182	319(26), 193(6.2), 205(3), 177(2.8), 265(1.9)	93(100), 69(96), 81(73), 80(50), 121(33), 136(31)	605(2), 492(1.2)
(*S*)-Linalyl	4	1.193	319(21), 193(5.8), 205(2.7), 177(2.7), 265(1.7), 291(0.4), 547(0.3)	93(100), 69(99), 81(56), 80(49), 121(33), 136(19)	605(2), 492(1.2)
Unknown 1[e]	5	1.210	319(100), 193(7.9), 205(6.8), 177(6.1), 547(4.8), 265(1.3)	71(83), 93(39), 69(32), 81(30), 107(23), 109(21), 135(18), 79(13)	
Unknown 2[e]	6	1.221	319(99), 193(16), 177(7.9), 205(7.7)	71(100), 93(95), 135(65), 69(63), 81(58), 107(56), 109(34), 79(29)	
Diol-3,6 (3*RS*,6*S*)[f]	7	1.250	319(100), 193(12), 205(9.2), 177(7.4), 547(4.8), 265(2.8), 291(0.9)	93(100), 71(61), 135(56), 69(52), 107(45), 81(43), 68(31), 109(30), 67(28), 79(21), 97(13), 119(12)	716(12.6), 603(1.6), 490(1)
2-Phenylethyl	8	1.259	319(5.3), 177(1), 193(0.9), 265(0.2), 291(0.1)	105(100), 104(40), 91(35), 106(19)	686(100), 460(32), 668(1)
Neryl	9	1.274	319(3.2), 193(1), 205(0.5), 177(0.5), 265(0.3), 291(0.06)	69(100), 81(22), 93(7.9), 68(7.2), 95(6.4)	718(11), 492(9.8), 604(6.3)
(*S*)-Citronellyl	10	1.324	319(70), 193(1.4), 177(1.4)	69(100), 81(44), 83(26), 95(18), 82(17), 70(16), 97(7.6), 123(7.5), 109(7.4)	494(4.7), 720(2.3), 608(1.4)
Geranyl	10	1.327	319(3.6), 193(1), 177(0.5), 205(0.4), 265(0.3), 547(0.07), 291(0.06)	69(100), 81(13), 123(8.2), 68(6.6), 93(5.6)	718(100), 492(7.9),
(*R,S*)-α-Terpinyl	11	1.387	319(21), 193(4.7), 265(2.7), 177(2.7), 205(2.4), 547(0.6), 291(0.5)	136(100), 81(69), 93(59), 121(49), 137(41), 69(40)	492(4.3)
4-Nitrophenyl	12	1.634	319(100), 177(13), 205(12), 547(9.6), 193(5.3), 291(1.9), 265(1.6)	139(16), 76(3.8), 122(2.6)	

[a] Retention time relative to phenyl β-D-glucopyranoside (for TFA and GC conditions, see Experimental).
[b] See footnote *b* in Table I.
[c] A small portion of *m/z* 69 can be accounted for by CF_3^+.
[d] From CI-MS with ammonia as reactant gas.
[e,f] See footnotes *e* and *f* in Table I.

TABLE V

MASS SPECTRA OF TFA DERIVATIVES OF 6-O-(α-L-RHAMNOPYRANOSYL)-β-D-GLUCOPYRANOSIDES

Aglycone residue	Peak No. in Fig. 2	Relative retention time[a]	EI-MS: characteristic fragment ions[b] of	
			Sugar moiety	Aglycone moiety[c]
Benzyl	12	1.634	193(9.4), 207(4.6), 278(1.9), 179(1.2), 265(0.7), 292(0.7), 435(0.6), 177(03), 319(0.2)	91(100), 107(23), 92(13), 108(6.4)
(*R*)-Linalyl	12	1.634	207(30), 435(4.8), 179(4), 265(2.6), 193(1.9), 177(1.4), 292(0.9), 319(0.6), 278(0.5)	69(100), 81(77), 93(77), 136(68), 80(55), 137(29), 92(24)
(*S*)-Linalyl	13	1.643	207(25), 193(5.3), 179(3.7), 435(3.4), 265(2.4), 177(1.3), 292(0.8), 319(0.5), 278(0.4)	69(100), 93(84), 81(79), 80(59), 136(37), 92(25), 127(23)
Neryl	14	1.713	207(11), 435(2.6), 179(1.7), 193(1.2), 265(0.8), 292(0.5), 177(0.4), 319(0.3), 278(0.2)	69(100), 81(24), 68(15), 93(13), 137(12), 123(11)
2-Phenylethyl	16	1.742	207(12), 179(2), 435(1.6), 193(1.1), 265(0.9), 292(0.8), 319(0.4), 177(0.4), 278(0.2)	105(100), 104(60), 106(23), 91(12)
Geranyl	17	1.778	207(6.8), 435(2), 193(1.2), 179(1.1), 265(0.6), 177(0.4), 292(0.4), 278(0.2), 319(0.2)	69(100), 81(24), 68(13), 123(11), 93(9.2), 95(8.6)
(*R,S*)-α-Terpinyl	19	1.810	207(14), 435(3.6), 193(2.7), 179(2.4), 265(1.4), 177(0.7), 292(0.7), 319(0.4), 278(0.3)	136(100), 81(47), 137(43), 93(30), 121(23), 69(22)

[a-c] See footnotes in Table IV.

TABLE VI

MASS SPECTRA OF TFA DERIVATIVES OF 6-O-(α-L-ARABINOFURANOSYL)-β-D-GLUCOPYRANOSIDES

Aglycone residue	Peak No. in Fig. 2	Relative retention time[a]	EI-MS: characteristic fragment ions[b] of		CI-MS: characteristic fragment ions[d]
			Sugar moiety	Aglycone moiety[c]	
Benzyl	14	1.713	193(21), 278(0.9), 265(0.9), 279(0.3), 177(0.3), 165(0.3), 421(0.2)	91(100), 107(26), 92(12), 108(5.2)	770(1.1)
(*R*)-Linalyl	14	1.713	193(28), 265(2.8), 177(1.2), 421(1), 165(0.9), 278(0.9), 319(0.35), 279(0.1)	136(100), 69(89), 81(79), 93(63), 137(51), 80(31)	
(*S*)-Linalyl	15	1.726	193(31), 265(2.3), 421(1.6), 165(1.3), 278(0.8), 177(0.7), 319(0.1)	69(100), 93(58), 81(54), 80(35), 136(20), 137(14)	
Neryl	18	1.796	193(22), 421(1.9), 265(1.7), 278(0.8), 165(0.7), 177(0.3), 279(0.25), 319(0.2), 307(0.03)	69(100), 81(35), 68(16), 93(16), 95(14)	
2-Phenylethyl	20	1.818	193(17), 265(1.5), 278(1), 421(0.9), 165(0.4), 279(0.4), 177(0.2), 319(0.07)	105(100), 104(55), 106(21), 91(12)	784(28), 1010(20)
Geranyl	21	1.863	193(17), 421(1.7), 265(0.9), 278(0.6), 165(0.5), 177(0.2), 279(0.1), 319(0.1)	69(100), 81(20), 68(14), 123(12), 93(9.3), 95(8)	
(*R,S*)-α-Terpinyl	22	1.889	193(31), 265(1.9), 421(1.4), 165(1.2), 278(0.9), 177(0.6), 279(0.2), 319(0.1)	136(100), 81(60), 137(49), 93(38), 121(26)	

[a-d] See footnotes in Table IV.

With regard to diastereoisomers, β-D-glucopyranosides of (*R,S*)-linalool and glycosides of (*R,S*)-α-terpineol were well or partially resolved (Tables I–III). The elution order of the glycosides of (*R,S*)-linalool was determined using synthetic (*S*)-linalyl glycosides. Each diastereoisomer of the glycosides of (*R,S*)-α-terpineol was easily identified from the significantly different relative concentrations (1:2) for each pair [6].

Gas chromatographic behaviour of glycosides TFA-esters

Trifluoroacetylation has been widely used to derivatize many functional groups [18]. Sullivan and Schewe [10] showed that N-methylbis(trifluoroacetamide) (MBTFA) cleanly produced the trifluoroacetates of some mono-, di-, tri- and tetrasaccharides, with high volatility, enabling single-run analyses of sugar mixtures to be performed. This reagent produced cleanly, rapidly and under mild conditions the TFA derivatives of the synthetic glycosides studied (Fig. 2). These TFA derivatives were more polar and far more volatile than the corresponding TMS derivatives. Consequently, the best separation was achieved when using a more polar capillary column than with the TMS derivatives [1]. Further, their distribution constants are probably low in moderately polar phases, as shown by the peak distortion when large amounts were injected. Thus good results were achieved when using a capillary column with a low phase ratio, a situation that was possible owing to the greater volatility of the TFA derivatives compared with the TMS derivatives.

Under these conditions, all the glycosides injected (injection mode as above) were detected and their separation was satisfactory except in a few instances (Fig. 2). The elution temperatures of the monoglucosides were between 185 and 209°C and those of the diglycosides between 224 and 240°C. The diastereoisomers of the glycosides of (*R,S*)-linalool were better resolved than their TMS derivatives, in contrast to those of (*R,S*)-α-terpineol (Tables IV–VI). Indeed, only a slight separation was observed for the (*R,S*)-α-terpineyl β-rutinosides and for the (3*RS*,6*S*)-2,6-dimethyl-3-hydroxy-1,7-octadien-6-yl β-D-glucopyranosides. The elution order of the glycosides of (*R,S*)-linalool was determined using synthetic (*S*)-linalyl glycosides and those of the glycosides of (*R,S*)-α-terpineol were determined as described above for the TMS derivatives.

Improvement of this method might be achieved with electron-capture detection, allowing detection on a submicrogram scale for these fluorinated derivatives [20], but difficulties such as an unstable baseline in the chromatogram of the natural glycoside extract at high ECD sensitivity need to be overcome.

As regards internal standards, phenyl β-D-glucopyranoside was well separated from the glycosides of interest, as it was eluted before the β-D-glucopyranosides. In contrast, 4-nitrophenyl β-D-glucopyranoside was co-eluted with (*R*)-linalyl β-rutinoside and benzyl β-rutinoside and could not be used for this purpose.

General mass spectrometric behaviour of glycosides of TMS and TFA derivatives

EI mass spectra of the TMS (Tables I–III) and TFA (Tables IV–VI) derivatives of the glycosides were complex, consisting of peaks resulting from the aglycone and from the sugar moieties; the molecular ion was never detected. However, as the peaks assignable to a given sugar moiety or to a given aglycone moiety were obtained from every compound containing that sugar or that aglycone, it was relatively easy to distinguish between them. Interestingly, sugar moieties gave characteristic fragment ions, of medium to strong intensities, in the range $m/z > 190$. In this mass range there were few, if any, fragment ions derived from the aglycones studied. Fragment ions resulting from the sugar moiety were only assignable to osidic units, even in the case of the diglycosides studied for which no fragment ion assignable to the disaccharide moiety was detected. In addition to fragment ions derived from the aglycone, the mass spectra of the TMS derivatives indicated fragment ions due to the aglycone bound to a fragment of the sugar moiety: these were very useful for characterization.

CI mass spectra of the TMS derivatives with ammonia as reactant gas allowed determination of their molecular mass from the medium or strong intensity pseudo-molecular ion peak (M + 18). Further, they often indicated fragment ions due to the cleavage of the interosidic linkage in addition to the ion due to the breakage of the bond between the oxygen atom O-1 and the aglycone carbon atom C-1″.

With the TFA derivatives, GC–CI-MS (with ammonia as reactant gas) gave unsatisfactory results (owing to weak peaks against a strong background), except for some terpenyl monoglucosides and for benzyl and 2-phenylethyl 6-O-(α-L-arabinofuranosyl)-β-D-glucopyranosides: the pseudomolecular ion, if any, was weak, replaced with or accompanied by weak fragment ions at m/z = M − 208 (M + NH_4^+ − 2TFAO) or at m/z = M − 95 or 96 (M + NH_4^+ − TFAO or TFAOH).

EI-MS of TMS derivatives: aglycone moiety (Tables I–III)

The fragment ions of a given aglycone were very similar, regardless of the structure of the sugar moiety. Indeed, they were similar, although with different abundance, to those observed for the corresponding acetates (NBS Library), making their identification easy. However, some unspecific fragment ions resulting from the sugar part (m/z 169, 147, 129, 117, 103, 89, 73; see below) were observed in the mass range of the fragment ions given by the aglycones, and showed intensities generally higher than those obtained for the latter moieties; this could make the identification of some natural aglycones difficult.

EI-MS of TMS derivatives: sugar moiety

β-D-glucopyranosides (Table I). The glucose moiety gave characteristic fragment ions at m/z 361, 331, 271 and 243. These have also been reported for the EI-MS of penta-O-(trimethylsilyl)-α-D-glucopyranoside [21] and TMS derivatives of glycosides of flavonoids, terpenoids and saponin [18]. Other significant peaks correspond to a fragment of the reducing sugar bound to aglycones such as benzyl, 2-phenylethyl, phenyl, 4-nitrophenyl and (*R,S*)-citronellyl (aglycone-O-CH^+-OTMS). These fragment ions were not obtained with the other terpenyl aglycones which gave fragment ions at m/z 233 and 263 with a higher abundance than that observed for the former compounds [1].

Along with these characteristic peaks, numerous other peaks at m/z 305, 217, 204, 191, 169, 147, 129, 117, 103, 89 and 73 were found in the mass spectra of the glucosides and of rutinosides and 6-O-(α-L-arabinofuranosyl)-β-D-glucopyranosides [1,21].

β-Rutinosides (Table II) and 6-O-(α-L-arabinofuranosyl)-β-D-glucopyranosides (Table III). The only fragment ions characteristic of the disaccharide moiety were those arising from each osidic unit, *i.e.*, ions at m/z 361, 331, 271 and 243 for the glucose unit, at m/z 363, 333, 273 and 244 for the rhamnose unit and at m/z 349, 259 and 230 for the arabinose unit. In addition, the fragment ions at m/z 217, 204 and 73 were the most abundant of the unspecific ions resulting from the disaccharide moiety. The other significant but very weak peaks observed in the mass spectra of both classes of diglycosides were due to breakage of the interosidic bond, at m/z 515, 483 and 469 depending on the aglycone, and due to a fragment of the reducing sugar unit bound to the aglycone moiety (aglycone-O-CH^+-OTMS), observed only for the benzyl and 2-phenylethyl derivatives (m/z 209 and 223).

EI-MS of TFA derivatives: aglycone moiety (Tables IV–VI)

It was interesting that fragment ions resulting from the sugar moiety were far less numerous and abundant than those from the aglycone moiety. This made assignment to the peaks of the aglycone easier than with the TMS derivatives. Further, as no peak resulting from the aglycone moiety was present in the mass range of the characteristic fragment ions of the sugar moiety, identification of the sugar moiety was as easy as for the TMS derivatives. However, part of the ion at m/z 69, which is abundant in the terpenyl glycosides (pentenyl ion, $C_5H_9^+$), could be accounted for by CF_3^+, found in all mass spectra of TFA derivatives (note the weakness of this peak for TFA derivatives of the phenyl, 4-nitrophenyl, benzyl and 2-phenylethyl glycosides). As with the TMS derivatives, fragment ions from a given aglycone were very similar, regardless of the structure of the sugar moiety, and were also similar, although with different abundance, to those observed for the corresponding acetates.

EI-MS of TFA derivatives: sugar moiety

β-D-glucopyranosides (Table IV). The glucose moiety gave characteristic fragment ions at m/z 547, 319, 291, 205, 193 and 177 and fragment ions at m/z 265, 157, 127, 113, 97 and 69 not specific to a hexose unit, similar to those reported earlier [22] for glucopyranoside TFA derivatives. The latter peaks were far weaker than the aglycone peaks, found mostly in the same mass range, making identification of the aglycones easier than for the TMS derivatives.

TABLE VII

MEAN EXTRACTION COEFFICIENTS, CALIBRATION FACTORS (INCLUDING XAD-2 EXTRACTION AND GC) AND REPRODUCIBILITY FOR ALCOHOLS FROM WINE MODEL MIXTURE WITH PENTANE AND AZEOTROPE PENTANE–DICHLOROMETHANE AS ELUTION SOLVENTS

Compound	Starting amount (mg)[a]	Pentane			Pentane–Dichloromethane (2:1)		
		Mean extraction coefficient (%)	Mean calibration factor	Reproducibility[b] (%)	Mean extraction coefficient (%)	Mean calibration factor	Reproducibility (%)
4-Nonanol[c]	6.66	70.4	1	0	81.5	1	0
Geraniol	0.49	65.5	1.1	7.4	79.8	1.1	1.2
Nerol	0.44	69.8	1.1	6.6	81.2	1.1	1.2
(*R*,*S*)-Linalol	0.47	74.8	0.9	2.7	82.3	1	0.4
(*R*,*S*)-α-Terpineo	0.53	75.9	1.1	5.5	82.7	1.1	0.7
Benzyl alcohol	0.48	49.8	1.3	4.7	84.9	0.9	0.7
2-Phenylethanol	0.42	56.4	1.2	6.8	87.9	0.9	0.4
Diol-3,6[d]	0.46	0.2	374	–	60.6	1.4	5.2
Diol-3,7[e]	0.48	0.1	712	–	33.7	2.6	10.6

[a] Amount used (mg) in 50 ml of synthetic wine for each repetition.
[b] Calculated as relative standard deviation ($n = 3$) for the calibration factor.
[c] Internal standard.
[d] Diol-3,6 = 2,6-dimethyl-1,7-octadien-3,6-diol.
[e] Diol-3,7 = 2,6-dimethyl-3,7-octadien-2,6-diol.

β-Rutinosides (Table V) and 6-O-(α-L-arabinofuranosyl)-β-D-glucopyranosides (Table VI). As observed for the TMS derivatives, the only fragment ions characteristic of the disaccharide residue were those arising from each osidic unit, *i.e.*, ions at *m/z* 319, 193 and 177 for the glucose unit, at *m/z* 435, 292, 207 and 179 for the rhamnose unit and at *m/z* 421, 193 and 165 for the arabinose unit. These fragment ions are similar to those reported earlier for glucopyranose, rhamnopyranose and aldopentofuranose TFA derivatives [22]. The fragment ion at *m/z* 193, although in the mass spectra of both classes of diglycosides, had a higher relative abundance with the 6-O-(α-L-arabinofuranosyl)-β-D-glucopyranosides. In addition to these fragment ions, the unspecific ions mentioned above were also detected, with weak relative abundance, in addition to fragment ions at *m/z* 279, 278 (di-TFA-butenyl) and 265 (di-TFA-propenyl), which were used to distinguish the 6-O-(α-L-arabinosyl)-β-D-glucopyranosides from the 6-O-(β-D-apiofuranosyl)-β-D-glucopyranosides, another class of diglycosides recently found in grapes [1,23].

Quantitative analysis of a synthetic mixture of wine: extraction yields, calibration and reproducibility

The procedure described here complements the technique published earlier [7] to determine the free and glycosidically bound fractions of grapes and wine. The earlier technique used XAD-2 resin to extract free terpenes and their glycosides from an aqueous or aqueous–alcoholic solution and allowed the recovery of the free and bound monoterpenes by successive elution of the resin with pentane and ethyl acetate, respectively.

The free fraction was analysed directly by GC and the bound fraction was analysed indirectly through enzymic hydrolysis. Now we can concomitantly directly analyse the bound fraction by GC and thus determine the extraction yields of a mixture of the available synthetic glycosides, in addition to the influence of the elution solvents (see Experimental) and the reproducibility of the whole method.

For this experiment we used a synthetic solution imitating wine, *i.e.*, an aqueous–alcoholic solution, to provide the least favourable conditions expected to be required for retention of the solutes by the

TABLE VIII

MEAN EXTRACTION COEFFICIENTS, CALIBRATION FACTORS (INCLUDING XAD-2 EXTRACTION, DERIVATIZATION AND GC) AND REPRODUCIBILITY FOR GLYCOSIDES FROM WINE MODEL MIXTURE

Compound[a]	Starting amount (mg)[b]	Mean extraction coefficient (%)	Mean calibration factor	Reproducibility[c] (%)
Phenyl GLU[d]	1	31	1	0
Neryl GLU	0.4	71.4	0.3	7.8
(*S*)-(+)-Linalyl GLU	0.65	88.1	0.4	9
(*R,S*)-α-Terpineyl GLU	0.8	97.6	0.5	9.3
(*R,S*)-Citronellyl GLU	0.5	76.3	0.4	8.5
Benzyl GLU	0.55	40	0.7	7.2
2-Phenylethyl GLU	0.5	65	0.4	8.1
Geranyl RG	0.75	84.5	0.4	6.8
Neryl RG	0.7	71.1	0.5	14.3
(*R,S*)-Linalyl RG	0.65	87.3	0.6	6.5
(*R,S*)-α-Terpinyl RG	0.85	99.7	0.7	10.4
Benzyl RG	0.4	15	2.1	8.2
2-Phenylethyl RG	0.8	13	1.9	9.6
Geranyl AG	0.7	80.5	0.6	7.5
Neryl AG	0.6	72.3	0.7	8.8
(*R,S*)-Linalyl AG	0.75	77.2	1.1	7.6
(*R,S*)-α-Terpinyl AG	0.85	98	0.8	13.5
Benzyl AG	0.6	15.3	2.7	7.9
2-Phenylethyl AG	0.75	10.8	2.4	12.1

[a] GLU = β-D-glucopyranoside; RG = 6-O-(α-L-rhamnopyranosyl)-β-D-glucopyranoside; AG = 6-O-(α-L-arabinofuranosyl)-β-D-glucopyranoside.
[b] Amount used (mg) in 50 ml of synthetic wine for each repetition.
[c] Calculated as relative standard deviation (n = 3) for the calibration factor.
[d] Internal standard.

resin. The compounds analysed for (Tables VII and VIII) were the glycosides studied above (except for the glucoside of the monoterpendiol, owing to an insufficient amount) together with their corresponding alcohols and two monoterpenediols that occur naturally in grapes; synthesized by previously reported methods [8].

The results are summarized in Table VII for the alcohols and in Table VIII for the glycosides. The azeotrope pentane–dichloromethane (2:1) provided better elution of alcohols than pentane, particularly for the more polar monoterpenediols (as previously reported [24]) and the aromatic alcohols, without partial elution of the glycosides. On the other hand, ethyl acetate allowed a good recovery of monoterpenylglycosides but only a low recovery of the glycosides of aromatic alcohols; elution with methanol increased the extraction yields (*ca.* 55%) but, owing to its low selectivity, it proved unsatisfactory for natural extracts [1]. Phenyl β-D-glucopyranoside, chosen as an internal standard, exhibited an extraction yield intermediate between those of the glycosides of the aromatic alcohols and those of the monoterpenyl glycosides. Finally, the reproducibility of the whole method (XAD-2 extraction, concentration, TFA derivatization and GC analysis) was tested by calculating the relative standard deviations of the calibration factors, *i.e.*, response relative to the internal standards. This showed fairly good results for both free and bound compounds (Tables VII and VIII).

CONCLUSION

Synthetic glycosides were used to demonstrate the potential of XAD-2 extraction [7] and GC–MS analysis in investigation of naturally occurring mono- and diglycosides of volatile compounds.

TFA derivatization proved more suitable for the qualitative and quantitative analysis of monoterpenyl diglycosides but TMS derivatization, as well as recent HPLC methods [4,5], could provide complementary information. The XAD-2 extraction/GC–MS analysis method has been successfully applied to extraction and determination of glycosides in some grape cultivars; these results will be reported in a future paper.

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