Isolation of daphneticin (**1**). 700 g air-dried and powdered roots and stems of *Daphne tangutica* were extracted with CH_2Cl_2 in a Soxhlet for 2 days. The extract was evaporated to a syrup and the residue (20 g) fractionated on a column of Si gel (70 × 7 cm) with toluene–Me_2CO (3:1). The fractionation, monitored by TLC, yielded 10 main fractions [1].

Fraction 9 was rechromatographed on a Si gel column with $CHCl_3$–MeOH (20:1). The fraction containing daphneticin (R_f 0.22 in toluene–Me_2CO, 3:1, R_f 0.32 in $CHCl_3$–MeOH, 20:1, Liebermann–Burchard reagent) was evaporated and the residue crystallized from MeOH–Me_2CO, (0.07 g). Mp 235–238°; $[\alpha]_D^{25}$ = 0° (C_5H_5N; *c* 0.92); $C_{20}H_{18}O_8$ (MS, M^+ 386.1006); UV λ_{max}^{MeOH} nm (ε): 242 (9170), 260 (8892) and 317 (11 230); IR $\bar{\nu}_{max}^{KBr}$ cm^{-1}: 3480, 3210, 1730, 1610, 1570, 1450, 1340, 1270, 1115, 1055, 835; MS *m/z* (rel. int.): 386 (6), 368 (5), 354 (9), 311 (5), 210 (50), 178 (100), 167 (60), 150 (58); ^1H NMR (DMSO-d_6): 8.01, 6.36 (AB pattern, *J* = 10 Hz, H-4, H-3), 7.25, 7.01 (AB pattern, *J* = 9 Hz, H-5, H-6), 6.81 (2H, *s*, H-2″,H-6″), 5.10 (1H, *d*, J = 7.5 Hz, H-1′), 4.34 (1H, *m*, H-2′), 3.81 (6H, *s*,–OMe × 2), 3.59 (2H, *m*, H-3′).

Daphneticin acetate (**3**). 40 mg daphneticin were acetylated with 6 ml C_6H_5N and 6 ml $(MeCO)_2O$ at room temp. for 12 hr and processed in the usual way. Recrystallization from Me_2CO yielded 30 mg **3** mp 208–209°; $C_{24}H_{22}O_{10}$ (MS *m/z* 470, M^+); R_f 0.5 ($CHCl_3$–Me_2CO, 10:1); IR $\bar{\nu}_{max}^{KBr}$ cm^{-1}: 1730, 1610, 1570, 1450, 1360, 1200, 1055, 835; ^1H NMR (DMSO-d_6): 8.02, 6.38 (AB pattern, *J* = 10 Hz, H-4, H-3), 7.28, 7.04 (AB pattern, *J* = 9 Hz, H-5, H-6), 6.97 (2H, *s*, H-2″, H-6″), 5.25 (1H, *d*, *J* = 7.5 Hz, H-1′), 4.79 (1H, *m*, H-2′), 4.22 (2H, *m*, H-3′), 3.83 (6H, *s*, –OMe × 2), 2.31 (3H, *s*, Ar–OAc), 2.09 (3H, *s*, $-CH_2$–OAc); 90 MHz, in $CDCl_3$: 7.67, 6.33 (AB pattern, *J* = 10 Hz, H-4, H-3), 7.02, 6.93 (AB pattern, *J* = 9 Hz, H-5, H-6), 6.62 (2H, *s*, H-2″, H-6″), 4.98 (1H, *d*, *J* = 7.5 Hz, H-1′), 4.40 (1H, *m*, H-2′), 4.18 (2H, *m*, H-3′), 3.84 (6H, *s*, –OMe × 2), 2.33 (3H, *s*, Ar–OAc), 2.07 (3H, *s*, aliph. OAc).

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REFERENCES

1. Zhuang, L. G., Seligmann, O., Jurcic, K. and Wagner, H. (1982) *Planta Med.* **45**, 172.
2. Ray, A. B., Chattopadhyay, S. K., Konno, C. and Hikino, H. (1980) *Tetrahedron Letters* **21**, 4477.
3. Ray, A. B., Chattopadhyay, S. K., Konno, C. and Hikino, H. (1982) *Heterocycles* **19**, 19.
4. Arisawa, M., Handa, S. S., McPherson, D. D., Lankin, D. A., Cordell, G. A., Fong, H. H. S. and Farnsworth, N. R. *J. Natural Prod.* (in press)
5. Das Graças, M., Zoghbi, B., Rogue, N. F. and Gottlieb, O. R. (1981) *Phytochemistry* **20**, 180.

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ASTILBIN AND ENGELETIN IN GRAPES AND WINE

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Key Word Index—*Vitis vinifera*; Vitaceae; grapes; dihydroquercetin 3-rhamnoside; dihydrokaempferol 3-rhamnoside; flavanonols; wines.

Abstract—Astilbin (dihydroquercetin 3-rhamnoside) and engeletin (dihydrokaempferol 3-rhamnoside) were isolated for the first time from grapes. Details of their identification include nonderivatized ^1H NMR spectra. These flavanonol glycosides were concentrated in the skins of white grapes, and were also present in white wines as shown by HPLC. Amounts and relative amounts differed by cultivar. They may be involved in certain discoloration problems during wine processing.

INTRODUCTION

A case can be made that the grape and the products made from it constitute the most important fruit crop of the world [1, 2]. Furthermore, phenols are not only important factors in characterizing the different grapes and grape products, but also through these products enter the human diet in large total amounts. For these reasons and because different natural phenols can have quite different roles in browning and other discoloration reactions [3], flavor contribution [4] and oxidative reactions in foods including wines, considerable interest attaches to the qualitative and quantitative phenol composition of grapes.

In the course of HPLC studies of white grapes and their wines, two phenols not previously reported from grapes or wines were isolated and identified as the flavanonol rhamnosides astilbin and engeletin. While these compounds are known from a few other plant sources, none of these is important as foods. In fact, dihydroflavonols

(flavanonols) appear to be rare in plants, or plant parts, used for food and usually have been found in wood and as free aglycones [5]. Ampelopsin (dihydromyricetin), the only dihydroflavonol previously reported from the Vitaceae of which we are aware, was from leaves of a species, *Ampelopsis meliaefolia*, of no commercial importance [6].

RESULTS AND DISCUSSION

Isolation and identification

The two compounds, astilbin and engeletin, were isolated in chromatographically pure form from Chardonnay grape berry skins and also detected by HPLC in wines and skin extracts from the cvs French Colombard, Thompson Seedless, Sémillon and Chenin blanc. Their UV spectra with standard spectral shift and other test reagents [7, 8] indicated they were flavanon(ol)s. Their structures were confirmed by ^{1}H NMR and direct comparisons where possible. The spectral data were considered sufficient for assignment of configuration as *trans*-2(*R*):3(*R*) in both compounds [9]. The coupling constants for H-2 and H-3 in both compounds and the chemical shifts for both the rhamnose H-1 proton and the C-5 methyl are consistent with those reported by other workers.

^{1}H *NMR data*

Much of the ^{1}H NMR data available on flavonoids is on trimethylsilyl derivatives and, therefore, the following data in dimethylsulfoxide from a 200 MHz instrument have reference value. Astilbin: ^{1}H NMR in DMSO-d_6: δ1.05 (3H, *d*, $J_{5'',6''}$ = 6.2 Hz, H-6''), 4.02 (1H, *s*, H-1''), 4.65 (1H, *d*, $J_{2,3}$ = 9.9 Hz, H-3), 5.23 (1H, *d*, $J_{3,2}$ = 9.9 Hz, H-2), 5.88 (1H, *s*, H-6), 5.90 (1H, *s*, H-8), 6.73 (2H, *s*, H-5' and H-6'), 6.88 (1H, *s*, H-2'), 9.08 (1H, *s*, OH-3'), 9.11 (1H, *s*, OH-4'), 10.75 (1H, *s*, OH-7), 11.81 (1H, *s*, OH-5). Engeletin: ^{1}H NMR in DMSO-d_6: δ1.05 (3H, *d*, $J_{5'',6''}$ = 6.1 Hz, H-6''), 3.95 (1H, *s*, H-1''), 4.76 (1H, *d*, $J_{2,3}$ = 10.6 Hz, H-3), 5.28 (1H, *d*, $J_{3,2}$ = 10.5 Hz, H-2), 5.90 (1H, *s*, H-6), 5.91 (1H, *s*, H-8), 6.78 (2H, *d*, J = 8.4 Hz, H-3' and H-5'), 7.33 (2H, *d*, J = 8.5 Hz, H-2' and H-6'), 9.63 (1H, *s*, OH-4'), 10.83 (1H, *s*, OH-7), 11.83 (1H, *s*, OH-5).

Amounts and possible importance

Based upon HPLC peak areas by *A* at 291 nm in comparison with known amounts, the respective amounts of astilbin and engeletin in white wines made from clarified juice was 0.1–2.0 mg/l. and 0.04–0.2 mg/l. for the four cvs Thompson Seedless, Sémillon, French Colombard and Chenin blanc, with the latter the highest in astilbin. Both compounds were higher in extracts of skin than in wines made from juice (up to 10.7 and 2.4 mg/kg fr. wt of berries, respectively) and wines made by fermentation of the skins with the juice had increased contents up to 10.3 and 3.2 mg/l., respectively, of astilbin and engeletin. These compounds are expected to be present in red wines as well, but this has not yet been satisfactorily investigated owing to increased complexity of the chromatograms. The chemical nature and amount of these two compounds have suggested them as a possible source of a specific type of oxidative discoloration that sometimes occurs in white wine [10]. This is under active investigation.

EXPERIMENTAL

The seeds and pulp from ripe Chardonnay grapes (30 kg) collected in Oct. 1979 were removed by extrusion by hand rubbing in a stream of H_2O through 6.3 mm mesh wire hardware cloth. The skins (*ca* 10%) were ground and extracted × 3 with *ca* 10 l. 95% EtOH and once with 8 l. 50% aq. EtOH. The extracts were concd, tartaric acid (0.1%) added and the soln mixed with 1.5 kg dry, acid washed Celite 545. After eluting with light petrol the compounds were eluted with Et_2O satd with 0.1% tartaric acid in H_2O (3.67 g of phenols as gallic acid [11]). The combined Et_2O was removed and the residue triturated with 40% MeOH and separated on Sephadex LH-20 (twice) followed by semi-prep. HPLC with a Whatman M9 C_8 column and aq. 35% MeOH (0.2% formic acid) as the mobile phase. Astilbin was separated in > 99% purity but engeletin had to be further purified by prep. PC with 50% HOAc.

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REFERENCES

1. Singleton, V. L. and Esau, P. (1969) *Adv. Food Res. Suppl.* **1**, 1.
2. Constant, G. (1981) *Bull. Offic. Int. Vin.* **54**, 723.
3. Singleton, V. L. (1972) *Adv. Food Res. Suppl.* **3**, 143.
4. Singleton, V. L. and Noble, A. C. (1976) *Am. Chem. Soc. Symp. Ser.* **26**, 47.
5. Bohm, B. A. (1975) in *The Flavonoids* (Harborne, J. B., Mabry, T. J. and Mabry, H., eds.) p. 561. Academic Press, New York.
6. Kotake, M. and Kubota, T. (1940) *J. Liebigs Ann. Chem.* **544**, 253.
7. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids*. Springer, Berlin.
8. Seikel, M. K. (1962) in *The Chemistry of Flavonoid Compounds* (Geissman, T. A., ed.) p. 34. Macmillan, New York.
9. Gaffield, W., Waiss, A. C., Jr. and Tominaga, T. (1975) *J. Org. Chem.* **40**, 1057.
10. Simpson, R. F. (1977) *Vitis* **16**, 286.
11. Slinkard, K. and Singleton, V. L. (1977) *Am. J. Enol. Vitic.* **28**, 49.