Aromatic Congener Formation in Maturation of Alcoholic Distillates

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Vanillin, syringaldehyde, coniferaldehyde, sinapaldehyde, scopoletin, and ethanol lignin were found in both whiskey and neutral spirits aged in various types of white oak cooperage. Data obtained indicate that the mode of development of these congeners is similar for both whiskey and spirits, the amount formed depending upon the type of cooperage used and proof of distillate being aged. Experimental evidence was obtained which supports that from the literature in indicating that these aromatic congeners can arise from chemical reaction between the ethanol and components of the oak wood (charred or uncharred)—i.e., lignin—under the acidic conditions (pH 4 to 5) imposed by the barrel. The concentrations of other congener groups formed in both types of alcoholic products stored for 18 months in new charred barrels were similar.

Whiskey, brandy, and other alcoholic distillates undergo certain organoleptic and chemical changes during contact with the barrel. The distillates develop a new, pleasing aroma and flavor and chemical analysis reveals a change in composition. During the aging of whiskey and brandy distillates, color is acquired and esters, aldehydes, furfural, tannins, and dissolved solids increase in concentration (*4*, *5*, *20*, *21*, *36*, *37*). These changes cannot be duplicated by storage in glass. There are several possibilities to account for the changes that occur during maturation—e.g., chemical interactions between the distillate and the (charred) wood, chemical interactions among the constituents of the distillate, and physical extraction of barrel constituents (*4*, *21*). It is now generally accepted that the maturation process is due to a combination of chemical interaction and extraction.

Since government regulations specify that whiskey barrels must be made of white oak (*Quercus alba*), a knowledge of the composition of this wood is essential to a study of maturation. Analyses of white oak heartwood at the Forest Products Laboratory, USDA (*27*), showed: cellulose, 49 to 52%; lignin, 31 to 33%; pentosan (hemicellulose), 22%; and hot water extractives plus ether extractives, 7 to 11%. The extractives are a complex group of substances containing, but not limited to, volatile oils and acids, resins, fats, waxes, pigments, tannins, phlobaphenes, and carbohydrates. Extractives include material soluble in alcohol. Many such substances, not present in distillates, are in matured whiskey.

These extractives were once thought to be exclusively responsible for the changes occurring during aging. When charred oak wood sawdust is directly extracted with water or 192° proof ethanol, however, the resulting unaged extracts differ markedly in odor from aged whiskey. Also, none of the various fractions of alcohol-soluble oak wood extractives contains flavors resembling mature whiskey (*14*). Therefore, some process in addition to simple extraction must be contributing to maturation.

The congener composition of brandies has been investigated by workers in Russia (*7–9*, *11*, *13*, *19*, *30–34*), while that of malt whiskey has been studied by workers in Japan (*24*, *25*, *38*). Products of this type are aged in re-used charred and uncharred, new charred, and new uncharred oak barrels. There is apparently nothing in the literature on the identification of barrel-derived congeners in American whiskey—i.e., bourbon and rye—except for a recent disclosure (*22*). Vanillin, however, has been reported as a component in the fusel oil concentrate from an unaged whiskey distillate (*3*). Lignin-derived congeners probably contribute in large measure to flavor improvement in aged products (*7–9*, *11*, *13*, *19*, *24*, *25*, *30–34*, *38*). Previous work from this laboratory revealed that aromatic aldehydes could be detected in white oak hardwood (*2*). Therefore, this class of congeners in aged products has been investigated further and is reported here.

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Procedure

Identification of Aromatic Aldehydes. After preliminary flushing with prepurified nitrogen, 1 liter of each product listed in Table I was concentrated in a rotary film evaporator (30 mm. of Hg) to about 300 ml. at 35° to 45° C. bath temperature. The residues had woody odors characteristic of aged whiskey and a brown, cloudy appearance.

A 1-liter sample of 140-proof unaged spirits similar to those stored in new charred barrels (Table I, samples 5 and 6) was concentrated in a similar manner to less than 1 ml. There was no visible solid residue and a phloroglucinol test (see below) on the concentrate was negative for aromatic aldehydes. Since the concentrate did not fluoresce when exposed to ultraviolet light, absence of scopoletin (see below) was indicated.

Each residue from aged products was centrifuged to remove a small amount of solids (ethanol lignin; see below), but the supernatant liquid remained cloudy. The concentrate was then saturated with sodium chloride, whereupon a brownish solid separated upon standing. The solid was removed by filtration and the clear filtrate extracted with three 50-ml. portions of ether. The combined ether extract was washed with 20 ml. of water and allowed to evaporate to dryness. All residues had woody, maple sirup like odors, while that from an uncharred barrel (Table I, sample 4) had, in addition, an intense phenolic odor with wax-like overtones. The residue was dissolved in 5 to 10 ml. of 96% ethanol and subjected to paper chromato-

Table I. Identification of Samples

Spirits	Age, Years	Cooperage[a]	Barrel Proof
1	3 1/2	Bourbon dumper (B.D.)	136.6
2	3 1/2	Recharred unscraped B.D.	136.0
3	6	Spirit dumper	137.0
4	6	New uncharred	136.7
5	1 1/2	New charred	109.7
6	1 1/2	New charred	140.8
Bourbon			
7	5	New charred	102.5
8	4	New charred	107.6
9	4	New charred	104.8
10	4	New charred	106.3

[a] Bourbon dumper, first refilling of new charred barrel that originally contained bourbon.
Spirit dumper, B.D. refilled one or more times with spirits.

graphic analysis using the procedure described previously (*2*); however, different solvents were employed:

Ligroin–Eastman practical grade, redistilled, b.p. 100° to 110° C. Its labeled boiling range was 100° to 120° C.; that of the previous solvent was 90° to 120° C.

Heptane–Eastman pure. This solvent was used as received and could be substituted for ligroin with comparable results.

Methylcyclohexane–Eastman pure, used as received. It is the preferred solvent because it gave the best resolution and most reproducible R_f values. Development time with this solvent was 12 to 14 hours.

n-Butyl ether–Eastman practical grade. Eastman pure grade is no longer available. This solvent was purified as follows: To remove peroxides, 1 liter of solvent was shaken with two 100-ml. portions of 30% aqueous sodium sulfite in a separatory funnel. A peroxide test (HCl–2% KI) on the purified solvent was negative while the solvent as received gave a positive test. The solvent was distilled and that distilling at 139–41° C. collected and stored in an amber bottle.

The sensitivity of detection of vanillin and syringaldehyde under ultraviolet light could be increased by first fuming the chromatogram with ammonia. This treatment caused a large absorption of ultraviolet light with resulting dark zones for these two aldehydes. The sinapaldehyde and coniferaldehyde zones turned yellow in visible light when fumed with ammonia. Indicator sprays were also used (*2*). Phloroglucinol, 2.5% in 3*N* ethanolic HCl, could be sprayed without clogging the nozzle. The 3*N* ethanolic HCl was prepared by diluting 25 ml. of concentrated HCl to 100 ml. with 96% ethanol.

Identification of 6-Methoxy-7-hydroxycoumarin (Scopoletin). Chromatograms of whiskey concentrates revealed a prominent unidentified spot which developed a blue-white fluorescence under ultraviolet light (*2*). To obtain a larger amount of this material for identification, 16 sheets of Whatman No. 1 filter paper were processed in a Chromatocab unit (*2*). The blue-white fluorescent zones were cut out, and the material was extracted with 96% ethanol, evaporated to small volume, and rechromatographed by descending method on Whatman No. 1 filter paper. Development time was 3 hours with water saturated with 1-butanol (9%). Prior to development the system was equilibrated for 18 hours with both phases in the bottom of the jar.

The blue-white fluorescent zones were again cut out, and the material was extracted with 96% ethanol. Ultraviolet spectra of the extract were determined with a Beckman DU spectrophotometer using 1-cm. cells:

$\lambda_{max}^{96\%\ EtOH}$ 229, 253 (sh), 300, 345mμ;

$\lambda_{max}^{96\%\ EtOH/(CH_3)_4NOH}$ 398 mμ

These values and the fluorescent behavior of the material corresponded to literature descriptions of two compounds: 6,7-dihydroxycoumarin (esculetin) and 6-methoxy-7-hydroxycoumarin (scopoletin) (*6*, *12*).

Comparative paper chromatograms were then run on the unknown compound simultaneously with authentic esculetin and scopoletin (K and K Laboratories, Inc., Plainview, N. Y.). The nitromethane-benzene–water system described by Dieterman *et al.* (*6*) was employed, except for the substitution of Whatman No. 1 filter paper. This method gave a wide divergence of R_f value for the two compounds, 0.09 for esculetin and 0.80 for scopoletin. The unknown had the same R_f value as scopoletin. The fluorescent properties of scopoletin and the unknown were identical under ultraviolet light, both directly and after exposure to ammonia fumes. In the latter case, the fluorescence changes to a lighter shade of blue as reported by other workers (*6*, *12*). Comparative paper chromatography with the 9% 1-butanol–water system was also used to verify the identity of scopoletin.

A second nonfluorescent zone which appeared below the scopoletin area during rechromatography gave positive tests with phloroglucinol and 2,4-dinitrophenylhydrazine, indicative of a phenolic carbonyl compound. This substance has not yet been characterized.

Identification of Ethanol Lignin. The solids which separated from all barreled products as a result of concentration were washed with water until the filtrates were colorless. After air-drying, they varied in color from light brown to light tan. Infrared spectra (samples mulled in mineral oil) were obtained with a Perkin Elmer 221 spectrophotometer. The spectra of all samples were identical: ν_{max}^{mull} 3311 (—OH stretching, hydrogen-bonded), 1704 (C=O stretching), 1592^{max} (aromatic ring skeletal vibration), 1499 (C=C stretching vibration of benzene ring), 1321 (O—H in plane bending vibration), 1266 (C—O—C asymmetric stretching vibration of aryl ether linkages), 1208 (C—O—C asymmetric stretching vibration of aryl-alkyl ether linkages), 1115 (C—O—C asymmetric stretching vibration in dialkyl ether linkages), 1026 cm.$^{-1}$ (C—O—C symmetric stretching vibration in dialkyl ether linkages). The ultraviolet spectrum gave $\lambda_{max}^{95\%\ EtOH}$ 280 mμ.

All of the isolated solids gave a positive Zeisel test

for methoxyl groups and a positive purple-red color test with phloroglucinol, indicating the presence of coniferaldehyde or sinapaldehyde moieties. These solids also gave a positive test for ethoxyl groups (*11*). The material isolated from sample 10 was oxidized with nitrobenzene (*15–17*), and the products were chromatographed with the methylcyclohexane–*n*-butyl ether–water system described above. The only spots obtained correspond to vanillin and syringaldehyde, which are the oxidation products expected from hardwood lignin. Analyses of white oak ethanol lignin were performed by the Clark Microanalytical Laboratory, Urbana, Ill.: C, 61.4; H, 5.7; —OCH_3, 15.7. Total alkoxyl—i.e., ethoxyl plus methoxyl—is reported as methoxyl. Apparently, there are no analytical data for white oak ethanol lignin in the literature.

Semiquantitative Analysis of Aromatic Aldehydes. The procedure described above for the identification of aromatic aldehydes was followed with these modifications: A 500-ml. sample of alcoholic product was evaporated under vacuum to 125 ml., quantitatively transferred to a separatory funnel, and extracted three times with 50-ml. portions of ethyl ether. The combined ether extracts were washed with 20 ml. of water, and the ether was removed in a gentle stream of nitrogen. The residue was dissolved in 96% ethanol, transferred to a 10-ml. volumetric flask, and made to volume with 96% ethanol.

An accurately measured aliquot was deposited from a micropipet on the base line of several sheets of Whatman No. 1 filter paper. An additional guide spot was placed to one side of each sheet, separate from the measured sample. After development, a vertical strip containing the guide spot chromatogram was cut from the sheet and sprayed with phloroglucinol reagent. The corresponding zones on the sheet were cut out, and the material was quantitatively eluted and diluted to known volume with 96% ethanol, and made alkaline with KOH (0.016%). Each extract was then analyzed by ultraviolet absorption with a Beckman DU spectrophotometer employing the constants:

	$\lambda_{max}^{EtOH/KOH}$	$E_{1\ cm.}^{1\%}$
Vanillin	353[a]	1980[a]
Syringaldehyde	370[a]	1530[a]
Coniferaldehyde	421	2100
Sinapaldehyde	443	1650

[a] Reference (*18*).

The concentration of each aldehyde was calculated from the absorbancies of the aliquots and expressed as grams per 100 liters at 100° proof. A preliminary evaluation with a mixture of two knowns indicated that recovery of vanillin was 85% and syringaldehyde 74%. The data obtained, uncorrected for incomplete recovery, are listed in Table II. Although recoveries are not complete, the data indicate the relative concentrations of these congeners in the products analyzed.

Analysis of Other Congener Groups. Analyses for the usual congeneric groups, except esters, were performed on some of the samples by AOAC procedures (*1*). After adjustment to 100° proof, the color of the aged samples was determined on a Klett colorimeter with a No. 54 filter (green) using a cobalt sulfate color standard; pH values were determined with a Beckman Model G pH meter, and the data are listed in Table III. Esters were determined by color development of ferric hydroxamate (*23*). The data are presented in Table IV.

Results and Discussion

Different types of alcoholic distillates stored in different types of white oak cooperage were analyzed to determine qualitative and quantitative changes that occurred as a result of the aging process. The authors found that the aromatic aldehydes—sinapaldehyde, coniferaldehyde, syringaldehyde, and vanillin—were present in all of the aged products listed in Table I, as well as in rye whiskey (*14*). Their occurrence here is consistent with similar investigations on brandies (*7–9, 11, 13, 19, 30–34*) and malt whiskey (*24, 25, 38*) which are aged in continually re-used barrels and casks. Recently, others reported the presence of vanillin and syringaldehyde in bourbon whiskey (*22*).

A substance which is responsible for the bluish white fluorescence of aged products when exposed to ultraviolet light was isolated and identified as scopoletin. It has not yet been established whether this congener arises exclusively by extraction of barrel wood or by chemical interaction as well. In working with a variety of wood extracts, this fluorescent substance has been found only in white oak and maple (*14*). Scopoletin is present in all samples listed in Table I.

The possibility was considered that the aromatic congeners found in neutral spirits samples 1, 2, and 3

Table II. Concentration of Aromatic Aldehydes in Aged Whiskey and Aged Spirits[a,b] (G./100 liters at 100° proof)

Sample No.	Vanillin	Syringaldehyde	Coniferaldehyde	Sinapaldehyde	Total
1	0.10	0.12	0.06	0.05	0.33
2	0.20	0.15	0.06	0.07	0.48
3	0.06	0.14	0.03	0.04	0.27
4	0.12	0.12	0.03	0.02	0.29
5	0.10	0.76	0.13	0.29	1.28
6	0.07	0.45	0.12	0.13	0.77
8	0.15	0.22	0.17	0.34	0.88
9	0.15	0.26	0.18	0.34	0.93

[a] Estimated recovery of 74 to 85%.
[b] Samples 7 and 10 were not analyzed quantitatively.

Table III. pH of Bourbon and Spirits at 100° Proof[a]

Age, Years	Bourbons	Spirits
0	4.7–5.3	6.8–7.0
1	4.3–4.5	4.8–5.0
2	4.2–4.4	4.6–4.8
3	4.1–4.4	4.6–4.7
4	4.1–4.4	4.4–4.6
5	4.1–4.4	4.2–4.4

[a] Aged in new charred and used charred cooperage, respectively.

Table IV. Comparison of Congener Development in Spirits and Bourbon Aged in New Charred Barrels

(All determinations based on 100° proof)

	Spirits			Bourbon			
Age, months	18			12	24		
Barrel proof	109.7			108.1	107.5		
Color, Klett units	113			99	126		
pH	4.3			4.5	4.3		
				Aged Bourbon		Contribution of Aging[b]	
Congeners[a]	Distillate	Aged Spirits	Contribution of Aging[b]	Distillate	12 months	24 months	12 months	24 months
Solids	0[c]	199	119	0[c]	93	124	93	124
Ash	0[c]	4	4	0[c]	5	5	5	5
Acids, total	0.8	39.8	39.0	2.0	36.0	46.5	34.0	44.5
Acids, fixed	0[c]	7.5	7.5	0[c]	5.0	5.5	5.0	5.5
Acids, volatile[d]	0.8	32.3	31.5	2.0	31.0	41.0	29.0	39.0
Esters	0.4	12.6	12.2	8.0	17.0	21.0	9.0	13.0
Fusel oil	1	1	0	190	190	200	0	10
Aldehydes	0.1	2.8	2.7	0.5	3.5	4.5	3.0	4.0
Furfural	0[c]	0.8	0.8	0[c]	1.4	1.4	1.4	1.4
Tannins	0[c]	34	34	0[c]	29	36	29	36

[a] In g./100 liters as follows: acids as acetic acid, esters as ethyl acetate, aldehydes as acetaldehyde, and tannins as tannic acid. [b] Aged product value minus distillate value. [c] Not determined, but assumed to be essentially absent in distillate. [d] Calculated as difference between total acids and fixed acids values.

may be due to leaching of residual whiskey from the previous filling of the barrel. However, samples 4, 5, and 6 also contained these congeners, showing that spirits stored in new uncharred barrels and in new charred barrels can duplicate the behavior of bourbon or rye whiskeys in forming these congeners. As mentioned previously, there are no aromatic congeners in unaged spirits prior to storage in barrels.

Semiquantitative data for the aromatic aldehydes in all but two of the aged whiskey and aged spirits samples, which were analyzed qualitatively, are listed in Table II. The total concentration of these congeners in spirits aged in used cooperage (Nos. 1 and 3) is about one third that of bourbon whiskey stored in new charred cooperage (Nos. 8 and 9). Charring of both new and used cooperage (Nos. 2 and 6 compared with Nos. 1 and 4, respectively) apparently causes an increase in the amount of aromatic congeners formed. The role of charring is being investigated further. The total concentration of aromatic aldehydes in spirits aged in new charred cooperage (Nos. 5 and 6) is comparable to that of bourbon whiskey. Also, in comparing Nos. 5 and 6, apparently 110° proof spirits are maturing at a faster rate than 140° proof spirits. If these congeners were developed by simple extraction, equal or greater amounts would be expected in higher proof spirits. Therefore, an extraction-reaction sequence for congener development is probably involved. Other workers have drawn similar conclusions from their studies on maturation (*8, 24, 26, 29, 31*).

A substance which is soluble and present in large amount in aged products, including bourbons, ryes (*14*), and aged spirits, has been identified as ethanol lignin. The qualitative and quantitative chemical evidence for the presence of ethanol lignin in whiskeys and spirits has been detailed above. Its role in the maturation of brandies and malt whiskey has been noted above. The exact structure of ethanol lignin has not been determined, but it is probably of lower molecular weight than the parent lignin from which it is derived. The analytical data obtained show that oak ethanol lignin isolated from both aged spirits and whiskeys is closely related to other forms of oak lignin (*15–17*). The nitrobenzene oxidation experiments show that ethanol lignin is a true hardwood lignin and is different from Nord's "native" oak lignin (*15–17*).

A possible pathway for the formation of ethanol lignin and other aromatic congeners is presented in Figure 1. These aromatic congeners may arise from other precursors in wood as well. The interrelationships shown are based on the available chemical evidence presented in this work and that taken from the literature (*8, 10, 28, 31, 32, 35*). Thus, under the acidic conditions (pH 4 to 5) imposed by the barrel on whiskey and spirits, Table III, ethanol can react with lignin in the barrel wood to produce an alcohol-soluble form of lignin, ethanol lignin, and ultimately the other congeners listed in Figure 1. This process is known as ethanolysis. Since lignin can be converted to coniferyl alcohol (*31*), it is reasonable to assume that ethanol lignin can undergo a similar transformation (*7, 8, 30, 32*). Also, a splitting of lignin into various building blocks under hydrogenation conditions can produce dihydroconiferyl alcohol, which can originate from coniferyl alcohol (*26*). Moreover, nitrobenzene oxidation of ethanol lignin yielded syringaldehyde and vanillin, which can come from sinapic alcohol and coniferyl alcohol, respectively. Under the mild oxidizing conditions in the barrel, aromatic alcohols are probably slowly converted into corresponding sinapaldehyde and coniferaldehyde.

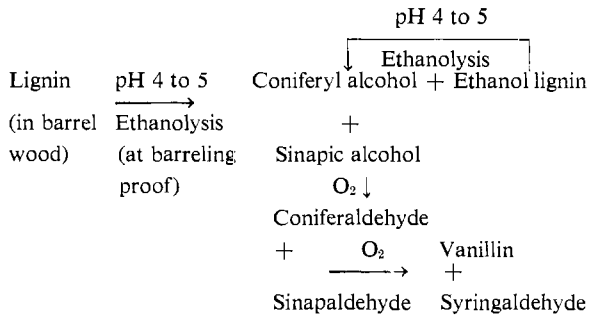

Figure 1. Postulated pathway for lignin-derived congener formation

Further oxidation of these aldehydes at the olefinic bond produces vanillin and syringaldehyde, respectively.

A comparison of conventional congener analyses of bourbon and spirits aged for a comparable time in new charred barrels is shown in Table IV. Data are included to demonstrate the congener increase during maturation, calculated by subtracting the distillate congener values from the aged product values. On this basis, the congener values for 18-month spirits generally fall between those for bourbon at 12 and 24 months. The exceptions are probably due to differences in barrels. The spirits and bourbons have similar pH values under these conditions; however, the data of Table III show higher values for spirits in used cooperage. These data are significant in showing that the amount of congener development in spirits is essentially the same as that in bourbons under comparable conditions. However, the proper balance of flavor is developed only when a distillate is aged in the type of barrel most suited to the character of the distillate.

The extent to which extraction and reaction participate in aging is not yet known. However, some congeners are probably derived essentially from extraction alone—e.g., ash and tannins—while ethanol lignin and the aromatic aldehydes represent a type of reaction-extraction with the barrel. The development of maturation congeners in re-used cooperage is qualitatively similar to but quantitatively less than new charred cooperage. Apparently, therefore, a distillate of whiskey or spirits can undergo fundamentally similar chemical and organoleptic changes when stored in oak wood. The aged alcoholic products acquire a mellowness and flavor (*14*) resulting from development of many congeners not present in the distillate at the time of barreling.

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