Studies on the Hydrolysis of *Vitis vinifera* Monoterpene Precursor Compounds and Model Monoterpene β-D-Glucosides Rationalizing the Monoterpene Composition of Grapes

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Synthetic neryl, geranyl, and linalyl β-D-glucopyranosides when hydrolyzed at pH 3.2 and pH 1.0 gave product distributions similar to those from natural monoterpene disaccharides of *Vitis vinifera*. At pH 3.2 linalool and α-terpineol were major products along with lesser amounts of nerol, 3,7-dimethyloct-1-ene-3,7-diol, 2,6,6-trimethyl-2-vinyltetrahydropyran, and hydrocarbons. At pH 1, 1,4- and 1,8-cineoles, isomers of 2,2-dimethyl-5-(1-methylprop-1-enyl)tetrahydrofuran and 2-(5,5-dimethyltetrahydrofuran-2-yl)butan-2-ol, 1- and α-terpineols, and lesser quantities of γ- and 4-terpineols and Z-ocimenol were obtained. Of the model glucosides studied, the α-terpinyl derivative was distinct, giving only cyclic products at each pH. These studies rationalize the pattern of free monoterpenes at the linalool oxidation level, found in muscat juice, wine, and distillates.

Recent studies in these laboratories have investigated several aspects of the hydrolytic enhancement of muscat grape monoterpenoids (Williams et al., 1980a, 1981). It has been established that the free monoterpenoids of the juice arise from nonvolatile precursor forms, either by direct pathways or by way of certain hydroxylated linalool compounds (polyols). While monoterpenes at the higher, linalool oxide oxidation state can come via polyols, the pathways by which those at the lower, linalool oxidation level are derived are less clear.

Experiments on both whole juice and monoterene precursor material isolated from juice (Williams et al., 1981, 1982a) have demonstrated that significantly different patterns of volatile monoterpenes are produced when each is hydrolyzed at different pH values. Furthermore, there appears to be a pH-dependent interrelationship between several of the grape monoterpenes. Thus, for example, the isomeric ocimenols and myrecenol appear to be formed hydrolytically in juice at pH 1 at the expense of linalool, nerol, and geraniol, the last three compounds being pH 3 products.

An understanding of these findings has of necessity required a knowledge of the composition of the grape monoterpene precursors. Structural studies on precursors of the linalool oxidation state monoterpenes of *Vitis vinifera* have shown these to be a glycosidic mixture of rutinosides and 6-*O*-α-L-arabinofuranosyl-β-D-glucopyranosides of predominantly geraniol, nerol, and linalool (i.e. **53l**, **53m**, **52l**, **52m**, **33l**, and **33m**; see Figure 1) together with much smaller amounts of α-terpineol (**47l** and **47m**) (Williams et al., 1982b). Because of the complexity of this mixture, resulting from both the heterogeneity and nature of the glycosides, it has not been possible to investigate the chemical behavior of individual precursor constituents. Accordingly, the hydrolysis reactions of geranyl, neryl, linalyl, and α-terpinyl β-D-glucopyranosides (**53k**, **52k**, **33k**, and **47k**) have been studied as models for the natural products in an effort to rationalize the observed monoterpenoid hydrolysis products of wines and juices. This paper reports the results of these hydrolysis studies on the mixture of natural grape monoterpene glycosides and on the model glycosidic compounds.

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EXPERIMENTAL SECTION

Glycosides. Acetates of monoterpene β-D-glucosides were prepared and characterized as previously described (Williams et al., 1982b). These derivatives were deacetylated at room temperature with dilute $NaOCH_3$ in methanol and structures of the free glucosides confirmed by ^1H NMR and ^{13}C NMR spectroscopy.

Grape monoterpene precursor material was isolated from *V. vinifera* L variety Muscat of Alexandria (syn. Muscat Gordo Blanco) by chromatography of the juice on a C_{18} reversed-phase adsorbent (Williams et al., 1982a).

Hydrolysis Procedure. Monoterpene β-D-glucoside samples (1–4 mg) were each dissolved in 3–5 mL of either tartrate buffer at pH 3.2 or water to which perchloric acid was added to give pH 1.0 (glass electrode). Each solution was extracted with cold Freon F11 (2 × 10 mL) to ensure removal of any volatiles prior to hydrolysis. The solutions were then heated on a steam bath for 15 min, cooled, and reextracted with Freon F11 (3 × 12 mL). The solvent extracts of the hydrolyses were made up to 40 mL. Portions (5 mL) of each extract were concentrated in a sharply tapered flask by distillation of the solvent through a glass column (1 × 15 cm) of Fenske's helices (bath temperature 35 °C). The residue was cooled and injected into the GC or GC–MS.

Grape monoterpene precursor material from 4 L of juice was taken up in water (5 mL), and an aliquot (250 μL) was hydrolyzed as detailed above.

Gas Chromatography and Mass Spectrometry. Analytical GC and GC–MS were carried out on a SP-1000 SCOT glass column. Conditions and instrumentation used for these analyses were as described previously (Williams et al., 1982a).

Nuclear Magnetic Resonance Spectroscopy. ^1H and ^{13}C NMR spectra were recoreded at 80 and 20.1 MHz, respectively, on a Bruker WP80 Fourier transform instrument, with $CDCl_3$ as the solvent and Me_4Si as the reference standard.

Reference Compounds. The compounds **3**, **16**, **18**, **42**, **45**, and **55** were synthesized previously in this laboratory (Williams et al., 1980a,b), while **33j**, **47j**, **52j**, **53j**, and **57** were commercially available and **11**, **25**, **38**, **40**, **41**, and **48** were donated samples.

1-Terpineol (**35**) was isolated from a large-scale acid hydrolysis of *cis*-1,8-terpen (**57**) by spinning band distil-

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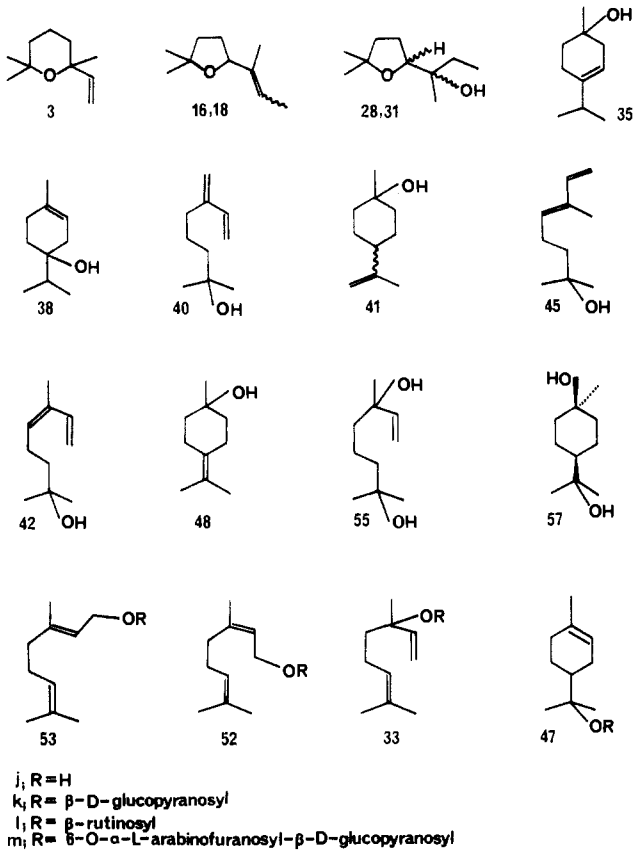

Figure 1. Monoterpenoids and derivatives referred to in this work.

lation (bp 60–64 °C, 2.5 mmHg) and subsequent preparative GC. The ^{1}H NMR spectrum (80 MHz, $CDCl_3$, Me_4Si) showed δ 1.0 (d, 6 H, CH_3CHCH_3), 1.23 (s, 3 H, CH_3COH), 1.69 (m, 2 H, CH_2CH_2COH), 1.78 (s, 1 H, –OH), 2.11 [br, 5 H, $-CH_2C{=}C$, $HC(CH_3)_2C{=}C$], and 5.31 (br, 1 H, HC=C). The MS was identical with that reported by Heller and Milne (1978).

γ-Terpinene (19) was similarly isolated from the products of acid hydrolysis of linalool carried out at 80 °C as described by Strickler and Kovats (1966). The structure of the hydrocarbon was confirmed by ^{1}H NMR, and its MS was consistent with that reported by Thomas and Willhalm (1964).

A mixture of diastereoisomers of 2-(5,5-dimethyltetrahydrofuran-2-yl)butan-2-ol (**28** and **31**) was prepared by Corbier and Teisseire (1974), but no physical data for the products were given. In the present work a mixture of **28** and **31** was synthesized in two steps from 2,2-dimethyl-5-(1-methylprop-1-enyl)tetrahydrofuran (**18**). First, olefin (**18**) (96 mg) was epoxidized with *m*-chloroperbenzoic acid in ether at room temperature. This was followed, after workup, by reduction over 90 min with $LiAlH_4$ in refluxing dry ether. The purified product (69 mg) was obtained by liquid chromatography on silica gel with pentane/acetone (70/30) as the solvent.

The diastereoisomeric mixture of **28** and **31** showed the following ^{1}H NMR spectrum (80 MHz, $CDCl_3$, Me_4Si): δ 0.92 (t, 3 H, J = 7 Hz, CH_3CH_2–), 1.04 and 1.17 (2 s, 3 H, CH_3COH), 1.23 [s, 6 H, $(CH_3)_2CO$–], 1.50 (m, 2 H, CH_3CH_2COH), 1.77 [m, 4 H, $C(CH_3)_2\ CH_2CH_2C$], 2.0 (br s, 1 H, –OH), and 3.84 (br t, 1 H, J = 7 Hz, $-CH_2CHO$–). Compound **28** had the following GC–MS (70 eV) m/z (rel intensity): 143 (3), 139 (1), 125 (5), 116 (15), 99 (26), 81 (55), 73 (94), 69 (15), 57 (20), 55 (33), 43 (100), 41 (25). Compound **31** under the same conditions showed [m/z (rel intensity)] 139 (3), 125 (5), 116 (13), 99 (27), 81 (57), 73 (100), 69 (10), 57 (21), 55 (35), 43 (96), and 41 (23).

γ-Terpineol (**48**) was donated as a 1/1 mixture with α-terpineol (**47j**) and its structure confirmed by difference ^{1}H NMR spectroscopy. Diagnostic features of the difference spectrum of **48** included a sharp singlet at δ 1.22 (CH_3COH), a broad singlet at δ 1.67 [$(CH_3)_2C{=}C$], a broad triplet at δ 2.27 [$(CH_2CH_2)_2C{=}C(CH_3)_2$], and the absence of any vinyl protons. γ-Terpineol had the following GC–MS (70 eV) m/z (rel intensity): 154 (3), 136 (48), 121 (100), 107 (22), 93 (79), 81 (29), 79 (30), 67 (23), 55 (32), 43 (95), 41 (55).

RESULTS AND DISCUSSION

Table I shows the results of GC analysis of volatile products given by the various monoterpene glycosides when each was hydrolyzed at either pH 1.0 or pH 3.2. The latter value is typical for the pH of juice from mature grapes, while pH 1.0 was used to ensure complete hydrolysis of all precursor components isolated by C_{18} reversed-phase chromatography (Williams et al., 1982a).

On hydrolysis at pH 3.2 linalyl, geranyl, and neryl β-D-glucoside (**33k**, **53k**, and **52k**) each gave the same major products, linalool (**33j**) and α-terpineol (**47j**). Nerol (**52j**), 3,7-dimethyloct-1-ene-3,7-diol (**55**) and 2,6,6-trimethyl-2-vinyltetrahydropyran (**3**) were lesser components from all of these reactions as were the hydrocarbons limonene (**11**) and terpinolene (**25**). Additionally, several compounds were observed in the hydrolysis of linalyl and geranyl glucosides (**33k** and **53k**) that were not given by the neryl derivative (**52k**). These include geraniol (**53j**), (*E*)- and (*Z*)-ocimene (**20** and **17**), α-terpinene (**9**), and myrcene (**7**). Not surprisingly α-terpinyl β-D-glucoside (**47k**) gave no acyclic monoterpenes, and a α-terpineol (**47j**) was its predominant hydrolysis product at pH 3.2.

The mixture of naturally occurring monoterpene disaccharides, which make up the linalool oxidation state precursors of muscat grapes, was also hydrolyzed at pH 3.2. This material gave all of the products mentioned above and in comparable proportions to those given by the model substrates. Furthermore, a similar pattern of linalool oxidation state monoterpenes had been obtained from Rhine Riesling precursor material (Williams et al., 1982a) or when whole muscat juice had been heated at pH 3 (Williams et al., 1981).

Hydrolytic studies at pH 1 on precursor fractions from grapes have shown a very different pattern of volatiles to that seen at pH 3 (Williams et al., 1982a). The contribution of linalool oxidation state terpene precursors to this pH 1 hydrolysis pattern can be seen from the data in Table I.

In contrast with the reaction at pH 3.2 the precursor material at low pH gave little linalool (**33j**), and no geraniol (**53j**), nerol (**52j**), or 3,7-dimethyloct-1-ene-3,7-diol (**55**) was detected. While α-terpineol (**47j**) was still a significant product at pH 1, it was now accompanied by many other monoterpenoids not given by the precursors or model substrates at pH 3.2, namely, 1,4- and 1,8-cineoles (**8** and **13**), the isomeric 2,2-dimethyl-5-(1-methylprop-1-enyl)-tetrahydrofurans (**16** and **18**), the hydrated forms of these two oxides (**28** and **31**), *p*-cymene (**23**), and 1-terpineol (**35**). Additionally, myrcenol (**40**), the isomeric ocimenols (**42** and **45**), 4-terpineol (**38**) and γ-terpineol (**48**) were formed from the precursor material at low pH as were several other unidentified products. Members of this latter group appear from their MS fragmentation patterns (see Table II) to include hydrocarbons, oxides, and alcohols.

All of the major products from the precursor at pH 1 were also given by geranyl, neryl, and linalyl β-D-glucosides

Table I. Volatile Products from Acid Hydrolyses of Monoterpene Glycosides

compd no.	ret. index[a]	compd	evidence for assignment[b]	ref	rel proportion[c] given by the substrate at pH 3.2: grape precursor	pH 3.2: lin Glcp, 33k	pH 3.2: ger Glcp, 53k	pH 3.2: ner Glcp, 52k	pH 3.2: α-terp Glcp, 47k	pH 1.0: grape precursor	pH 1.0: lin Glcp, 33k	pH 1.0: ger Glcp, 53k	pH 1.0: ner Glcp, 52k	pH 1.0: α-terp Glcp, 47k
1	400	unknown								++	++	+++	++	
2	419	unknown										+	+	
3	446	2,6,6-trimethyl-2-vinyltetrahydropyran	C, D, E	*d*	+	+	+	+			+	+	+	
4	468	unknown								+		+	+	
5	477	unknown								+		+	+	
6	483	unknown								+		+	+	
7	496	myrcene	D, E	*e, f*	+	++	+					+	+	
8	511	1,4-cineole	D, E	*g, h*						++	++	+	++	++
9	514	α-terpinene	D, E	*e, f*	+	+	+			++	++	++	++	+++
10	530	unknown								++	+	++	+	
11	535	limonene	A, B		++	++	+	+	+			+		
12	539	unknown								+		+	+	
13	544	1,8-cineole	D, E	*g, h*						++	++	+	++	+++
14	546	β-phellandrene	D, E	*e, f*		+								
15	550	unknown								++	+	++	+	
16	562	an isomeric 2,2-dimethyl-5-(1-methylprop-1-enyl)tetrahydrofuran	C, D, E	*d*						+		+	++	
17	587	(*Z*)-ocimene	D, E	*e, h*	+	+	+					+		
18	599	an isomeric 2,2-dimethyl-5-(1-methylprop-1-enyl)tetrahydrofuran	C, D, E	*d*						+++	++	+++	+++	
19	600	γ-terpinene	A, B		+	+				+	+	+	+	+
20	606	(*E*)-ocimene	D, E	*e, h*	+	++	+					+		
21	610	unknown								++	++	++	++	
22	615	unknown									+	+	+	+
23	618	*p*-cymene	D, E	*e, h*						++	+	+	+	+
24	626	unknown												+
25	629	terpinolene	A, B		++	++	+	+	+			+	+	+
26	634	unknown								+	++	++	++	++
27	717	an alloocimene	D, E	*e, f*								++	+	
28	732	an isomeric 2-(5,5-dimethyltetrahydrofuran-2-yl)butan-2-ol	A, B							+++	+	+++	+++	
29	739	an alloocimene	D, E	*e, f*						+		++	++	
30	756	unknown								+			+	
31	760	an isomeric 2-(5,5-dimethyltetrahydrofuran-2-yl)butan-2-ol	A, B							+++	+	+++	+++	
32	878	unknown										+	+	
33j	924	linalool	C, D, E	*d*	+++	+++	+++	+++		+		+	+	
34	940	unknown										+	+	
35	951	1-terpineol	A, B							+++	+++	++	+++	+++
36	960	unknown										+		
37	967	unknown										+	+	+
38	977	4-terpineol	A, B			+			+	+	+	+	++	+
39	991	unknown										+		
40	1002	myrcenol	A, B			+				+		+	++	
41	1011	an isomeric β-terpineol	A, B									+	+	+
42	1036	(*Z*)-ocimenol	A, B			+				+	+	+++	+++	
43	1051	unknown											+	
44	1052	unknown									+	+		
45	1054	(*E*)-ocimenol	A, B			+		+		+		++	++	
46	1061	unknown										+	+	
47j	1063	α-terpineol	C, D, E	*d*	+++	+++	++	+++	+++	++	++	++	+++	+++
48	1067	γ-terpineol	A, B						+	+	+	+	++	+
49	1082	unknown								+		++	++	
50	1100	unknown										+	+	
51	1118	unknown								+		+	+	
52j	1148	nerol	C, D, E	*d*	+	++	+	+						
53j	1185	geraniol	C, D, E	*d*	++	+++	+++							
54	1190	unknown					+							
55	1285	3,7-dimethyloct 1-ene-3,7-diol	C, D, E	*i*	+	+	+	+						
56	1329	unknown										+	+	
57	1366	*cis*-1,8-terpin	C, D, E	*d*		+		+				+	+	

[a] Van Den Dool and Kratz (1963). [b] 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 cochromatographed on the SP-1000 column; C = proven previously in this laboratory by spectral and chromatographic comparison with reference material; D = the mass spectrum was consistent with that of published data; E = the retention time was consistent with that of published data. [c] Peak sizes were ranked from (+++) denoting a major component to (+) denoting a minor component. [d] Williams et al. (1980a). [e] Klouwen and Ter Heide (1962). [f] Thomas and Willhalm (1964). [g] Jennings and Shibamoto (1980). [h] Heller and Milne (1978). [i] Williams et al. (1980b).

Table II. Mass Spectral Data for Unidentified Products Given on Acid Hydrolysis of Monoterpene Glycosides

compd no.	eight most prominent ions (intensities, %)	comments
1	41 (100), 57 (95), 69 (30), 97 (60), 125 (15), 126 (30), 139 (5), 154 (20)	an oxide
2	43 (100), 81 (18), 83 (15), 96 (5), 107 (7), 121 (5), 125 (40), 139 (5)	an oxide (M_r 154?)
4	39 (20), 41 (20), 77 (15), 79 (15), 91 (15), 105 (45), 121 (100), 136 (25)	a hydrocarbon
5	41 (30), 77 (30), 79 (60), 91 (35), 93 (100), 107 (70), 121 (60), 136 (40)	a hydrocarbon
6	39 (35), 41 (40), 79 (45), 91 (25), 93 (65), 105 (25), 121 (100), 136 (35)	a hydrocarbon
10	41 (90), 43 (100), 55 (55), 57 (35), 69 (98), 125 (10), 139 (60), 154 (40)	an oxide related to compound **15**
12	43 (100), 55 (25), 69 (25), 81 (30), 83 (50), 96 (45), 111 (20), 154 (10)	an oxide
15	41 (90), 43 (90), 55 (50), 57 (35), 69 (100), 97 (25), 139 (60), 154 (30)	an oxide related to compound **10**
21	41 (100), 43 (95), 57 (98), 69 (65), 97 (65), 139 (20), 154 (25), 155 (15)	maybe a hydrated form of compound **1**
22	41 (25), 77 (10), 79 (100), 91 (10), 93 (45), 107 (30), 121 (20), 136 (20)	a hydrocarbon
24	43 (100), 55 (45), 69 (40), 81 (40), 95 (25), 96 (30), 97 (40), 139 (40)	an oxide
26	41 (50), 77 (35), 79 (45), 91 (40), 93 (95), 107 (20), 121 (100), 136 (50)	a hydrocarbon
30	41 (45), 42 (5), 53 (5), 57 (100), 58 (5), 67 (5), 69 (10), 85 (20)	
32	41 (70), 56 (70), 57 (100), 69 (60), 70 (55), 83 (65), 139 (45), 154 (10)	
34	43 (100), 67 (30), 69 (50), 71 (40), 81 (40), 111 (15), 121 (35), 136 (20)	
36	41 (20), 43 (45), 55 (15), 79 (10), 81 (70), 96 (100), 121 (25), 136 (20)	
37	41 (70), 43 (100), 79 (45), 81 (80), 93 (80), 108 (25), 111 (55), 139 (55)	
39	43 (100), 59 (20), 79 (65), 93 (30), 107 (20), 121 (25), 136 (20), 139 (15)	
43	43 (100), 58 (25), 68 (35), 71 (50), 107 (30), 109 (30), 121 (30), 136 (25)	
44	41 (75), 43 (75), 68 (60), 71 (90), 81 (80), 93 (50), 109 (100), 136 (45)	
46	69 (65), 70 (10), 83 (15), 98 (5), 109 (10), 123 (100), 124 (15), 154 (5)	an alcohol
49	43 (100), 69 (40), 71 (15), 89 (20), 109 (35), 127 (25), 139 (5), 157 (5)	a hydroxy oxide (M_r 172?)
50	41 (100), 69 (70), 79 (25), 93 (35), 95 (40), 107 (35), 109 (45), 121 (35)	
51	41 (40), 69 (10), 93 (10), 105 (10), 121 (100), 136 (10), 139 (15), 154 (15)	an alcohol
54	41 (100), 67 (20), 68 (30), 69 (35), 81 (25), 94 (10), 107 (15), 121 (20)	
56	43 (85), 55 (45), 93 (15), 100 (10), 111 (100), 125 (5), 129 (10), 135 (5)	

(**53k**, **52k**, and **33k**) under these conditions while most of the minor products were also formed from the first two of these model substrates. Again, α-terpinyl β-D-glucoside (**47k**) gave no recognizable acyclic products at pH 1.

Arising out of these experimental observations are several points of importance to the terpene composition of grapes and wines. (1) Acid hydrolysis products are not diagnostic of the monoterpene aglycon composition of the grape precursors. Thus, while the grape glycosides are made up predominantly of geranyl, linalyl, and neryl derivatives and only trace quantities of α-terpinyl glycosides, the hydrolysis products at pH 3.2 are dominated by linalool and α-terpineol, with geraniol relatively less abundant. Furthermore, the experiments demonstrate that the model substrates geranyl, neryl, and linalyl β-D-glucosides each gave similar product distributions at pH 3.2 (and to a lesser extent at pH 1.0). (2) The acid hydrolyses as reported here appear to be major pathways to free monoterpenoids of the grapes. This follows from the abundance of α-terpineol seen in juices (Williams et al., 1980a), indicating a carbocationic genesis from the glycosidic precursors rather than an enzymatic one. (3) Because acid hydrolysis is a significant route to grape monoterpenes, the studies here rationalize the origin of the free monoterpenes of the juice. Thus, the observed relationship between linalool, geraniol, and nerol on the one hand and 3,7-dimethyloct-1-ene-3,7-diol with its hydrolysis products on the other (Williams et al., 1981) is now recognized as a circumstance involving alternative products derived from the same precursors and influenced by the acid conditions of the hydrolysis. Similarly, *cis*-terpin (**57**), a product observed in heated muscat grape juice (Williams et al., 1980a, 1981), is now understood as an end product of monoterpene glycoside hydrolysis. (4) The glycosidic precursors are in fact masked flavorants of the fruit, being nonvolatile and without significant aroma. However, facile hydrolytic reactions give rise to potent, highly volatile, fruit flavor compouds. (5) The ability of the glycosidic precursors to hydrolytically yield the compounds seen in Table I draws attention to the fact that all of these products are actually or potentially grape or wine volatiles. Most of the compounds given under hydrolytic conditions at pH 3.2 are the free terpenes of the juice (Cordonnier and Baynonove, 1974). However, many of those products developed at pH 1.0 were seen in the headspace of muscat juice heated for 15 min at 70 °C and pH 3.2 (Williams et al., 1980a). It has been observed that prolonged heating of juice at pH 3.0 ultimately altered the sensory character by imparting a eucalyptus-like aroma, attributable to the presence of excessive quantities of 1,8-cineole in the headspace composition of the juice. The occurrence of pH 1.0 products 1-terpineol, 4-terpineol, β-terpineol, γ-terpineol, and myrcenol in Cognac as reported by Ter Heide et al. (1978) can also be accounted for by hydrolytic degradation of grape monoterpene glycosides during wine distillation.

Numerous studies on the nonenzymatic solvolyses of geraniol, nerol, and linalool (Pickett et al., 1975; Baxter et al., 1978) as well as their pyrophosphate esters (Coates, 1976) and other derivatives (Bunton et al., 1972, 1979) have been made. The major products reported for these many reactions, linalool, α-terpineol, geraniol, and nerol, are the same as those given by the glycosides at pH 3.2. Notably, however, Baxter et al. (1978) observed the occurrence of 3,7-dimethyloct-1-ene-3,7-diol when geraniol, nerol and linalool as well as their acetates were hydrolyzed at pH 2.4 and 24 °C for several days. These workers attributed the formation of this enediol to hydration of the 6,7 double bond of the starting alcohols and esters, at a rate competitive with that for allylic rearrangement.

It is somewhat surprising that for the wide range of conditions used for the solvolytic studies on these monoterpene alcohols and their various derivatives, such a consistent pattern of products has been observed. The work of Strickler and Kovats (1966) is exceptional and reports that treatment of linalool with 30% sulfuric acid at 80 °C yielded some of the products given by the glycosides at pH 1.0. Thus, the change in reaction course, which could only be obtained with very strong acid on linalool, can be induced under relatively mild conditions on the glycosides.

While a detailed investigation into the mechanisms of monoterpene glycoside hydrolyses has not been undertaken, it would seem that the water solubility of the compounds as well as the presence of an allylic glycosidic linkage greatly influences the reactivity of these substrates. The latter property would facilitate formation of a carbocation, which in turn appears to be an important early step in the hydrolytic process. In support of this it was found that when geranyl β-D-glucopyranoside was hydrogenated to 3,7-dimethyloctyl β-D-glucopyranoside, and also when the grape precursor material was hydrogenated, these reduced products were resistant to acid hydrolysis. Similarly, Croteau and Martinkus (1979) found that menthyl glucosides were also relatively stable and showed no aglycon rearrangement on acid hydrolysis.

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Metabolism and Fate of Diflubenzuron in Swine

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^{14}C-Labeled diflubenzuron, *N*-[[(4-chlorophenyl)amino]carbonyl]-2,6-difluorobenzamide, was administered at 5 mg/kg to a female Duroc-Poland China pig as an oral dose. Analysis of feces for radioactivity revealed 82% of the administered dose, identified as diflubenzuron, while 5% of the dose was recovered in the urine. The highest [^{14}C]diflubenzuron residue present in pig tissues was 0.43 ppm in the gallbladder. Identification of the metabolic products found in the urine revealed (4-chlorophenyl)urea, 2,6-difluorobenzoic acid, 4-chloroaniline, and 2,6-difluorobenzamide. Cleavage of the urea moiety between the benzoyl carbon and urea nitrogen is indicated as the primary degradation pathway in swine.

Diflubenzuron, *N*-[[(4-chlorophenyl)amino]carbonyl]-2,6-difluorobenzamide, is a potent broad spectrum insect growth regulator. Diflubenzuron interferes with insect cuticle formation (VanDaalen et al., 1972) and is effective in controlling immature stages of insects. Diflubenzuron has been shown to be effective in controlling diptera larvae in the manure of cattle and chickens when incorporated into their diet (Miller, 1974; Miller et al., 1975, 1976; Wright, 1974, 1975; Wright and Spates, 1976).

The metabolism of diflubenzuron has been reviewed by Ivie (1977) and Schooley and Quistad (1979). Diflubenzuron metabolism by sheep (Metcalf et al., 1975; Ivie, 1978), cattle (Ivie, 1978), chickens (Opdycke et al., 1982), and rats (Willems et al., 1980) indicates that hydroxylation, conjugation, and cleavage of the urea moiety of the diflubenzuron molecule are possible metabolic pathways.

This study is concerned with the amounts of diflubenzuron retained, metabolized, and/or excreted by a pig. Analysis of the metabolism of diflubenzuron by this economically important animal will help to clarify its potential as an insect feed-additive larvicide.

MATERIALS AND METHODS

Chemicals. [^{14}C]Diflubenzuron, uniformly radiolabeled in both rings (specific activity 17.42 mCi/mmol), and technical diflubenzuron were supplied by the Thompson-Hayward Chemical Co., Kansas City, KS. Compounds that are possible metabolites, (4-chlorophenyl)urea, 4-chloroaniline, 2,6-difluorobenzoic acid, *N*-[[(4-chloro-2-hydroxyphenyl)amino]carbonyl]-2,6-difluorobenzamide,

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