[CONTRIBUTION FROM THE INSTITUTE OF PAPER CHEMISTRY]

Studies on the Chemistry of Aspenwood. VII.[1] Further Studies on the Ether Extractives of Commercial Aspen Spent Sulfite Liquor[2,3]

IRWIN A. PEARL AND DONALD L. BEYER

Received June 6, 1960

The ether extractives of a commercial spent sulfite liquor from the pulping of peeled mixed aspenwood (*Populus tremuloides, P. grandidentata,* and *P. tacamahaca*) were investigated. The following compounds were found in the alkali-soluble portion of the extractives by paper chromatographic, column chromatographic, and solvent distribution procedures: vanillin, syringaldehyde, *p*-hydroxybenzaldehyde, vanillic acid, syringic acid, *p*-hydroxybenzoic acid, *p*-coumaric acid, sinapic acid, dihydro-*p*-coumaric acid, dihydrosinapic acid, lirioresinol isomers, α-conidendrin, benzoic acid, and an unidentified organo-silicon compound.

In earlier papers in this series[4,5] the ether extraction of a commercial spent sulfite liquor from the pulping of mixed aspens (*Populus tremuloides, P. grandidentata,* and *P. tacamahaca*) and the isolation and identification of *p*-hydroxybenzoic acid and several isomers of syringaresinol were described. The present paper describes continuing studies on the ether extractives of this and similar commercial liquors and reports the isolation and identification of a number of compounds.

The previous publication[5] described the Craig machine distribution during 300 transfers between the phases of a 4:1:5 mixture of toluene, acetic acid, and water at 20° of a fraction of ether extractives of aspen spent sulfite liquor previously freed of a substantial portion of its *p*-hydroxybenzoic acid content. This fraction represented approximately 11% of the original ether extractives. Paper chromatography of the Craig machine fractions with the butanol saturated with 2% aqueous ammonia and with the benzene saturated with formic acid developers indicated in addition to the previously reported syringaresinol and isomers, the presence of vanillin, syringaldehyde, vanillic acid, syringic acid, *p*-hydroxybenzoic acid, *p*-coumaric acid, sinapic acid, dihydro-*p*-coumaric acid, and dihydrosinapic acid. Positive identification of these compounds was made by isolation and mixed melting points with authentic samples and by means of identity of ultraviolet and infrared absorption spectra with authentic samples.[6] Most of the compounds found had already been observed in alkaline hydrolyzates of the three *Populus* species present,[7,8] but sinapic, dihydrosinapic, and dihydro-*p*-coumaric acids had never been identified in hydrolyzates of these woods in the past.

A fresh batch of digester strength ammonia-base spent sulfite liquor from the pulping of peeled mixed aspen logs (*P. tremuloides, P. grandidentata,* and *P. tacamahaca*) was concentrated and extracted exhaustively with ether. The total ether extractives amounted to 2.1% of the total solids in the spent liquor. The ether extractives were fractionated into bisulfite-soluble, bicarbonate-soluble, alkali-soluble, and neutral fractions by successive extraction with 21% sodium bisulfite, 8% sodium bicarbonate, and 5% sodium hydroxide solutions in the Craig countercurrent distribution machine.

The alkali-soluble fraction, representing 23% of the original ether extractives, was shaken with an excess of a mixture of chloroform and ethylene glycol. The two phases of the system were separated, and the chloroform layer was evaporated to dryness to yield 41% of the alkali-soluble fraction as a dark solid. This solid was boiled with petroleum ether (b.p. 65–110°) and filtered. The petroleum ether–soluble material was a waxy mixture which, upon repeated recrystallization from acetone, yielded colorless crystals melting sharply at 60–61°. Emission spectroscopy indicated silicon, and ultimate analysis indicated 73.0% carbon and 12.6% hydrogen. The infrared absorption spectrum indicates that these crystals are a mixture of an organo-silicon compound probably mixed with a slightly oxidized paraffin hydrocarbon wax. Infrared absorption bands characteristic of silicon compounds were found at 3.50, 7.07, 7.94, 9.14, 9.85, 11.59 and 12.45 μ. Absorption bands at 5.75, 6.81, 7.25, 7.30, 7.45, 13.72, and 13.92 μ are attributed to hydrocarbon-type material. Neglecting the absorption bands associated with hydrocarbon material, the absorption pattern is very similar to those of the methylpolysiloxanes.[9]

(1) For paper VI of this series, see I. A. Pearl and D. L. Beyer, *Tappi*, **43**, 611 (1960).

(2) Presented before the Division of Cellulose Chemistry at the 138th meeting of the American Chemical Society, New York, New York, September 11–16, 1960.

(3) This paper represents a portion of the results obtained in the research program sponsored by the Sulphite Pulp Manufacturers' Research League and conducted for the League by The Institute of Paper Chemistry. Acknowledgment is made by the Institute for permission on the part of the League to publish these results.

(4) I. A. Pearl and D. L. Beyer, *Tappi*, **40**, 45(1957).

(5) I. A. Pearl, D. L. Beyer, and E. E. Dickey, *J. Org. Chem.*, **23**, 705 (1958).

(6) I. A. Pearl, *J. Org. Chem.*, **24**, 736 (1959).

(7) I. A. Pearl, D. L. Beyer, B. Johnson, and S. Wilkinson, *Tappi*, **40**, 374 (1957).

(8) I. A. Pearl, D. L. Beyer, and B. Johnson, *Tappi*, **41**, 255 (1958).

The exact nature of the organo-silicon material melting at 60–61° is unknown. If the hydrocarbon material could be separated from the organo-silicon compound, further spectral-structure correlations might be possible. On the other hand, it is also possible that the organo-silicon compound present is actually unknown, but pure, and its infrared absorption spectrum combines the bands of the hydrocarbon wax and known organo-silicon compounds. The origin of this organo-silicon material in the aspen spent sulfite liquor became of great interest. A check of the mill which produced the liquor indicated that no silicone product, defoamer or otherwise, was used in the mill. The large yield of purified material (14 g.) precluded the possibility of contamination by the small amount of stopcock grease employed during laboratory processing. Thus, except for the possibility of unknown contamination, it appears that the organo-silicon compound may have originated in the wood of one or more of the *Populus* species employed in the production of this ammonia base aspen spent sulfite liquor. If this last case is true, it will be the first instance, to our knowledge, of an organo-silicon compound being isolated from a botanical product. Silicon dioxide has been found *per se* in many plants, and it is entirely possible that its organic derivatives may be present in some plants, but have never been isolated. This possibility is being checked by starting with fresh peeled aspenwood and taking it through the same processes in the laboratory under conditions in which no silicone material can enter the ether extractives of the spent sulfite liquor. Results will be reported in the future.

The residue left after extraction with boiling petroleum ether was fractionated by use of chloroform, tetrahydrofuran, and ethanol to yield two isomers of lirioresinol melting at 189–190° and 183–184°, respectively, whose infrared absorption spectra were identical with that of lirioresinol reported earlier.[5] The crystalline lirioresinols melting at 189–190° and 183–184° had optical rotations $[\alpha]_D^{25}$ −9.31° and +13.18° (in chloroform), respectively. As noted earlier[5] the differences in melting points of the several lirioresinol products obtained from aspen spent sulfite liquor and from *Liriodendron tulipifera* are probably due to differences in ratios of optical antipodes present in the crystalline products.

The ethylene glycol layer of the original chloroform–ethylene glycol distribution was diluted with ten volumes of water and extracted with chloroform. The chloroform layer was concentrated to a small volume under reduced pressure and allowed to evaporate to dryness at room temperature. The residue of crystals and viscous oil, representing 34% of the original alkali-soluble fraction, was filtered. The crystals were washed with butyl acetate and recrystallized from ethanol to yield pure colorless crystals melting at 237–238° and not depressing the melting point of a mixture with authentic α-conidendrin.[10] Infrared absorption curves of the 237–238° crystals and of α-conidendrin were identical.

The isolation of α-conidendrin from the spent sulfite liquor of aspenwood is the first recorded instance of conidendrin being isolated from a dicotyledenous wood source. In the past it has been assumed[11,12] that conidendrin could be obtained only from coniferous spent sulfite liquors or woods. Failure of Hintikka[11] to find conidendrin in the spent sulfite liquor of aspen (presumably *P. tremula*) may have resulted because the conidendrin found in the present study represents only a small fraction of the percentage composition of conidendrin in coniferous spent liquors. In the present study, it was found only after interfering materials were removed by procedures unknown forty years ago. Although lack of coniferous contamination was assured by the supplier, the possibility still exists, and the present findings are being checked along with the organo-silicon compound in a controlled laboratory experiment on fresh peeled aspenwood.

The aqueous ethylene glycol layer after chloroform extraction was extracted with ether, and the ether extract was evaporated to yield a residue representing 14% of the alkali-soluble fraction. Paper chromatography indicated that no phenolic acids were present. The product was fractionated by column chromatography on acid-washed Magnesol[13] with petroleum ether–ethanol mixtures to yield benzoic acid and *p*-hydroxybenzoic acid identified by mixed melting point. Benzoic acid has never been reported in hardwood spent liquors, and the finding of benzoic acid in aspen spent sulfite liquor may be of botanical or taxonomic importance. The nature of the precursor of benzoic acid in the original wood should have important biosynthetic implications. Benzoates of salicin have been found in aspen barks,[14,15] but the instant spent sulfite liquor was produced from only peeled aspenwood, and, therefore, the benzoic acid must have originated in the wood.

The finding of *p*-hydroxybenzoic acid in quantity in one of the fractions from acid-washed Magnesol chromatography of a material which contained no phenolic acid *per se* led to a paper chromatographic evaluation of all fractions obtained from the Magnesol column. The paper chromatograms indicated

(9) N. Wright and M. J. Hunter, *J. Am. Chem. Soc.*, 69, 803 (1947).

(10) I. A. Pearl, *J. Org. Chem.*, 10, 219 (1945).

(11) S. V. Hintikka, *Cellulosechemie*, 2, 87 (1921).

(12) H. Erdtman, *Svensk Papperstidn.*, 47, 155 (1944).

(13) I. A. Pearl and E. E. Dickey, *J. Am. Chem. Soc.*, 73, 863 (1951).

(14) I. A. Pearl and S. F. Darling, *J. Org. Chem.*, 24, 731 (1959).

(15) I. A. Pearl and S. F. Darling, *J. Org. Chem.*, 24, 1616 (1959).

the presence of vanillic, syringic, and *p*-coumaric acids in addition to *p*-hydroxybenzoic acid. Thus, passage through the acid-washed Magnesol column caused hydrolysis or some other decomposition of materials originally present in the alkali-soluble fraction to yield these free phenolic acids, and probably the benzoic acid as well.

The bicarbonate-soluble fraction, representing 20% of the original ether extractives was submitted to qualitative paper chromatography, and the diazo spray reagents employed indicated *p*-hydroxybenzoic acid as the chief component along with smaller amounts of vanillic and syringic acids and very small amounts of *p*-coumaric and dihydro-*p*-coumaric acids. Quantitative analysis of the three major components by the paper chromatographic and ultraviolet spectrophotometric procedures described previously[7,8] indicated 65% *p*-hydroxybenzoic acid, 3.4% vanillic acid, and 3.4% syringic acid. The finding of benzoic acid in the alkali-soluble fraction, together with other studies in our laboratory on hardwood extractives, suggests that a substantial portion of the approximately 30% of unaccounted for materials in this fraction comprises benzoic and aliphatic acids which do not give spots with the diazo spray reagents employed.

The bisulfite-soluble fraction amounted to only 1.7% of the original ether extractives. Qualitative paper chromatography indicated only vanillin, syringaldehyde, and *p*-hydroxybenzaldehyde as known components of this fraction. Quantitative chromatography and spectrophotometry[8] indicated 12.5% vanillin, 12.5% syringaldehyde, and 1.7% *p*-hydroxybenzaldehyde in the bisulfite-soluble fraction. Thus, approximately 75% of this fraction still remains unidentified. It is interesting to note that *p*-hydroxybenzaldehyde was found in this ether extract. This aldehyde was also found in the alkaline hydrolyzate of quaking aspen (*P. tremuloides*), but not in those of the other two *Populus* species present in this liquor.[8] This earlier study[8] also reported that *p*-hydroxybenzaldehyde was found in the alkaline hydrolyzates of only those woods which also yielded *p*-coumaric acid under the same conditions. This confirms our finding of *p*-coumaric acid in the bicarbonate-soluble fraction of the present study.

EXPERIMENTAL[16]

Identification of compounds in previously reported fractionation. The 300-transfer Craig machine distribution between the phases of a 4:1:5 mixture of toluene, acetic acid, and water reported previously[6] was monitored by paper chromatography employing the butanol saturated with 2% aqueous ammonia and benzene saturated with formic acid developers. Spots were located by means of bisdiazotized benzidine, diazotized *p*-nitroaniline,[17] 2,4-dinitrophenylhydrazine, and Mäule spray reagents. On the basis of these chromatograms, the tubes of the Craig machine were combined as follows with the indicated percentage solids of the original fraction: A, 0–4, 21%; B, 5–24, 21.4%; C, 25–34, 3.9%; D, 35–50, 6.9%; E, 51–70, 7.6%; F, 71–99, 5.2%; G, 100–202, 6.2%; H, 203–286, 24.2%; and I, 287–299, 3.6%. From the paper chromatograms in the benzene–formic acid system at 20° the following phenolic acids were identified by means of color reactions and R_{va} values (*va* = vanillic acid): *p*-hydroxybenzoic acid (R_{va} 0.25), dihydro-*p*-coumaric acid (R_{va} 0.41), dihydrosinapic acid (R_{va} 0.57), sinapic acid (R_{va} 0.68), syringic acid (R_{va} 0.77), and vanillic acid (R_{va} 1.00). From the chromatograms in the butanol-aqueous ammonia developer, syringaldehyde (R_f 0.38) and vanillin (R_f 0.44) were identified. Chromatograms in quantity were prepared from the separate fractions, and the individual compounds were eluted from the chromatograms as follows in the manner described previously[7]: B and C, *p*-hydroxybenzoic acid; D, dihydro-*p*-coumaric acid and dihydrosinapic acid; E, sinapic acid, syringic acid, and vanillic acid; F, *p*-coumaric acid and vanillin; G, syringaresinol[5]; and H, syringaldehyde. Eluted compounds were compared with authentic compounds by means of ultraviolet and infrared spectra.[6]

Preliminary fractionation of ammonia-base aspen spent sulfite liquor. Digester strength spent sulfite liquor (90 gallons) from the ammonia-base cooking of peeled mixed aspen logs (*Populus tremuloides, P. grandidentata,* and *P. tacamahaca*) was obtained from the Charmin Paper Products Co., Green Bay, Wis., in the summer of 1957. The liquor was evaporated in a circulating evaporator to about half volume and extracted in 5-l. batches in the air-agitated liquid-liquid extractor.[18] The ether was evaporated to yield a total of 778 g. solids representing 2.1% of the total solids of the original liquor.

The entire ether extractives were fractionated in eighteen identical Craig machine fractionations. A solution of 42 g. of ether extractives in 160 ml. of *n*-butyl acetate was added to the first four tubes of the 100-tube 40 ml./40 ml. Craig machine filled as follows: tubes 0–4, water; tubes 5–24, aqueous 21% sodium bisulfite solution; tubes 25–49, water; tubes 50–69, aqueous 8% sodium bicarbonate solution; and tubes 70–99, water. After 120 transfers with *n*-butyl acetate as the upper phase, the tubes were monitored by paper chromatography and grouped into the fractions noted in Table I. All fractions were treated in the following manner.

TABLE I

PRELIMINARY CRAIG MACHINE FRACTIONATION OF ETHER EXTRACTIVES

Fraction	Tubes	Contents	Yield G.	Yield %
A	5–49	Bisulfite-solubles	12	1.5
B	50–80	Bicarbonate-solubles	156[a]	20
C	81–120	Alkali-solubles	178[b]	23
D	81–120	Neutrals	271	35

[a] In addition, 13 g. (1.7%) of ether-insoluble material was recovered from this fraction. [b] In addition, 20 g. (2.6%) of ether-insoluble material was recovered from this fraction.

The two phases were separated in a separatory funnel. The butyl acetate layer was extracted with 4% sodium hydroxide

(16) All melting points are uncorrected. Analyses were performed by Huffman Microanalytical Laboratories, Wheatridge, Col. Infrared spectra were determined by Mr. Lowell Sell.

(17) I. A. Pearl and P. F. McCoy, *Anal. Chem.*, **32**, 132 (1960).

(18) I. A. Pearl, *Ind. Eng. Chem., Anal. Ed.*, **16**, 62 (1944).

solution and washed with a little water. The alkaline extracts were added to the corresponding aqueous phases, and the mixtures were acidified with dilute sulfuric acid. The acidified aqueous mixtures were evaporated a short while under reduced pressure to remove any dissolved sulfur dioxide and/or butyl acetate and then exhaustively extracted with ether. The ether extracts were dried and concentrated for solids determinations. In the case of the butyl acetate layers from tubes 81–99 and the overflow, the butyl acetate was evaporated to dryness to give the "neutral" fraction. All other butyl acetate solutions were discarded even though they contained materials insoluble in dilute sodium hydroxide.

Analysis of alkali-soluble fraction (C). Preliminary paper chromatography indicated at least seven phenolic compounds, two of which were syringyl in nature. Fraction C (97 g.) was dissolved in 2500 ml. of chloroform, shaken with an equal volume of ethylene glycol, and separated. The chloroform layer was evaporated to yield 40 g. of dark solid. This dark solid was extracted with boiling petroleum ether (b.p. 65–110°), and the petroleum ether extract was evaporated to yield a waxy residue. Paper chromatography indicated very little phenolic material *per se*, but alkaline hydrolysis liberated small amounts of phenolic compounds. The product on standing deposited crystals. The entire product was covered with cold acetone, allowed to stand 1 day at room temperature, and filtered to yield 14 g. of crystals melting at 60–61°. Repeated recrystallizations from acetone did not change the melting point. Emission spectroscopy indicated silicon, and the infrared absorption spectrum ($\lambda_{max(\mu)}^{KBr}$ 3.45, 3.51, 6.77, 6.82, 7.93, 9.13, 9.78, 12.45, 13.72, 13.92) indicated an organo-silicon compound. *Anal.* Found: C, 73.0; H, 12.6; mol. wt. 888.

The residue left after extraction of the dark solid with petroleum ether was covered with ethanol and allowed to stand at 20° for several days. The separated crystals were filtered and washed with cold ethanol to yield 2.4 g. of colorless solid melting at 173–176°. Paper chromatograms and infrared absorption spectrum indicated that this material was an isomer of syringaresinol and essentially identical with the materials isolated previously.[5] The crude product was covered with chloroform, allowed to stand a few days at 20° and filtered. The clear filtrate was evaporated to dryness under reduced pressure, and the residue was boiled with tetrahydrofuran in the presence of decolorizing carbon and filtered. The tetrahydrofuran was evaporated to dryness under reduced pressure, and the residue was boiled with ethanol and allowed to stand at 20°. The precipitate was filtered and recrystallized several times from ethanol to yield colorless crystals melting at 189–190°, $[\alpha]_D^{25}$ −9.31° (*c* 1.7 in chloroform). The infrared absorption spectrum was identical with that of lirioresinol.[5]

The mother liquors from the crystallization of this compound were evaporated to dryness, and the residue recrystallized several times from ethanol to give colorless crystals melting at 72–73° with no phenolic or syringyl activity. The infrared absorption spectrum ($\lambda_{max(\mu)}^{KBr}$ 2.94, 3.45, 3.52, 5.75, 6.83, 7.92, 8.6, 13.72, 13.92) indicated that the compound was probably a long-chain aliphatic alcohol with some carbonyl-containing and unsaturated impurities. Nothing further was done with this fraction at this time.

The ethanolic filtrate from the original separation of the two above compounds was evaporated to dryness, and the residue was recrystallized from ethanol several times to yield colorless crystals melting at 183–184°, $[\alpha]_D^{25}$ +4.2° (*c* 1.7, chloroform), and having an infrared absorption spectrum identical with that of lirioresinol.[5] Acetylation with acetic anhydride and pyridine and recrystallization of the product from ethanol yielded lirioresinol diacetate melting at 186–187°, $[\alpha]_D^{25}$ +13.18°, and not depressing a mixed melting point with authentic lirioresinol diacetate.[5]

Analysis of the ethylene glycol-soluble portion of fraction (C). The ethylene glycol layer of the original chloroform–ethylene glycol distribution was diluted in batches with ten volumes of water and extracted first with chloroform and then with ether. The chloroform extract was concentrated to a small volume under reduced pressure and allowed to evaporate to dryness at room temperature. The yield of mixed crystalline precipitate and viscous oil was 33 g. or 34% of the alkali-soluble fraction C. The crude mixture was filtered through a sintered funnel, and the crystals were washed with cold butyl acetate to yield 3.5 g. of colorless crystals melting at 237–238°. Recrystallization twice from ethanol did not change the melting point. The compound did not depress the melting point of a mixture with authentic α-conidendrin,[10] its infrared absorption spectrum was identical with that of authentic α-conidendrin ($\lambda_{max(\mu)}^{KBr}$ 2.95, 3.42, 3.52, 5.67, 6.17, 6.29, 6.60, 7.86, 8.22, 9.02, 9.73, 10.05, 10.49, 11.44, 12.05, 12.80, 13.35, 13.63, 14.02, 14.51) and it gave a negative test for syringyl groups with the Mäule reagents.

The ether extract of the diluted ethylene glycol layer was evaporated to dryness to yield 14 g. (14%) of solids. Paper chromatograms indicated no syringyl or carbonyl activity in this fraction, and no recognizable phenolic acids. A sample containing 13.3 g. was adsorbed on a column of acid-washed Magnesol[13] 33 mm. in diameter and 42 inches in length. The column was developed as a flowing chromatogram with 4000 ml. of 50:1 petroleum ether (b.p. 65–110°)–ethanol followed by 2500 ml. of 20:1 petroleum ether–ethanol. Fractions (500 ml.) were taken in the effluent, and these were monitored by paper chromatography in the butanol-2% aqueous ammonia and 10:3:3 butanol–pyridine–water systems. Chromatograms were examined under ultraviolet light and by spraying with bisdiazotized benzidine reagent. The first 1000 ml. were discarded, and the next fraction appeared to contain only one compound giving a rejection spot with the diazo reagent at R_f 0.31 and 0.79, respectively in the above noted developing systems at 20°. The solvent was evaporated, and the white residue (0.11 g.) was recrystallized twice from water to give shiny white platelets melting at 119–120° and not depressing a mixed melting point with authentic benzoic acid.

The fraction comprising 3000 to 4000 ml. of effluent was evaporated to yield 0.43 g. solids. The residue was covered with cold methanol, and the mixture filtered to yield colorless crystals melting at 208–210°. Recrystallization from methanol yielded colorless crystals melting at 209–210° and not depressing a mixed melting point with authentic *p*-hydroxybenzoic acid. The methanol mother liquors were chromatographed in the butanol-aqueous ammonia and butanol–pyridine–water developers, and the spots were located by means of diazotized *p*-nitroaniline and Mäule reagents. In addition to *p*-hydroxybenzoic acid, the following acids were present: vanillic (butanol-pyridine-water R_f 0.44), syringic (butanol-aqueous ammonia R_f 0.08), and *p*-coumaric (butanol-aqueous ammonia R_f 0.12). The presence of these acids was confirmed by chromatography in the benzene–formic acid system.[7,8] All of these acids were also found in the fraction comprising 4000 to 4500 ml. of effluent (0.20 g. solids), and all but syringic acid were found in the fraction comprising 4500 to 5500 ml. of effluent (0.29 g.). In addition to these compounds, many spots for unknown compounds were also found.

Analysis of bicarbonate-soluble fraction (B). Paper chromatography of this fraction in the butanol-2% aqueous ammonia, 10:3:3 butanol–pyridine–water, and benzene–formic acid developers with location of spots by means of of bis-diazotized benzidine, diazotized-*p*-nitroaniline, and Mäule reagents in accordance with previously described procedures[7,8] indicated the presence of *p*-hydroxybenzoic acid as the chief component, vanillic and syringic acids as other major components, and small amounts of *p*-coumaric and dihydro-*p*-coumaric acids. Quantitative determination of the major components by previously described procedures[7] indicated 65% *p*-hydroxybenzoic acid, 3.4% vanillic acid, and 3.4% syringic acid.

Analysis of the bisulfite-soluble fraction (A). Qualitative

paper chromatography[7,8] indicated that vanillin, syringaldehyde, and *p*-hydroxybenzaldehyde were the only identifiable phenolic aldehydes present in this fraction, and quantitative paper chromatography and spectrophotometry[8] gave the following results: vanillin, 12.5%; syringaldehyde, 12.5%; and *p*-hydroxybenzaldehyde, 1.7%.

APPLETON, WIS.

[CONTRIBUTION FROM THE INSTITUTE OF PAPER CHEMISTRY]

Studies on the Chemistry of Aspenwood. VIII.[1] An Investigation of the Neutral Extractives of Commercial Aspen Spent Sulfite Liquor[2]

IRWIN A. PEARL AND PATRICIA F. McCOY

Received June 6, 1960

The "neutral" portion of the ether extractives of a commercial spent sulfite liquor from the pulping of mixed aspenwood (*Populus tremuloides*, *P. grandidentata*, and *P. tacamahaca*) was found to contain esters of long-chain fatty alcohols, sterols, glycerol, and phenolic acids with saturated and unsaturated long-chain fatty acids and phenolic acids together with some of their constituent components.

In the previous paper in this series[1] the large scale fractionation into bisulfite-soluble, bicarbonate-soluble, alkali-soluble, and neutral fractions of the ether extractives of a commercial spent sulfite liquor from the ammonia-base pulping of peeled mixed aspenwood (*Populus tremuloides*, *P. grandidentata*, and *P. tacamahaca*) was described. The present paper describes studies on the "neutral" fraction which were those materials which passed through the Craig machine countercurrent to 21% sodium bisulfite, 8% sodium bicarbonate, and 4% sodium hydroxide solutions, and which represented 35% of the original ether extractives.

The crude "neutral" fraction was evaporated to dryness, and the residual dark brown viscous oil was boiled with excess petroleum ether (b.p. 30–60°), and the clear petroleum ether extract was decanted. Evaporation of this petroleum ether extract yielded 90% of the "neutral" fraction as a yellow oily product. Subsequent extraction of the residue from the petroleum ether boiling with boiling benzene and then with boiling ether yielded 8% benzene-soluble and 1% ether-soluble fractions, respectively. The present study was concerned only with the petroleum ether-soluble portion. This crude fraction was chromatographed on alumina and eluted successively with petroleum ether (b.p. 65–110°), benzene, chloroform, 95% ethanol, and 1% acetic acid in ethanol. Fractions were analyzed by reverse-phase chromatography on mineral oil-impregnated paper in aqueous acetic acid and "peracid" developers and spots were located and identified by means of ultraviolet examination before and after exposure to iodine vapors, phosphomolybdic acid spray reagent, and the mercury stain.[3,4] The petroleum ether eluate representing 27% of the recovered fractions appeared to contain saturated compounds of relatively high molecular weight, probably alcohols or sterols. A small amount of fatty acid was also indicated. The benzene eluate (35%) appeared to comprise mainly saturated compounds, probably fatty alcohols, glycerides, and triglycerides. Some unsaturated material was indicated along with carboxylic acids. The chloroform eluate (17%) appeared to comprise predominantly sterol materials similar to β-sitosterol. The 95% ethanol eluate (7%) appears to be a mixture of unsaturated fatty acids, mostly linoleic and linolenic. The fraction eluted with 1% acetic acid in ethanol (13%) was composed of a mixture of saturated and unsaturated long-chain aliphatic acids. Linoleic, palmitic, stearic, arachidic, behenic, and lignoceric acids were identified. In addition, data indicated saturated fatty acids even higher than C_{24}.

Thus, the presence of saturated fatty acids with even chain lengths from 16 to 24 carbon atoms and higher was demonstrated in the so-called "neutral" fraction of the extractives of aspen spent sulfite liquor. Therefore, it appears that the fraction is actually not neutral at all, but contains a sizable amount of acidic materials. The finding of these acids in this "neutral" fraction indicates that probably much larger amounts are to be found in the bicarbonate-soluble and alkali-soluble fractions reported earlier.[1] To date, these other fractions have only been investigated for their aromatic constituents and not for their aliphatic components.

The paper chromatographic analysis of the fractions eluted from the above alumina column indicated that considerable overlap of individual fraction composition took place and that many ma-

(1) For paper VII of this series, see I. A. Pearl and D. L. Beyer, *J. Org. Chem.*, 26, 546 (1961).

(2) This paper represents a portion of the results obtained in the research program sponsored by the Sulphite Pulp Manufacturers' Research League and conducted for the League by The Institute of Paper Chemistry. Acknowledgment is made by the Institute for permission on the part of the League to publish these results.

(3) M. A. Buchanan, R. V. Sinnett, and J. A. Jappe, *Tappi*, 42, 578 (1959).

(4) M. A. Buchanan, *Anal. Chem.*, 31, 1616 (1959).