Identification of 2,3-Dihydroxy-1-guaiacylpropan-1-one in Brandies

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We have purified and identified a new phenolic compound in brandies aged in wood. Spectral structural analysis by HPLC-DAD suggested that the unknown compound corresponded to a 3-methoxy-4-hydroxybenzoic ketone with an aliphatic chain containing hydroxyl groups. ^{1}H NMR analysis confirmed its structure as 2,3-dihydroxy-1-guaiacylpropan-1-one. Concentration of this ketone in brandies is related to the genuine aging of these beverages in oak and, therefore, to their market value.

INTRODUCTION

The method of aging in wood has been known and used in the storage and mellowing of wines and spirits (brandy, cognac, whisky, rum, etc.) since antiquity. As a result of hydrolysis and ethanolysis reactions while in the cask, the wine or spirit undergoes a series of chemical changes in composition that are responsible for the organoleptic qualities of the final product. These changes have been ascribed to solubilization of substances leaching out of the wood and to reactions between those substances and the chemical components present in the wine or spirit.

Oak is the wood most commonly used for this type of aging. It is composed mainly of polysaccharides (cellulose and hemicelluloses) and lignin (Fengel and Wegener, 1984), along with small quantities of phenols and tannins, basically ellagitannins (Scalbert et al., 1988). The lignin of the inner surface of the cask leaches out of the wood into the alcoholic medium, where it forms ethanol−lignin complexes that are later broken down, giving rise to aromatic aldehydes. These substances later undergo oxidation reactions yielding aromatic acids. Vanillin and syringaldehyde are the main aldehydes in spirits aged in oak, though large amounts of the corresponding cinnamic aldehydes (coniferaldehyde and sinapaldehyde) and the respective acid forms, vanillic acid and syringic acid, are also present (Gómez-Cordovés et al., 1980; Puech, 1981; Reazin, 1981; Delgado and Gómez-Cordovés, 1987; Puech and Moutounet, 1992; Clyne et al., 1993). Other cinnamic and benzoic acids have also been detected, though in very small amounts (Puech, 1981; Clyne et al., 1993). (±)-Lyoniresinol is another breakdown product of lignin that has been identified in spirits aged in wood (Nabeta et al., 1987), though the mechanism of formation for that substance is unknown.

In addition to the above-mentioned substances formed from lignin, other phenolic compounds present in the wood that leach into the spirit during aging have also been identified as follows: scopoletin (Otsuka and Zenibayashi, 1974; Gómez-Cordovés et al., 1978; Tricard et al., 1987) along with gallic acid and ellagic acid and various hydrolyzed tannins, which may subsequently undergo hydrolysis to form the respective acids referred to above (Viriot et al., 1993). The concentrations of all these substances in the spirits vary with the type of oak and cask age and are directly correlated with aging time (Singleton et al., 1971; Puech and Moutounet, 1988; Piggott et al., 1993). For that reason these phenolic compounds are regarded as indicators of genuine aging in the oak and of the commercial quality of the final product, which is related to the amount of time the spirit is aged and to the addition of enhancers (Delgado et al., 1990; Piggott et al., 1993).

Other phenolic compounds that may originate as breakdown products of lignin were detected in brandies aged in oak in an earlier study (Delgado, 1986), where some features of the chemical structure of two unknown compounds were determined as follows: a guaiacyl substitution in one case and a syringyl substitution in another. The present paper describes the purification and identification of the former, 3,4-dihydroxy-1-guaiacylpropan-1-one, in spirits aged in oak. In addition, the content of this compound in commercial Spanish brandies was determined, and its presence was related to the value of these beverages in the market.

MATERIALS AND METHODS

Samples. Brandies of three different qualities—low (B_1), medium (B_2), and high (B_3)—aged by the "solera" system from a cave were supplied by a distillery. Spirits "holandas", liquor obtained by distillation from wines (65% alcohol, pH = 2.5), were macerated with 4% oak chips (O) and a mixture (O + A) with 2% oak chips and 2% almond shells (A) during 11 months in the laboratory.

Sample preparation was carried out as previously described (Fernández de Simón et al., 1992). Samples (100 mL) were concentrated to 25 mL with a rotatory evaporator and then extracted two times with 15 mL of diethyl ether and two times with 15 mL of ethyl acetate; fractions were combined and dried for 30 min with anhydrous sodium sulfate, filtered, and evaporated to dryness. The residue was redissolved in 2 mL of methanol/water (1:1, v/v).

TLC Analysis. Cellulose MN-300 and silica gel 60G were used as absorbents. Different solvent mixtures were used, and the detection of spots was carried out by UV light at 254 and 360 nm, before and after saturation with ammonia vapor, and by spraying with several reagents.

Purification of 2,3-Dihydroxy-1-guaiacylpropan-1-one. Purification was carried out in two steps (TLC and HPLC). Extracts were analyzed by semipreparative two-dimensional TLC using cellulose MN-300 as adsorbent and 2% formic acid

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Table 1. *R$_f$* Values of the Unknown Compound by TLC

solvent	absorbent	R_f
2-propanol/water/ammonium hydroxide (8:1:1, v/v/v)	cellulose MN-300	0.52
propanol/ammonium hydroxide (8 N) (3:1, v/v)	silica gel 60G	0.63
benzene/acetic acid (45:4, v/v)	silica gel 60G	0.12
benzene/dioxane/acetic acid (95:25:4, v/v/v)	silica gel 60G	0.27
2% formic acid	cellulose MN-300	0.76

Table 2. Detection of the Unknown Compound with Different Spraying Reagents

| detection	color
fluorescence under UV light, 360 mn	light
fluorescence under UV light with ammonia vapor	brilliant violet
fluorescence under UV light with lead acetate (basic)	mauve
diazotized 4-nitroaniline + 15% Na$_2$CO$_3$	nd
1% potassium ferricyanide + 2% ferric chloride (1:1, v/v)	blue
2.5% phloroglucinol in HCl (12 N) + ethanol (1:3, v/v)	pink
0.4% catechin in acetone/water/sulfuric acid (50:37.5:12.5, v/v/v)	light pink

and isopropyl alcohol/ammonium hydroxide/water (8:1:1, v/v/v) as solvents. Spots corresponding to the unknown compound were scraped from the TLC plates and washed with hot methanol three times. Wash solutions were pooled and evaporated to dryness. The residue was redissolved in methanol/water (1:1, v/v) and subjected to HPLC analysis. Pure compound was collected from the chromatograph oulet.

HPLC Analysis. HPLC was performed with a liquid chromatograph (Waters Associates, Inc., Milford, MA) equipped with a pump system controller (model 600E), an injector (model U6K), and a photodiode array detector (DAD) (model 991) operating from 240 to 360 nm. The column used was a reversed-phase Nova-Pack C18 (300 × 3.9 mm i.d.) with a 4 μm packing (Waters Associates, Inc., Milford, MA). Elution of phenolic compounds was carried out with a methanol/water gradient as described previously (Bartolomé et al., 1993). Spectrum parameters [wavelengths of maxima of the original spectrum and of the second-derivative spectrum, width of convexity interval (Bartolomé et al., 1993)] were determined by the software of the photodiode array detector.

NMR Analysis. ^{1}H NMR spectra of the unknown compound and two phenolic standards (vanillin and acetovanillone) were recorded at 20 °C on a Varian UNITY-500 spectrometer operating at 500 MHz and using acetone-d_6 as solvent. Spectra were collected in 20 800 data points over a 5490 Hz spectral width (1.9 s acquisition time) with a pulse width of 7 μs (90° flip angle) and zero filled to 32K before Fourier transformation. Double-quantum-filtered COSY 2D NMR spectra were acquired in the phase-sensitive mode. Data were collected in a 1024 × 256 matrix with a spectral width of 4383 Hz and a 2 s relaxation delay and processed in a 1024 × 1024 matrix. The NOESY spectrum was acquired in the same conditions and optimized for a mix time of 400 ms.

RESULTS AND DISCUSSION

Preliminary Studies. TLC analysis of phenolic extracts from brandies showed the presence of an unknown compound whose R_fs under different chromatographic conditions (absorbent and solvent) are listed in Table 1. This compound exhibited different responses after being spraying with several reagents (Table 2). All these results had already been reported in an earlier work (Delgado, 1986). The unknown compound was also detected in wines aged in wood and in bark formation processes in the pine *Pinus silvestris* L. from the bract to the bark itself; indeed, it was one
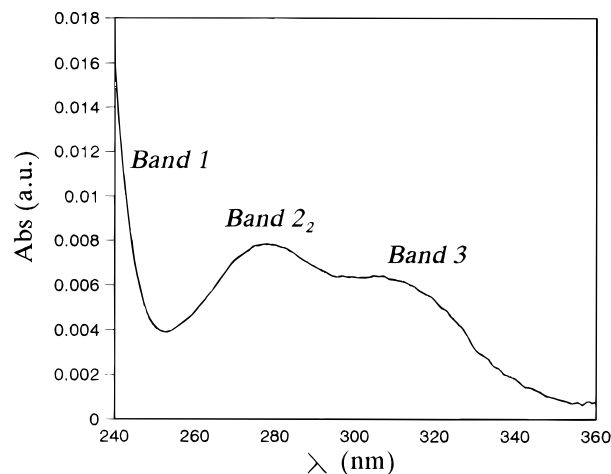
Figure 1. UV spectrum of the unknown compound.

of the components that underwent the greatest variation in concentration during the bark formation process (Gómez-Cordovés, unpublished results). Extraction and purification (see Materials and Methods) of the unknown compound from spirits "holandas" macerated with 2% oak chips and 2% almond shells allowed us to carry out futher investigations about its chemical structure by HPLC-DAD and ^{1}H NMR.
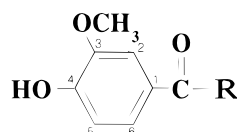
Spectral Structural Analysis. The UV spectrum of this compound recorded by the photodiode array detector (Figure 1) exhibited three absorption bands, suggesting a structure consisting of a hydroxy- or methoxy-substituted benzene ring with one functional group (COOH, CO, or C=C) attached (Bartolomé et al., 1993).

The spectral parameter values for this compound were compared with those for different benzoic acids and aldehydes and cinnamic acids (Bartolomé et al., 1993), and the results showed that the values of the spectral peaks (243.7, 278.9, and 304.9 nm) and the second derivatives (249.0 and 294.5 nm) were slightly lower than the values for 3-methoxy-4-hydroxybenzaldehyde (vanillin), although the values for the convexity intervals (a measure of spectral band width) were similar (25.3 and 24.4 nm) to the values for vanillin.

Studies of UV spectral patterns for some 3,4-disubstituted benzoic methyl ketones revealed that inclusion of the methyl group shifted the spectrum toward lower wavelengths without altering spectral band width. The methyl group also delayed elution during chromatography, i.e., in the case of the 3-methoxy-4-hydroxy substitution, 43.1 min for benzoic methyl ketone (acetovanillone) as opposed to 36.4 min for the benzaldehyde (vanillin).

Since retention time for the compound under investigation was relatively short (20.5 min), its structure was inferred to be that of a 3-methoxy-4-hydroxybenzoic ketone with an aliphatic chain (causing the observed hypsochromic shift in the spectrum as compared to vanillin) containing hydroxyl groups (which counteracted the delay in elution caused by the chain without altering the UV spectrum). Figure 2 depicts the proposed structures for the unknown compound together with the structures of the reference standards vanillin and acetovanillone.

Verification of the Structure. Structure determination was carried out by ^{1}H NMR spectroscopy (Figure 3). The 500 MHz ^{1}H NMR spectra of the unknown compound, in deuterioacetone at room temperature, showed signals for three coupled aromatic protons [δ
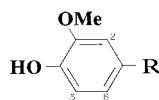
R = -CH$_2$OH Structure A

R = -CHOH-CH$_2$OH Structure B

R = H Vanillin

R = -CH$_3$ Acetovanillone

Figure 2. Proposed structures for the unknown compound after spectral structural analysis.

Compound	H2	H5	H6	OMe	R
Vanillin	7.44	7.00	7.45	3.92	C*H*O (9.82)
Acetovanillone	7.52	6.90	7.56	3.89	COC*H$_3$*(2.49)
Ketone	7.49	6.89	7.56	3.93	C*H*OH (5.06), CHO*H* (4.13) CH$_2$OH (3.86, 3.77), CH$_2$O*H*(3.91)

Figure 3. ^{1}H NMR chemical shifts (δ, ppm) of the unknown compound and the reference standards vanillin and acetovanillone.

6.89 (d, J = 8.3 Hz, 1H), 7.49 (d, J = 1.9 Hz, 1H), 7.56 (dd, J = 8.3, 1.9 Hz, 1H)], which implied the presence of a 1,3,4-substituted benzene ring. The presence of an aryl Me ester function [δ 3.93 (s)] and an exchangeable phenolic OH at δ 5.73 (broad signal, 1H) confirmed the guaiacyl substitution. Further, the deshielding of the two low-field protons and the comparison with chemical shifts of the model compounds vanillin and acetovanillone indicate that the aliphatic chain must be a carbonyl substituent (Figure 3). The spectrum of the unknown compound also exhibited five aditional signals at δ 5.06 (m, J = 3.4, 5.4, 6.3 Hz, 1H), 4.13 (d, J = 6.3 Hz, 1H), 3.91 (m, 1H), 3.86 (m, J = 3.4, 5.7, −11.7 Hz, 1H), 3.77 (m, J = 5.4, 5.1, −11.7 Hz, 1H).

When a drop of D$_2$O was added, the signals at 5.73, 4.13, and 3.91 ppm dissapeared, whereas the splitting pattern of the remainder signals in this region simplified to an ABX system [δ 5.06 (dd, J = 3.4, 5.4 Hz), 3.86 (dd, J = 3.4, −11.7 Hz), 3.77 (dd, J = 5.4, −11.7 Hz)]. A single COSY experiment confirmed the interprotonic connectivities, showing correlation signals between the multiplet at 5.06 ppm and the two multiplets at 3.86 and 3.77 ppm. These results were only compatible with a CO−CHOH−CH$_2$OH structure of the aliphatic chain (structure B, Figure 2). Therefore, the unknown compound was identified as 2,3-dihydroxy-1-guaiacylpropan-1-one. This structure was further supported by a NOESY experiment. This spectrum showed correlation signals between the methine proton at 5.06 ppm and the ortho aromatic protons at 7.49 and 7.56 ppm, corroborating the presence of only one carbonylic carbon in this structure. It was not possible to confirm these results by ^{13}C NMR spectroscopy because of the small amount of the unknown compound available.

Role of This Ketone in Aging in Brandies. The authors' work on aging of wine and spirits in oak commenced with research on the low molecular weight components. In a previous paper, Delgado et al. (1990) put forward the ratios Sr/Vn (total syringyl compounds/total vanillyl compounds), G/V (gallic acid/vanillin), V/C (vanillin/coniferaldehyde), V/Va (vanillin/vanillic acid),

Table 3. Concentration Relationships in the Aging with Oak and Almond Shells and in Commercial Brandies[a]

	O	O + A	B$_1$	B$_2$	B$_3$
Sr/Vn	3.35	0.42	0.67	1.02	1.42
G/V	7.37	0.58	1.08	1.68	1.71
S/V	1.35	0.24	0.48	0.32	1.24
G/Ke	21.23	2.26	6.54	10.75	18.10
S/Ke	3.88	0.92	2.9	2.03	13.13

[a] O, spirit "holanda" macerated with 4% oak chips; O + A, spirit "holanda" macerated with 2% oak chips and 2% almond shells; B$_1$, brandy of low quality; B$_2$, brandy of medium quality; B$_3$, brandy of high quality; Sr/Vn, total syringyl compounds/total vanillyl compounds; G/V, gallic acid/vanillin; S/V, syringaldehyde/vanillin; G/Ke, gallic acid/2,3-dihydroxy-1-guaiacylpropan-1-one; S/Ke, syringaldehyde/2,3-dihydroxy-1-guaiacylpropan-1-one.

and S/V (syringaldehyde/vanillin) as indicators of the quality of spirits and genuine aging in wood. It was established that a total content higher in syringyl compounds than in vanillyl compounds was indicative of genuine aging in oak. Similarly, a higher percentage G/V (gallic acid/vanillin) ratio value compared to the other ratios also indicates medium to high brandy quality. A high vanillin content indicates the presence of woods other than oak.

Table 3 presents the values for the most significant ratios and certain of these components included in the ratios in relation to the unknown compound identified in this paper. The ratios were calculated for macerated oak (O), macerated oak and almond shells (O + A), and three brandies of differing quality—low (B$_1$), medium (B$_2$), and high (B$_3$)—from the same distillery. Samples were taken from the same distillery in an effort to minimize the impact of external environmental factors that affect aging. As expected, the previously established ratios Sr/Vn, G/V, and S/V indicated that the three brandies had been aged in oak (Delgado et al., 1990), though the ratio values varied with the brandy quality.

In view of the close structural relationship between the newly identified ketone (ke) and vanillin, the concentration ratio values might be expected to stay the same irrespective of the type of wood used. Effectively, the results in Table 3 show that the ratios G/V (gallic acid/vanillin) and G/Ke (gallic acid/2,3-dihydroxy-1-guaiacylpropan-1-one) and S/V (syringaldehyde/vanillin) and S/Ke (syringaldehyde/2,3-dihydroxy-1-guaiacylpropan-1- one) follow the same pattern when spirits were macerated wih oak chips and almond shells and, hence, that addition of vanillin as an aroma enhancer would be clearly discernible, in that it would not be accompanied by a corresponding increase in the ketone.

Furthermore, a number of workers have reported that gallic acid is indicative of aging in oak, though the concentration of this component may fall off with cask age. It has already been established that the G/V ratio is an indicator of brandy quality, and Table 3 shows that this was again the case in this study, though the differences in the values for the three brandies were not very large. In contrast, this was not the case for the G/Ke (gallic acid/2,3-dihydroxy-1-guaiacylpropan-1-one) ratio, for which the differences were much larger.

The findings of this study suggest that the ketone identified here is the same one Sakakibara (1983) proposed as the second structural unit in softwood lignin, though no identification was carried out. Accordingly, the concentration ratios of 2,3-dihydroxy-1-guaiacylpropan-1-one to gallic acid and vanillin should be indicators of genuine aging in oak and of possible

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adulteration through the addition of aroma enhancers like vanillin.

LITERATURE CITED

Bartolomé, B.; Bengoechea, M. L.; Gálvez, M.; Pérez-Ilzarbe, F. J.; Hernández, T.; Estrella, I.; Gómez-Cordovés, C. Photodiode array detection for elucidation of the structure of phenolic compounds. *J. Chromatogr. A* **1993**, *655*, 119–125.

Clyne, J.; Conner, J. M.; Paterson, A.; Piggott, J. R. The effect of cask charring on Scotch whisky maturation. *Int. J. Food Sci. Technol.* **1993**, *28*, 69–81.

Delgado, T. Compuestos fenólicos de bajo peso molecular en brandies: Identificación y relación con su añejamiento y calidad. B.Sc. Thesis, Facultad de Farmacia, University Complutense of Madrid, Madrid, Spain, 1986.

Delgado, T.; Gómez-Cordovés, C. Teneur des brandies commerciaux espagnols en aldeydes et acides phenoliques (Content of phenolic aldehydes and acids in commercial Spanish brandies). *Rev. Fr. Oenol.* **1987**, *107*, 39–42.

Delgado, T.; Gómez-Cordovés, C.; Villarroya, B. Relationships between phenolic compounds of low molecular weight as indicators of the aging conditions and quality of brandies. *Am. J. Enol. Vitic.* **1990**, *41*, 342–345.

Fengel, D.; Wegener, G. *Wood, Chemistry, Ultrastructure, Reactions*; Walter de Gruyter: Berlin, 1984.

Fernández de Simón, B.; Pérez-Ilzarbe, F. J.; Hernández, T.; Gómez-Cordovés, C.; Estrella, I. Importance of phenolic compounds for the characterization of fruit juices. *J. Agric. Food Chem.* **1992**, *40*, 1531–1535.

Gómez-Cordovés, C.; Garrido, D.; Diez, C. Etude des composés phénoliques des eaux-de-vie viellies en bois de chêne. (Study of phenolic compounds in eaux-de-vie aged in oak wood.) *Bull. Liaison Groupe Polyphenols* **1978**, *8*, 369–373.

Gómez-Cordovés, C.; Hernández, M. T.; de la Calera, J. G. Application de la CLHP a létude des eaux-de-vie. (Application of HPLC in the study of eaux-de-vie.) *Bull. Liaison Groupe Polyphenols* **1980**, *10*, 254–258.

Nabeta, K.; Yonekubo, J.; Miyake, M. Phenolic compounds from the heartwood of european oak (*Quercus robur* L.) and brandy. *Mokuzai Gakkaish* **1987**, *33*, 408–415.

Otsuka, K.; Zenibayashi, Y. On the determination of scopoletin in aged distilled liquors. *Agric. Biol. Chem.* **1974**, *38*, 1079–1080.

Piggott, J. R.; Conner, J. M.; Paterson, A.; Clyne, J. Effects on scotch whisky composition and flavour of maturation in oak casks with varing histories. *Int. J. Food Sci. Technol.* **1993**, *28*, 303–318.

Puech, J. L. Extraction and evolution of lignin products in Armagnac matured in oak. *Am. J. Enol. Vitic.* **1981**, *32*, 111–114.

Puech, J. L.; Moutounet, M. Liquid chromatographic determination of scopoletin in hydroalcoholic extract of oak wood and in matured distilled alcoholic beverages. *J. Assoc. Off. Anal. Chem.* **1988**, *71*, 512–514.

Puech, J. L.; Moutounet, M. Phenolic compounds in an ethanol-water extract of oak wood and in a brandy. *Lebensm. Wiss. Technol.* **1992**, *25*, 350–352.

Reazin, G. H. Chemical mechanisms of whiskey maturation. *Am. J. Enol. Vitic.* **1981**, *32*, 283–289.

Sakakibara, A. Chemical structure of lignin related mainly to degradation products. In *Recent Advances in Lignin Biodegradation Research*; Higuchi, T., Chang, H. M., Kirk, T. K., Eds.; UNI Publisher: Tokyo, 1983; pp 12–33.

Scalbert, A.; Monties, B.; Favre, J. M. Polyphenols of *Quercus robur*: adult tree and in vitro grown calli and shoots. *Phytochemistry* **1988**, *27*, 3483–3488.

Singleton, V. L.; Sullivan, A. R.; Kramer, C. An analysis of wine to indicate aging in wood or treatment with wood chips or tannic acid. *Am. J. Enol. Vitic.* **1971**, *22*, 161–166.

Tricard, C.; Salagoïty, M. H.; Sudraud, P. La scopoletine: un marqueur de la conservation en fûts de chêne (Scopoletin as a marker of aging in oak casks). *Connaiss. Vigne Vin* **1987**, *21*, 33–41.

Viriot, C.; Scalbert, A.; Lapierre, C.; Moutounet, M. Ellagitannins and lignins in aging of spirits in oak barrels. *J. Agric. Food Chem.* **1993**, *41*, 1872–1879.