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Proofs for the Occurrence of Homoprotocatechuic Acid and some Other Pyrocatechol and Guaiacol Derivatives in Pressure Heated Cellulose Spent Liquors

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Dedicated to Professor *Holger Erdtman* on his 60th birthday

Mixed melting point determinations on crystalline substances or their crystalline derivatives obtained from sodium sulfide enriched pressure heated spent kraft or sulfite liquors have yielded proofs for the occurrence of the following substances: guaiacol, pyrocatechol, *p*-methylcatechol, *p*-ethylcatechol, acetovanillone, vanillin, protocatechualdehyde, *p*-acetopyrocatechol, vanillic acid, and homoprotocatechuic acid. For the separation of the ketonic substances from the aldehydic ones a technique of stepwise acidification of an aqueous solution containing the Girard-P-derivatives of these substances was used.

During many years one of us (T.E.) together with his coworkers has studied the pressure heating of spent kraft or sulfite cellulose liquors to which sodium sulfide has been added. The results have been in part described in several papers [1–7]. Among other things, the occurrence of a series of pyrocatechol and guaiacol derivatives in these liquors has been reported in many cases, until now based on chromatographic evidence only. In the present report these findings have been substantiated by the determinations of mixed melting points of some of these substances or of their crystalline derivatives. The presence of the same substances has been shown also by paper chromatography in several different systems. Qualitative and quantitative chromatographic investigations and methods will be reported in another paper. In the present paper some R_F values only are given. The yields reported in the present paper have been surpassed in other experiments.

SPENT SULFITE LIQUOR

The pressure heated sulfite liquor deferred to in the present paper is identical with the liquor TU 10 described in Ref. [4], Tables 1 and 3. It was obtained by heating continuously a spent commercial calcium base sulfite liquor from the mill of Tainionkoski after addition of sodium sulfide (15.6 % calculated as Na_2S in the organic matter, 1230 g in 7050 ml liquor) for one minute (effective heating time) at about 320° and 155 atmospheres. After

the heating a sample of the liquor was acidified to a pH of about 2 with hydrochloric acid. A current of nitrogen was passed through the liquor which was kept at about 60° in order to remove hydrogen sulfide, the precipitate of "demethylated lignin" obtained was filtered off, and the filtrate extracted continuously with ethyl ether in a liquid-liquid extractor. The ether solution was dried with anhydrous sodium sulfate and the solvent was partly distilled off and finally evaporated *in vacuo* at room temperature.

5 g of the ether extract was dissolved in 75 ml of N sodium hydroxide and the solution extracted continuously with ether in order to remove neutral substances. The aqueous layer was separated from the ether and a stream of carbon dioxide passed through it until the pH had dropped to about 8. A phenolic fraction was isolated by continuous ether extraction of the solution in a yield of about 2.54 g. It was dissolved in 35 ml 99 % methanol. Girard "P" reagent (acethydrazide pyridinium chloride [8]) and several drops of formic acid were added, and the mixture was refluxed for 55 min. The cooled solution was then mixed with 100 ml ether with stirring, and the solution was decanted off from the precipitate consisting of derivatives formed by carbonyl compounds with the reagent. The solvent was evaporated from the solution, the residue was dissolved in 5 ml 75 % ethanol and added to 50 ml of a saturated solution of lead acetate in 50 % ethanol. The precipitate formed (lead salts of pyrocatechol and its derivatives) was separated by centrifugation, washed with alcohol and ether, suspended in acetone and decomposed with hydrogen sulfide. The lead sulfide was filtered off and the filtrate evaporated. The residue was treated with benzoyl chloride and pyridine according to the method of Einhorn [9]). The reaction product was recrystallized three times from aqueous ethanol; m.p. 84°C alone or admixed with an authenthic sample of *pyrocatechol dibenzoate*, prepared according to Ref. [10].

The aqueous-alcoholic supernatant obtained after separation of the lead salts of pyrocatechol and its derivatives was diluted with 2 volumes of water and extracted several times with ether. The ether solution was evaporated without drying and the water and alcohol dissolved in the ether were removed by addition of benzene and distillation through a Vigreux column. The benzene containing residue was treated with 3,5-dinitrobenzoyl chloride and pyridine. The reaction product was recrystallised from alcohol; m.p. 140°C alone or admixed with an autenthic sample of *guaiacol-3,5-dinitrobenzoate*, prepared according to Ref. [11].

The precipitate obtained by treatment with the Girard "P" reagent was extracted with boiling ether and dissolved in 50 ml of water and its separation into a ketonic and aldehydic part was performed by stepwise acidification in the following way:

The pH of the solution was brought to about 6 by the addition of hydrochloric acid and the solution continuously extracted with ether for 5 h. The ether was separated, the aqueous solution acidified to about pH 5.5 and again extracted with ether for 4 h. The fractionation was further continued in the same way, in lowering the pH 0.5 units with hydrochloric acid and extracting with ether for 4 h until pH 1 was reached. Each ether extract was evaporated separately and a sample of the residue analysed by paper chromatography in the ethanol-water-conc. ammonia (80:16:4) system. The fractions obtained at pH 5.5 – 3.5 gave the same two spots and were combined. At pH 3.5 – 1.5 4 spots could be observed but these fractions were not further analysed. The fraction, pH 1.5 – 1, and an additional fraction obtained by a further 4 h ether extraction at pH 1 gave 2 spots not identical with the former and were combined.

The residue, after evaporation of the ether from the fractions between pH 5.5 and 3.5 was dissolved in 1 ml of 75 % aqueous ethanol and precipitated by addition of 3 ml of a saturated solution of lead acetate in 50 % ethanol. The precipitate was centrifuged off, washed with acetone and decomposed in acetone suspension by a current of hydrogen sulfide. The precipitate of lead sulfide was filtered off, the filtrate was evaporated and treated with benzoylchloride in pyridine. The reaction product was recrystallized from aqueous ethanol; m.p. 117°C alone or admixed with an authenthic sample of *3,4-dihydroxyacetophenone-dibenzoate* (dibenzoate of *p*-acetopyrocatechol), prepared according to Ref. [12]

The aqueous alcoholic supernatant left after removal of the precipitate obtained on addition of lead acetate, was diluted with 2 volumes of water and extracted with ether. The ether layer was evaporated and distilled together with benzene as in the above mentioned case of guaiacol. The residue was treated with benzoyl chloride and pyridine as already described, and the reaction product was recrystallized from aqueous ethanol. It showed a m.p. of 106° and did not give any depression of m.p. on admixture with an authenthic sample of *acetovanillone benzoate* prepared according to Ref. [13].

Table 1. R_F values. Chromatography on Whatman No. 1 paper. Descending technique. Spraying with silver nitrate-ammonia, Folin-Denis reagent, diazotized sulfanilic acid or diazotized *p*-nitroaniline.

Eluent	2 % acetic acid	Xylene-butanone [16]	Butanol	Ethanol-ammonia	$CHCl_3$
Impregnation with	—	Formamide	Na_2MO_4 [20]	—	Formamide
Guaiacol	—	0.62	0.94	0.85	0.78
Pyrocatechol	0.72	0.24	0.25	0.81	0.046
p-Methylcatechol	0.72	0.40	0.36	0.81	0.11
p-Ethylcatechol	0.72	0.53	0.69	0.81	0.22
Vanillin	0.64	0.41	0.95	0.69	0.69
Acetovanillone	0.61	0.50	0.96	0.70	0.80
p-Acetopyrocatechol	0.55	—	0.11	0.63	0.79
Protocatechualdehyde	0.59	0.12	0.07	0.63	0.00
Vanillic acid	0.47	0.11	0.28	0.52	0.01
Homovanillic acid	0.77	0.07	0.25	0.61	0.01
Protocatechuic acid	0.46	0.051	0.03	0.45	0.00
Homoprotocatechuic acid	0.75	0.023	0.03	—	0.00
Dihydrocaffeic acid	0.67	0.035	0.03	—	0.00

The fraction obtained at pH 1.5—1 was separated in a similar way. The substance precipitated with lead acetate yielded a benzoate, m.p. 97°, alone or admixed with the *dibenzoate of protocatechualdehyde*, prepared according to Ref. [14]. The supernatant obtained after removal of the lead compound possessed a strong odor of vanillin and gave a benzoate, m.p. 75°, alone or admixed with the *benzoate of vanillin* [15].

The weakly alkaline solution, from which the phenolic fraction had been removed by extraction with ether, was acidified with hydrochloric acid and again extracted with ether, which dissolved a mixture of organic acids. Paper chromatography of this mixture with 2 % aqueous acetic acid as eluent gave three spots, two of which showed R_F identity with protocatechuic acid and homovanillic acid. The third acid was isolated by preparative paper chromatography on Whatman No. 1 paper using a mixture of ethanol-water-conc. ammonia (80:16:4) as eluent. The substance with an R_F value of about 0.5 was eluted from the paper with acetone. The solvent was evaporated and the residue sublimed. The sublimate gave no depression of m.p. on admixture with *vanillic acid*.

KRAFT BLACK LIQUOR

To 450 ml concentrated kraft black liquor from the mill of Enso Gutzeit, **Kotka**, containing 50 % of solids and 239.0 g of organic substance, 116.2 g of crystalline sodium sulfide ($Na_2S \cdot 9H_2O$), 58.41 g of sodium hydroxide and 450 ml of water were added. The mixture was heated in a stainless steel rocking autoclave for 10 min at 285°C.

Phenols. After cooling 1138 g of the heated liquor was neutralized to a pH of about 7 by the addition of 1.41 l of about 2 N sulfuric acid, 200 g sodium hydrogen carbonate was added and a first ether soluble fraction was obtained by continuously extracting the liquor with its suspended precipitate of demethylated lignin with ethyl ether in a liquid-liquid extractor in a slow current of nitrogen and evaporating the solvent. The residue was dissolved in a small amount of acetone and precipitated by addition of a solution of 25 g crystalline lead acetate ($Pb(OCOCH_3)_2 \cdot 3H_2O$) in 130 ml of water and 170 ml of acetone. The precipitate was filtered off, washed with acetone, and decomposed by the addition of dilute sulfuric acid. Pyrocatechol and its derivatives were extracted with ether and the ether solution on evaporation gave a dark syrup weighing 6.0 g. This mixture was separated by preparative paper chromatography on Whatman No. 3 paper impregnated with formamide and using chloroform as eluent (similar to the Freudenberg-Lehmann system

II [16], but using chloroform instead of xylene-methyl-ethyl ketone). Zones with R_F values of 0.046, 0.11. 0.22 and a weak zone with a higher R_F value were obtained. The zones could be located by comparison with parallel chromatograms sprayed with diazotized *p*-nitroaniline or silver nitrate-ammonia reagent and with difficulty, by their dark colour in ultraviolet light. The zones were cut out and eluted with acetone, which also dissolves the residual formamide. Part of this formamide could be removed *in vacuo*.

A sample (127 mg) of the substance from the zone with R_F = 0.22 was dissolved in 2 ml of acetone and refluxed for 30 min together with 440 mg of dinitrofluorobenzene and 0.23 ml of triethylamine (*cf.* Ref. [17]). The reaction mixture was diluted with water and extracted with 15 ml of ethyl ether and the ether layer washed successively with diluted hydrochloric acid, diluted sodium hydroxide solution and water. Evaporation of the ether solution gave a brown oil. Crystallization from ethanol yielded crystals, m.p. 95—97°. Recrystallization from aqueous methanol raised the m.p. to 105—108°. On admixture with an authenthic sample of *2,4-dinitrophenyl ether of p-ethylcatechol*, m.p. 106.5—108°, gave a m.p. of 105—108°. Analysis of the sample obtained from the black liquor (A. Bernhardt, Mülheim, Ruhr): Found: C 51.33; H 3.14; N 12.01. Calc. for $C_{20}H_{14}N_4O_{10}$: C 51.07; H 3.00; N 11.91.

The zone with R_F 0.11 treated in the same way gave a 2,4-dinitrophenyl ether, m.p. 124.5—125.5°, which did not show any depression of m.p. on admixture with an authenthic sample of *2,4-dinitrophenyl ether of methyl catechol*.

Acids. After the ether extraction of the phenolic fraction at pH 7 as described above, the precipitate of "demethylated lignin" was filtered off and the main part of the filtrate (80.2 % by volume) was acidified to a pH of about 2 by the addition of 180 ml sulfuric acid 1:1. A small precipitate was filtered off and the filtrate was extracted continuously with ether in a current of nitrogen. After evaporation of the solvent 51.12 g of liquid residue was obtained, having a strong odor of acetic and formic acids. On drying of a small sample on the water bath, the content of non volatile acids was found to be 46.6 %, corresponding to 23.9 g. Chromatography of a 5 g sample of the non volatile fraction on a column of polycaprolactam (perlon) powder suspended in water (*cf.* Ref.[18]) was carried out. The height of the polycaprolactam layer was 40 cm, the inner diameter of the tube 25 mm. Elution was performed with 1100 ml of water followed by 700 ml of a mixture of isopropanol-water-conc. ammonia 80:16:4. The eluate was collected in 370 test tubes using an automatic fraction collector. Paper chromatography of fractions 138—162 with water as eluent gave one main spot, the same substance was also found in fractions 163—140 although in a lower degree of purity. Ether extraction of fractions 138—162 gave 148 mg of an almost white substance, which crystallized on standing over night at room temperature. Fractions 163—140 gave 119 mg of an ether soluble syrup of brownish color.

A sample of the substance found in fractions 138—162 was recrystallized four times from benzene, once using decolorising carbon (Merck). The purified substance had a m.p. of 127° both alone or when admixed with an authenthic sample of ***homoprotocatechuic acid***, m.p. 127—128°, prepared by Dr. J. Halmekoski by demethylation of synthetic homovanillic acid with hydroiodic acid [19].

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