Phenolic Constituents, Furans, and Total Antioxidant Status of Distilled Spirits

David M. Goldberg,*,† Barry Hoffman,†,‡ Joseph Yang,§ and George J. Soleas§

Department of Laboratory Medicine and Pathobiology, Banting Institute, University of Toronto, 100 College Street, Toronto, Ontario, M5G 1L5 Canada, Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, 600 University Avenue, Toronto, Ontario, M5G 1X5 Canada, and Quality Assurance Department, Liquor Control Board of Ontario, 55 Lakeshore Boulevard East, Toronto, Ontario, M5E 1A4 Canada

The concentrations of 11 phenols and 5 furans were measured in 12 categories of distilled spirits by HPLC methodology, together with the total antioxidant status (TAS) of the same beverages. Ellagic acid was the phenol present in highest concentration in all beverages. Moderate amounts of syringaldehyde, syringic acid, and gallic acid, as well as lesser amounts of vanillin and vanillic acid, were measurable in most samples of whiskey, brandy, and rum but were largely undetectable in gin, vodka, liqueurs, and miscellaneous spirits. 5-(Hydroxymethyl)furfural was the predominant furan in the former three beverages, notably cognac, with 2-furaldehyde the next highest, but these were undetectable in most of the latter beverages. Highest TAS values were given by armagnac, cognac, and bourbon whiskey, all three of which tended toward the highest concentrations of phenols. Negative TAS values were exhibited by rum, vodka, gin, and miscellaneous spirits in line with the low or undetectable phenol concentrations in these beverages. Wood aging is the most likely source of phenols and furans in distilled spirits. Those beverages exposed to this treatment contain significant antioxidant activity, which is between the ranges for white and red wines, with the potential to augment the antiatherosclerotic functions attributable to the ethanol that they contain.

Keywords: *Phenols; furans; antioxidants; distilled spirits; gallic acid; vanillic acid; syringic acid; ellagic acid; syringaldehyde; 5-(hydroxymethyl)furfural; 2-furaldehyde*

INTRODUCTION

The regular consumption of alcohol in moderation is widely recognized as reducing overall mortality from all causes, predominantly by lowering the risk of coronary heart disease (CHD), the major cause of death in men aged 40 and over as well as among postmenopausal women (Goldberg et al., 1995; Criqui, 1996; Kannel and Ellison, 1996). These effects can largely be ascribed to the ability of ethanol to increase the plasma concentration of high-density lipoproteins, a negative risk factor for CHD (Suh et al., 1992; Gaziano et al., 1993) and to reduce blood coagulability (Renaud et al., 1992; Rubin and Rand, 1994).

In line with some epidemiological studies suggesting that red wine may impact mortality more favorably than other alcoholic beverages (Hegsted and Ausman, 1998; Renaud and De Lorgeril, 1992; Gronbaek et al., 1995), many laboratory experiments, predominantly using in vitro approaches, have shown that phenolic constituents present in red wine such as quercetin, resveratrol, and the catechins possess antioxidant, anticancer, and anti-inflammatory properties [see Soleas et al. (1997a) for review]. Some phenols (Kirby and Wheeler, 1980; Sakuma et al., 1995; McMurrough et al., 1996) and a number of furans with reducing properties (Brenner and Kahn, 1976; Lo Coco et al., 1995) have been described in beer, but only a few sporadic publications attest to the existence of these same constituents in distilled spirits (Guymon and Crowell, 1968; Jefford et al., 1989; Sanchez et al., 1990; Moutounet et al., 1995). Intuitively, one would expect them to be quite prominent components of beverages distilled from fruit sources and/or extracted from oak barrels during the many years of maturation frequently employed (Chatonnet et al., 1990, 1991, 1992).

As a chemical class, the furans have been less intensively studied, but certain members of this family have antioxidative and anticancer activities (Benjamin et al., 1991; Koga et al., 1998). They are leached from oak barrels during the storage and aging of wines and undergo complex chemical changes during these processes [see Spillman et al. (1998) for review].

The present paper describes the concentrations of a wide range of phenols and furans in a comprehensive selection of distilled spirits, together with the total antioxidant status (TAS) of these beverages.

MATERIALS AND METHODS

Standards. The following products, all from Aldrich (Milwaukee, WI), may be used with satisfactory results. The structural formulas of the principal components for which data are presented appear in Figure 1.

Phenols included gallic acid (39, 822-5), 3,4-dihydroxybenzoic acid (D10, 980-0), *p*-hydroxyphenethyl alcohol (60-12-8), 3,4,5-trimethoxyphenylacetic acid (T7, 060-2), vanillic acid (H3, 600-1), vanillin (V1110-4), syringic acid (S800-5), syringalde-

* Author to whom correspondence should be addressed [fax (416) 978-5650; e-mail david.goldberg@utoronto.ca].
† University of Toronto.
‡ Mount Sinai Hospital.
§ Liquor Control Board of Ontario.

10.1021/jf9811626 CCC: $18.00
Published on Web 09/28/1999

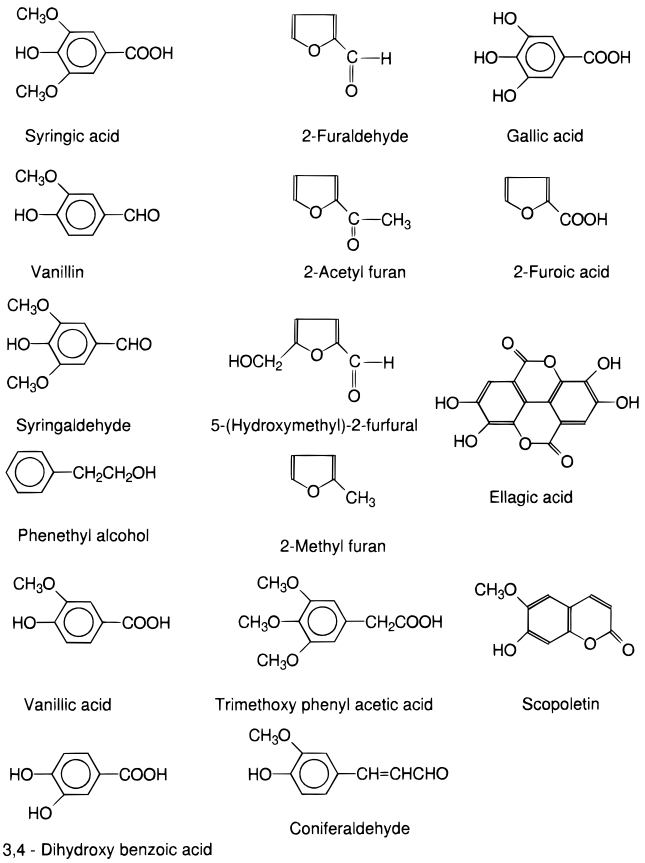

Figure 1. Structural formulas of principal constituents for which data are presented.

hyde (S760-2), ellagic acid (E40-1), coniferaldehyde (38, 205-1), scopoletin (24, 658-1), and catechin (86, 181-2).

Furans included 5-(hydroxymethyl)furfural (H4, 080-7), 2-furoic acid (F2, 050-5), 2-furaldehyde (18, 591-4), 2-methylfuran (M4, 684-5), and 2-acetylfuran (A1, 625-4).

Instrumentation. The HPLC equipment used was from Waters Associates, comprising the 600 controller, 717 autosampler, 616 pump, inline degasser, and 996 photodiode array detector. The system was interfaced with a Millennium 2010 Chromatography Manager. An ET 250/4 Nucleosil 100-5 C_{18} column (25 cm × 4 mm) from Macherey-Nagel, Duren, Germany, with a 5-μm particle size was used as the stationary phase and was maintained at 25 °C during operation. It was preceded by a guard column comprising KS 11/4 Nucleosil 120-5 C_{18} (2 cm × 4 mm) of 5-μm particle size from the same source.

Procedure. The method of Goldberg and Soleas (1998) was employed. Twenty microliters of the beverage being assayed was directly injected into the HPLC, and the phenols were eluted in reverse phase using a gradient mobile phase of 0.2 mL of phosphoric acid and 2 mL of acetic acid in 1 L of deionized water, pH 2.1 (solvent A), together with 0.2 mL of phosphoric acid and 2 mL of acetic acid in 1 L of acetonitrile, pH 2.4 (solvent B), according to the program defined in Table 1. The solvents were filtered through a Millipore 0.45-μm PFTBA membrane filter (Millipore, Canada) and degassed inline. The phenols and furans were monitored over a range of wavelengths from 250 to 390 nm.

Method Characteristics. Chromatographic resolution is demonstrated for two beverages in Figure 2. The method was linear over the range 0.24−500 mg/L except for ellagic acid, which was linear in the range 0.03−100 mg/L. Recovery ranged from 87% (syringaldehyde) to 106% (2-furoic acid), except for gallic acid (70%). Precision ranged from 1.6% (gallic acid) to 9.6% (catechin) except for 2-furoic acid (16.0%) and 5-(hydroxymethyl)furfural (16.2%). The detection limit was ≥0.01 mg/L for all compounds for which data are presented.

Table 1. Gradient Elution Program for Method 2[a]

time (min)	flow rate (mL/min)	A (%)	B (%)	curve
0	0.30	90	10	
20	0.30	87	13	8
50	0.30	85	15	11
70	0.30	84	16	11
80	0.33	80	20	11
90	0.30	78	22	11
110	0.30	75	25	11
120	0.30	73	27	10
155	0.60	50	50	6
157	0.65	90	10	4
165	0.65	90	10	

[a] Compositions of eluants A and B are described in the text.

Assay of TAS. This was measured on each sample in duplicate with the kit manufactured by Randox Laboratories Ltd., Mississauga, ON, Canada (catalog no. NX2332) using the Cobas Fara II centrifugal analyzer (Roche Analytical Instruments, Nutley, NJ) as previously described (Soleas et al., 1997c). The total reaction volume of 305 μL comprised the following: 250 μL of chromogen/enzyme reagent with buffer and cofactors; 5 μL of sample followed by 20 μL of diluent (water); 20 μL of initiator (H_2O_2) followed by 10 μL of diluent (water). The appearance of the blue-green radical cation $ABTS^{\bullet+}$ is monitored continuously at 600 nm. Antioxidants cause suppression of this color production to a degree that is proportional to their concentrations. Calibration was performed using 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) with results reported as millimoles per liter. All samples with concentrations >1.5 mmol/L were diluted and reassayed.

Samples Analyzed. Samples of 12 different categories of distilled spirits were randomly selected from among those routinely submitted for approval by the Quality Assurance Department of the Liquor Control Board of Ontario over a 6-month period, the analysis being undertaken within a short interval of opening the bottle. The number of samples and the categories are indicated in the following tables and figures.

Statistics. For ease of data presentation, the mean and SEM of individual samples within each category were compiled, but as the distribution of values within some categories was non-Gaussian, the nonparametric Mann−Whitney U-test (Sokol and Rohlfs, 1981) was used to test the significance of differences among the various beverages. To evaluate the relationship of dependent (e.g., TAS) and independent (e.g., duration of wood aging) variables, the Pearson product moment correlation coefficient r and the slope (regression coefficient, R) and intercept of the regression equation, together with their standard errors (SE), were calculated (Snedecor and Cochran, 1967).

RESULTS

Although a number of phenolic compounds could be measured consistently in the majority of the distilled spirits analyzed, the chemical spectrum was completely different from that of wine, either red or white (Soleas et al., 1997b; Goldberg et al., 1998). Notably absent or undetectable were flavonols (e.g., quercetin and myricetin), flavan 3-ols (e.g., catechin and epicatechin), hydroxystilbenes (e.g., resveratrol and polydatin), and hydroxycinnamates (e.g., *p*-coumaric acid). Those phenols that were present in the beverages analyzed manifested an association with oak wood and were found most commonly and in highest concentrations in those spirits in which wood aging is a major feature of production and maturation, suggesting that they were derived wholly or partly from this process.

Of the beverages submitted for analysis, most phenols failed to reach measurable levels in the majority of samples of the following: vodka, gin, liqueurs, and

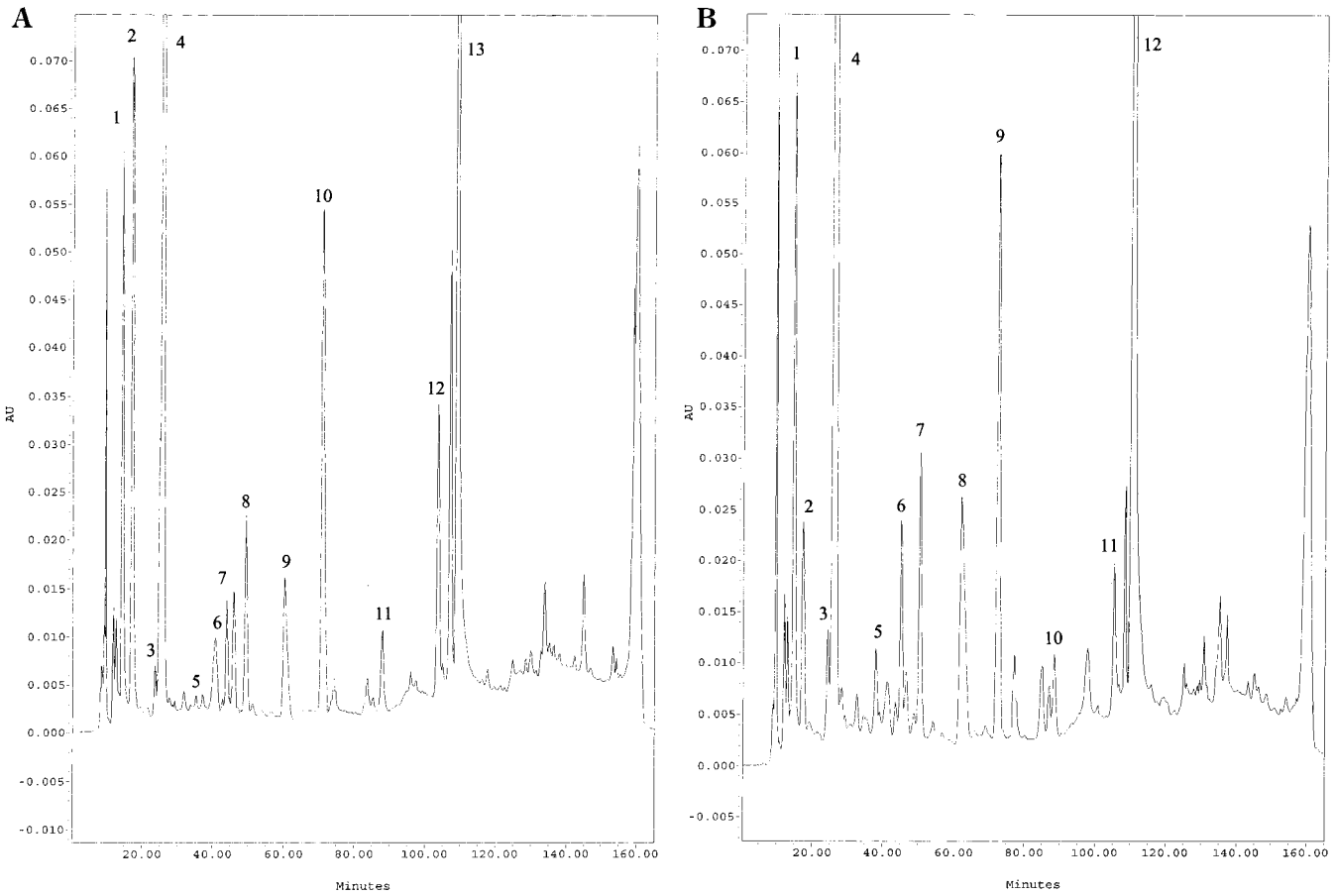

Figure 2. Typical chromatograms of bourbon (A) and single-malt Scotch whiskey (B) with output as integrated scan over wavelengths 250−350 nm are numbered as follows (figures in parentheses indicate wavelengths, essentially λ_{max}, at which each constituent was read): (A) 1, gallic acid (274.5 nm); 2, 5-(hydroxymethyl)furfural (284 nm); 3, 3,4-dihydroxybenzoic acid (260.4 nm); 4, 2-furaldehyde (279.3 nm); 5, 2-acetylfuran (274.5 nm); 6, vanillic acid (260.4 nm); 7, 2-methylfuran (293.5 nm); 8, syringic acid (274.5 nm); 9, vanillin (279.3 nm); 10, syringaldehyde (307.7 nm); 11, 3,4,5-trimethoxyphenylacetic acid (269.8 nm); 12, coniferaldehyde (341 nm); 13, ellagic acid (256.6 nm). (B) 1, gallic acid; 2, 5-(hydroxymethyl)furfural; 3, 3,4-dihydroxybenzoic acid; 4, 2-furaldehyde; 5, 2-acetylfuran; 6, vanillic acid; 7, syringic acid; 8, vanillin; 9, syringaldehyde; 10, 3,4,5-trimethoxyphenylacetic acid; 11, coniferaldehyde; 12, ellagic acid. Other peaks identified and quantitated in some samples were *p*-hydroxyphenethyl alcohol (260.4 nm), 2-furoic acid (255 nm), and scopoletin (345.8 nm).

Table 2. Concentrations of Six Polyphenols in Distilled Spirits[a]

beverage	gallic acid	vanillic acid	syringic acid	syringaldehyde	vanillin	ellagic acid
whiskies						
single-malt Scotch (23)	1.29 ± 0.19	0.21 ± 0.03	0.85 ± 0.11	1.00 ± 0.14	0.47 ± 0.06	10.04 ± 1.60
blended Scotch (12)	0.81 ± 0.15	0.34 ± 0.09	0.54 ± 0.23	1.23 ± 0.43	0.32 ± 0.09	5.09 ± 1.22
Canadian rye (19)	0.57 ± 0.09	0.21 ± 0.03	0.98 ± 0.15	1.14 ± 0.15	0.38 ± 0.05	6.09 ± 0.71
American bourbon (12)	1.28 ± 0.19	0.64 ± 0.17	2.06 ± 0.25	4.44 ± 0.49	0.94 ± 0.12	11.68 ± 2.10
brandies						
cognac (10)	3.23 ± 0.58	0.28 ± 0.09	1.07 ± 0.19	1.16 ± 0.22	0.64 ± 0.09	14.84 ± 1.72
armagnac (7)	4.77 ± 0.67	0.52 ± 0.22	2.15 ± 0.93	2.74 ± 1.00	1.50 ± 0.49	30.78 ± 5.57
regular brandy (19)	1.06 ± 0.32	0.04 ± 0.01	0.49 ± 0.23	0.22 ± 0.07	0.24 ± 0.10	2.31 ± 0.65
rum (21)	0.67 ± 0.43	0.11 ± 0.05	0.35 ± 0.18	0.43 ± 0.21	0.83 ± 0.53	2.13 ± 0.60

[a] Data are mean ± SEM (mg/L) with number of samples in parentheses.

miscellaneous spirits. Those beverages having the majority of samples with measurable concentrations of phenols are listed in Table 2 together with the mean ± SEM for each constituent. Data are not presented for individual compounds for which <50% of the samples analyzed contained measurable concentrations.

Phenolic Constituents. *Gallic Acid.* This was present in all of the beverages listed in Table 2, being highest in armagnac (P <0.01), followed by cognac (P < 0.025). Rum, rye whiskey, and blended Scotch had the lowest values.

Vanillic Acid. Here, the concentrations were highest in bourbon whiskey (P < 0.05) followed by armagnac (P < 0.05), and lowest concentrations were given by brandy and rum.

Syringic Acid. For this constituent, armagnac and bourbon gave the highest concentrations (P < 0.02). Values were low in rum, brandy, and blended Scotch.

Vanillin. With this constituent, armagnac had the highest mean concentration (P < 0.01) followed by bourbon and rum. Lowest concentrations were present in blended Scotch and rye whiskies.

Syringaldehyde. This was present in higher concentrations than syringic acid in most beverages, the highest being bourbon (P < 0.025), followed by armag-

nac. The lowest mean concentration was given by brandy ($P < 0.001$) and the next lowest by rum.

Ellagic Acid. This was the predominant phenolic constituent in distilled spirits and the highest by far in all beverage classes ($P < 0.001$). Once again armagnac had the highest mean concentration ($P < 0.01$), with cognac, bourbon, and single-malt Scotch whiskey next in that order. Rum and brandy contained the lowest concentrations.

Characteristics of Individual Beverages. *Single-Malt Scotch Whiskey*. In addition to the constituents listed in Table 2, this beverage was unique in having a rather high mean concentration of *p*-hydroxyphenethyl alcohol (2.47 ± 2.48 mg/L). 3,4-Dihydroxybenzoic acid and trimethoxyphenylacetic acid were present in mean concentrations of 0.30 ± 0.05 and 2.61 ± 0.51 mg/L, respectively, as well as coniferaldehyde (0.05 ± 0.01 mg/L).

Rye Whiskey. In addition to the compounds listed in Table 2, 3,4-dihydroxybenzoic acid was present at a mean concentration of 0.27 ± 0.06 mg/L and coniferaldehyde at 0.04 ± 0.01 mg/L.

Blended Scotch Whiskey. As in single-malt Scotch whiskey, this beverage contained trimethoxyphenylacetic acid (2.38 ± 0.77 mg/L) as its principal phenolic constituent after ellagic acid.

Rum. 3,4-Dihydroxybenzoic acid was present in the majority of the samples analyzed at a mean concentration of 0.15 ± 0.06 mg/L. For most phenolic constituents, this beverage gave the lowest or second lowest values of all those analyzed.

Brandy. No phenolic constituents additional to those included in Table 2 were detected in this beverage, which shared with rum the distinction of having the lowest concentrations of phenols among virtually all of the beverages presented in Table 2.

Cognac. Quite high values of trimethoxyphenylacetic acid (6.30 ± 1.81 mg/L), but not 3,4-dihydroxybenzoic acid, were found in this beverage that ranked second among all those tested for gallic acid and ellagic acid. Indeed, the concentrations of all phenolic constituents were in the top half of the ranges for all beverage classes as indicated in Table 2. Additionally, coniferaldehyde was present at a mean concentration of 0.19 ± 0.04 mg/L.

Armagnac. This had the highest concentrations of gallic acid, syringic acid, vanillin, and ellagic acid, as well as the second highest of vanillic acid, syringaldehyde, coniferaldehyde, and trimethoxyphenylacetic acid (0.34 ± 0.09 and 6.53 ± 7.26 mg/L, respectively, for the latter two constituents; not shown in Table 2).

Bourbon Whiskey. 3,4-Dihydroxybenzoic acid (0.37 ± 0.05 mg/L) was consistently measurable in the samples of this beverage analyzed. As well as the highest concentrations for vanillic acid, syringaldehyde, coniferaldehyde, and trimethoxyphenyl acetic acid (0.94 ± 0.13 and 7.95 ± 1.47 mgL, respectively, for the latter two constituents; not shown in Table 2), the concentrations of the other constituents of bourbon presented in Table 2 were consistently among the upper half of the ranges demonstrated by the different beverage classes. Additionally, scopoletin was consistently measurable in bourbon whiskey at a mean concentration of 0.57 ± 0.17 mg/L.

Furans. Two compounds, 5-(hydroxymethyl)furfural and 2-furaldehyde, were present in most of the beverages analyzed (Figure 3). The former yielded concentrations in line with those for ellagic acid, with which it shared the distinction of having the highest concentrations for any of the constituents that were analyzed in this study. The highest mean concentration of this compound was found in cognac ($P < 0.001$), with brandy and rum next in line. Canadian rye whiskey had the lowest mean concentration for this constituent ($P < 0.001$). With respect to 2-furaldehyde, single-malt Scotch whiskey gave the highest mean concentration ($P < 0.01$), followed by cognac and armagnac; rum and rye whiskey had the lowest mean concentrations. The other furans analyzed were present in only two to five of the eight beverages featured in Figure 3. 2-Methylfuran showed highest concentrations in bourbon whiskey (2.97 ± 1.47 mg/L, $P < 0.05$); lower levels were detected in armagnac and cognac, whereas few examples of rye whiskey, blended Scotch, and rum gave measurable values of this constituent. On the other hand, 2-acetylfuran was most concentrated in cognac (1.00 ± 0.58 mg/L, $P < 0.05$), whereas single-malt Scotch whiskey and armagnac contained lesser amounts. 2-Furoic acid was measurable only in rye whiskey and rum at mean concentrations of 0.83 ± 0.08 and 0.66 ± 0.19 mg/L, respectively.

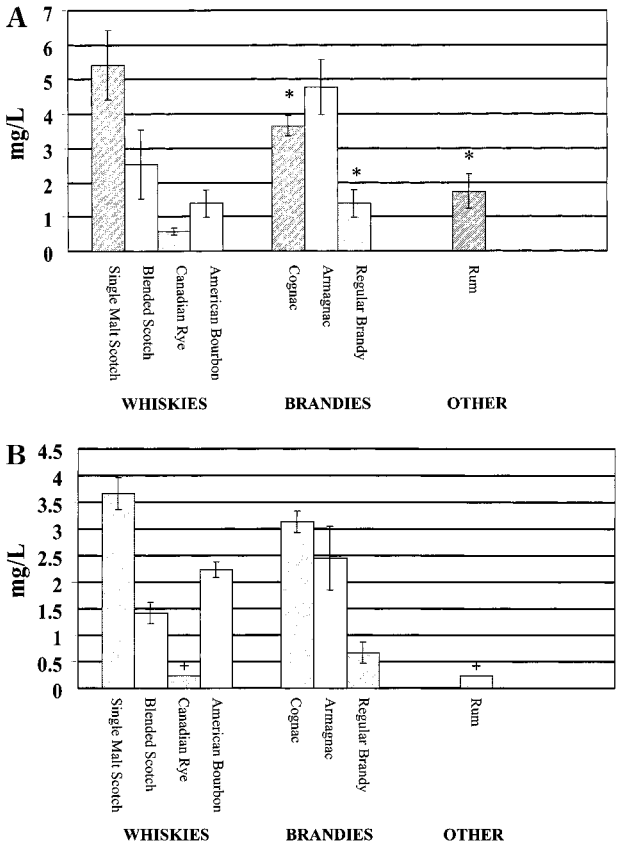

Figure 3. Mean concentrations (milligrams per liter) of 5-(hydroxymethyl)furfural (A) and 2-furaldehyde (B) of eight distilled spirits analyzed in this survey where the majority of samples contained measurable amounts of the two furans. Number of samples is as in Table 2. The bars represent 1 SEM. The asterisks refer to data that have been multiplied by 1×10^{-1}, and the + symbols indicate that the SEM was <0.04 mg/L.

TAS. The highest TAS values were seen for armagnac ($P < 0.001$) followed by cognac (Figure 4), with bourbon whiskey showing higher mean values than single-malt

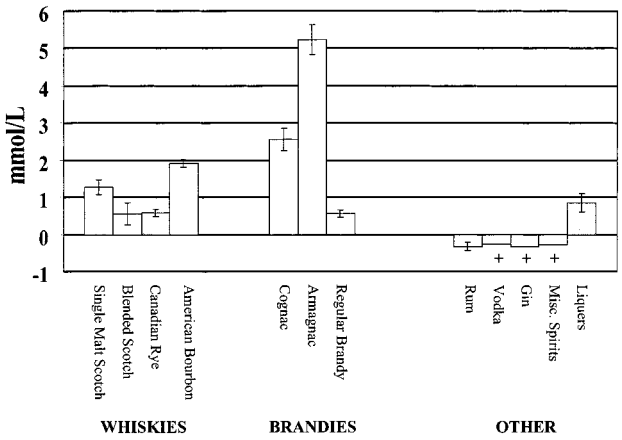

Figure 4. Mean TAS (mmol/L) of distilled spirits and beer analyzed in this survey. The bars represent 1 SEM. For the three beverages marked by the + symbol, the SEM was <0.01 mmol/L. In addition to the beverages enumerated in Table 2, the numbers for the other five were as follows: vodka (18), gin (8), miscellaneous spirits (12), and liqueurs (11). Two samples of tequila and two of ouzo, not included in Figure 4, had positive TAS values that averaged 2.11 mmol/L.

or blended Scotch whiskey, rye whiskey, and brandy ($P < 0.05$). The mean values for TAS were negative in vodka, gin, rum, and miscellaneous spirits (Figure 4). The latter category comprised 7 samples of grappa and 5 of eaux-de-vie for a total of 12 samples. Liqueurs spanned a wide range from negative to highly positive values with a mean of 0.86 ± 0.84 mmol/L. Of the 11 liqueurs analyzed, 7 gave small negative TAS values and the other 4 quite large positive values, so that the overall mean was 0.86 ± 0.84 mmol/L.

DISCUSSION

Role of Production Techniques. Distilled spirits are produced by processes that are specific to each beverage, the two principal variables being the starting material (mash or must) used for distillation and the aging process, if any (Lembeck, 1983).

For most constituents, both phenolic and furans, single-malt Scotch whiskey had higher mean concentrations, as well as higher TAS concentration, than blended Scotch. This is in line with the more intense enzymatic conversion of starches to low molecular weight carbohydrate in fully germinated malt barley than in the grains that form the basis for >50% of the spirit in blended Scotch whiskey. Barrel aging, usually in American oak or sherry casks, also proceeds for longer periods in single malt. Indeed there was a significant correlation ($r = 0.591$; $P < 0.05$) between the TAS and the age of the single-malt Scotch whiskies over the range of 8−17 years of wood aging.

Rye whiskey is made in Canada from up to four different grains, including rye and barley malt, and is produced by fermentation and distillation techniques similar to those used for blended Scotch; prolonged barrel aging does not usually occur. Its chemical composition in this study was similar to that of blended Scotch, in line with expectation.

Bourbon whiskey is made by rather different procedures. Two, in particular, are worthy of note. The distillation takes place at a quite low proof, not exceeding 160; this has the effect of allowing many congeners to pass over with the ethyl alcohol. The second is the use of charred oak barrels for aging, periods of up to 8 years not being uncommon. It appears that these two processes may account for the higher phenolic and furan contents and TAS of this whiskey compared with the previous three whiskies, many of these differences being statistically significant when compared with all of the other whiskies combined: vanillic acid ($P < 0.01$); syringic acid ($P < 0.005$); syringaldehyde ($P < 0.005$); vanillin ($P < 0.01$); coniferaldehyde ($P < 0.001$); 5-(hydroxymethyl)furfural ($P < 0.001$); TAS ($P < 0.05$).

Brandy, cognac, and armagnac are distilled from wine, the latter two from wines produced under rigorous control from a very limited range of areas of France and only from strictly defined white grape varietals. Many white wines contain significant amounts of phenols (Goldberg et al., 1999), consistent with the finding that cognac and armagnac combined have higher mean concentrations of such constituents (gallic acid, $P < 0.0001$; vanillic acid, $P < 0.01$; syringic acid, $P < 0.0005$; vanillin, $P < 0.01$; coniferaldehyde, $P < 0.01$; and ellagic acid, $P < 0.01$) than of all the other spirits in this survey collectively excluding bourbon whiskey, as well as the highest TAS values ($P < 0.001$). However, because their volatility is lower than that of ethanol, they are more likely to be derived from wood than from grapes. Wood aging of up to 10 years, in Limousin oak for cognac and black Monlezun oak for armagnac, is a notable feature. A major difference from most other distilled spirits is that these beverages undergo two distillation processes, which are batch operations in the case of cognac and continuous in the case of armagnac.

All of the phenols measured in these two spirits were significantly higher than those of brandy. The 19 samples of the latter in this survey were from various countries, although nearly 50% were from France. The soil conditions and the grapes used in production are different from those employed in making cognac and armagnac, and wood aging takes place for shorter periods of time. An analysis of the chemical composition and TAS of cognac showed a trend toward higher values according to the progression VO, VSOP, XO, in line with increasing quality as reflected by longer wood aging, but this did not reach statistical significance in the small number (10) included in this survey.

The miscellaneous spirits included in this survey comprised eau-de-vie and grappa. The latter are distilled from grape pomace but are not aged in wood. They failed to contain significant concentrations of grape phenols, in contrast to cognac and armagnac. The grape origin of these spirits seems to be unimportant, with wood contact as the likely major source of these phenols.

Most rums are produced by distilling fermented molasses, the residue of sugarcane juice once the crystallized sugar has been removed. The 21 rums in this survey included white, gold, and dark rums, the latter having significantly higher values for TAS ($P < 0.05$) and somewhat higher values for most phenols than the first two types combined. White rums do receive up to 6 years of aging in seasoned oak barrels but are then passed through a filtering and leaching vat. Caramel is added to gold or amber rums. Dark rums are aged in oak puncheons for up to 15 years before bottling, being full bodied and rich in congeners. Their main source is Jamaica, whereas white and gold rums are predominantly from Puerto Rico and the Bahamas.

TAS of Beverages Analyzed. The negative mean TAS values for gin, vodka, and miscellaneous spirits (as well as for white rum) were significantly different from

zero ($P < 0.001$). These negative values obtained with beverages low in phenols and furans might be thought to be attributable to their high (~40%) alcohol concentration. In vivo, ethanol has pro-oxidant activity and enhances free radical formation (Nordman, 1994), but we could find no evidence for this effect in vitro using the Randox assay. Catechin in concentrations of 25, 50, and 100 mg/L had similar values for TAS whether dissolved in 5%, 10%, or 40% ethanol. It is possible that traces of other alcohols found in distilled spirits, in the absence of phenolic antioxidants, could produce a beverage with overall pro-oxidant activity. Be that as it may, the TAS values in beverages such as cognac and armagnac are possibly lowered by their high alcohol content, the pro-oxidant activity of which may reduce those values due to phenols and furans in the milieu.

A wide range of values, both negative and positive, were found for TAS in liqueurs, the vast majority of which were devoid of any of the compounds that we assayed. Either the herbs and fruits used in their preparation do not lead to the presence of antioxidants in the finished spirit, or they generate antioxidants different from the polyphenols and furans that were measured in this investigation. Although many liqueurs are known to have a high sugar content, the reducing properties of these sugars do not contribute to the antioxidant activity of those liqueurs that gave positive TAS results. Neither sucrose (a nonreducing sugar) nor glucose (a reducing sugar) in final concentrations up to 20 g/L altered the TAS of catechin or Trolox, nor did they possess TAS activity of their own, and a scrutiny of these beverages did not reveal major differences of sugar content between liqueurs with negative and positive TAS values. Interestingly, the two with the highest values of 5.31 and 3.35 mmol/L were both from coffee, which appears to have significant anticancer and antimitogenic activities (Abraham, 1995; Nagasawa, 1996). The explanation for these properties is not known. There is little information about the chemical compositon of coffee, but it is possible that antioxidants, phenolic or otherwise, may be contributing factors as has been well established for the related beverage, tea, that has been reported to possess strong antimitogenic (Ruch et al., 1989; Bu-Abbas et al., 1994, 1996) and antiatherosclerotic (Ikeda et al., 1992; Yokozawa et al., 1995) activities.

An important issue in this investigation is the reliability of the TAS assay. This was developed by Rice-Evans and colleagues (Rice-Evans and Miller, 1991; Miller et al., 1993) and has been applied to clinical studies utilizing human serum (Miller et al., 1993; Sharpe et al., 1995). Its use with this matrix, where albumin, urate, and ascorbate are the major antioxidants (Maxwell, 1997), has been criticized because of nonlinear behavior with respect to the time course of the reaction and sample dilution (Schofield and Braganza, 1996; Cao and Prior, 1998), but we have found the method to work well with wine (Soleas et al., 1997c) and also with spirits in the present investigation. The main technical concern is the somewhat high imprecision that makes it advisable to perform duplicate assays on each sample for reliable results. Another concern is the validity of extrapolating the antioxidant activity measured in aqueous environments to that expected in vivo, where the action occurs in a lipid milieu or at an oil−water interface such as membranes or lipoprotein particles (Frankel et al., 1994, 1995).

Comparison with Literature Values. Few studies on the concentrations of polyphenols and furans in distilled spirits have been published. The handful to be found in the global literature are anectodal or experimental and do not cover defined finished commercially available beverages. Guymon and Crowell (1968) described the presence of vanillin, syringaldehyde, and coniferaldehyde in brandy extracts of oak woods that they attributed to the lignin fraction. In nine samples they found vanillin and syringaldehyde concentrations ranging from 7 to 13 mg/L and from 11 to 12 mg/L, respectively. Our values were very much less, but in good agreement with those reported by Baldwin et al. (1967) for extracts of American whiskey and by Sanchez et al. (1990) for the same two constituents of four unspecified rums. Lactones of methylfuran have also been reported as aroma constituents of whiskey and cognac (Jefford et al., 1989). The only paper describing hydroxy or methoxy derivatives of benzoic acid in an alcoholic beverage that we could identify was focused upon sherry musts and did not give actual concentrations (Guillen et al., 1993).

Conclusion. Compared with red and white wines with TAS values around 8−12 and 0.25−1 mmol/L, respectively (Soleas et al., 1997c), many distilled spirits have intermediate values that may confer some nutritional advantage through enhanced consumption of exogenous antioxidants. These possible benefits are restricted to beverages that receive wood aging and parallel their concentrations of phenols and furans measurable by our HPLC method. The former differ in large measure from those present in red wines, such as (+) catechin and (−) epicatechin, that have been well characterized with respect to their antioxidant activities (Frankel et al., 1995; Soleas et al., 1997c). Quantitatively, the most important phenolic constituent in these beverages was ellagic acid, a potent antimutagenic and anticancer agent (Josephy et al., 1990), which is a minor constituent of wines.

Doubtless, other phenols that we could neither detect nor quantitate are present in these beverages, but there do not appear to be many unknown peaks in our chromatograms (Figure 2). In projecting the potential health benefits of these beverages, one must take into account the quantities consistent with moderate and safe alcohol consumption (Doll et al., 1994). Clearly, the recommended daily volumes will be inversely related to their alcohol concentrations. On this basis, the permissible daily consumption of red wine will provide more antioxidants than that of distilled spirits. A recent report documented similar and significant increases in plasma total phenol content and antioxidant capacity within 30 min of drinking 100 mL of whiskey or red wine (Duthie et al., 1998). It remains to be determined whether the furans present in these beverages can offer the protection against cancer, atherosclerosis, and inflammatory diseases that have been ascribed to red wine constituents such as quercetin and resveratrol (Soleas et al, 1997a).

ACKNOWLEDGMENT

We thank Mrs. Pat Machado and Mrs. Rosy Moses for the preparation of the manuscript and Ms. Dorothea Vafiadis for bibliographical assistance. Valuable technical help was provided by Ms. Jasmine Wong.

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Received for review October 21, 1998. Revised manuscript received July 12, 1999. Accepted July 19, 1999. This work was performed with the aid of a grant from the Distilled Spirits Council of the United States.

JF9811626