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Title Separation of Some Common Phenols by Chromatographic Adsorption

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Chromatography is the term applied to the separation of mixtures, in solution, by selective adsorption in conjunction with a two phase equilibrium.

In studies on the possible utilization of tars obtained by destructive distillation of wood and lignin, attempts have been made to separate the phenols present by fractional distillation after separation of the neutral substances and acids. During the distillation, approximately one-half of the extracted material polymerizes and is thereby lost. This procedure has demonstrated that wood and lignin tars contain many different phenols including some of the more valuable dihydroxy compounds. Some method, other than fractional distillation, is needed to separate these phenols in order to avoid the polymerization and subsequent loss of a considerable quantity of valuable products. Chromatographic analysis was proposed as a possible method of solving the problem. Rather than to start with the tar extract, it was thought more feasible to separate increasingly complex mixtures of phenols known to be present in the extract. The problem was further simplified to the attempted separation of two different mixtures, each containing three or four phenols.

A flowing chromatogram was used and various fractions were collected and tested with ferric chloride for the presence of the phenols. The sensitivity of the ferric chloride test for some of the phenols was determined since it could not be found in the literature.

A mixture of phenol, p-cresol, pyrogallol, and catechol dissolved in benzene was chromatographed on an alumina column. The column was then eluted successively with four different eluents. 10 ml. fractions were collected and tested with saturated ferric chloride solution. The phenol and p-cresol were found together in two of the first fractions. The pyrogallol and catechol were found separated from the other two phenols and from each other, the catechol coming off the column after the phenol and p-cresol and before the pyrogallol. An intermediate fraction contained a mixture of these two latter phenols.

The aryloxyacetic acid derivative for phenol and p-cresol and the acetate derivative for catechol and pyrogallol were made and melting points determined to prove the presence of the phenols in various fractions.

o-, m-, and p-Cresol were separated in a similar manner. Various size fractions were collected and tested by odor and ferric chloride solution for the presence of the cresols. The three cresols were found to be separated from each other with intermediate fractions containing mixtures of two cresols. The o-cresol came off the column first, the m-cresol second, and the p-cresol last. The aryloxyacetic acid derivative was made for each of the fractions and the melting points determined to prove the presence of the cresols in the various fractions.

SEPARATION OF SOME COMMON PHENOLS BY CHROMATOGRAPHIC ADSORPTION

by

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SEPARATION OF SOME COMMON PHENOLS BY CHROMATOGRAPHIC ADSORPTION

I. INTRODUCTION

Chromatography is the term applied to the separation of mixtures, in solution, by selective adsorption in conjunction with a two phase equilibrium.

The first published use of chromatographic analysis was by Tswett in 1906 (16,pp.1-5; 20,pp.1-4), when he separated chlorophyll a and b. This work did not attract notice until after the war (1914-1918) due to lack of scientific communication. About 1930, the use of chromatographic analysis was revived, and since then has increasingly expanded.

The general procedure of chromatographic analysis involves the following steps: the uniform packing of an adsorbent into tubing, usually glass; the addition of the mixture in solution to the column; the addition of the eluents; the obtaining of separated solute by further elution and collection of fractions (flowing chromatogram), extruding the column and extracting the solutes, or a combination of these two methods.

The theory of chromatographic analysis is not yet completely understood. Apparently, chromatography is a dynamic process. Separation of the solutes depends upon a physical equilibrium existing between the solute adsorbed on

the solid adsorbent and the solute dissolved in the flowing solvent. It also depends upon the selective adsorption of the solutes, which have different degrees of adsorption activity. The substance with the strongest adsorption affinity apparently exhausts the adsorption surface so that those of lower affinity are adsorbed farther down the column. It is thought that, at first, all of the solutes are adsorbed at the top of the column, but that the solute with the strongest affinity displaces those less strongly adsorbed. With the addition of more solvent to the column the least adsorbed solute is desorbed most easily, and moves down the column faster than those with greater adsorption affinity. True adsorption (dependent upon surface forces), exchange reactions, and the formation of complexes all seem to take part in the sorption process. Probably, varying combinations of these forces take place in the formation of different chromatograms.

Many theoretical equations (2,6,11,13,17,19) and empirical relationships (1,8,18) have been formulated to assist prediction of chromatographic action on various mixtures. However, chromatographic analysis is still an empirical method.

Today chromatography has many uses. Some of these are: separation of mixtures; concentration of dilute solutions; purification; identification and control of

commercial products; and testing for homogeneity.

STATEMENT OF PROBLEM. In studies on the possible utilization of tars obtained by destructive distillation of wood and lignin, attempts have been made to separate the phenols present by fractional distillation, after separation of the neutral substances and acids (15). During the distillation, approximately one-half of the extracted material polymerizes and is thereby lost. This procedure has demonstrated that wood and lignin tars contain many different phenols including some of the more valuable dihydroxy compounds. Some method, other than fractional distillation, is needed to separate these phenols in order to avoid the polymerization and subsequent loss of a considerable quantity of valuable products. Chromatographic analysis was proposed as a possible method of solving the problem. Rather than to start with the tar extract, it was thought more feasible to separate increasingly complex mixtures of phenols known to be present in the extract. The problem was further simplified to the attempted separation of two different mixtures, each containing three or four phenols.

II. MATERIALS AND PROCEDURE

APPARATUS. The glass tubing used for the columns was of 18 mm. outside diameter and of various lengths.

The column was stoppered at the bottom with a one-hole rubber stopper. From this stopper, a piece of 6 mm. glass tubing made connection with the collecting vessel.

MATERIALS. The adsorbents used were: activated alumina of the Aluminum Co. of America; Aluminum Oxide Merck (anhydrous according to Brockman); Silica Gel 645200-R 6.0 of Davison Chemical Company. The solvents used were: Shell petroleum ether, commercial grade benzene; 95% ethyl alcohol; and C. P. propionic acid, chloroform, and ethyl ether. The phenols used were: Eastman white label catechol and o-, m-, p-cresol; U. S. P. phenol and resorcinol; Eimer and Amend tested purity pyrogallol; and C. P. phloroglucinol and hydroquinone.

METHOD. Except for deviations noted in descriptions of the individual experiments, the following procedure was used. A glass wool pad was placed above the bottom stopper to prevent the adsorbent from being washed out of the column. The adsorbent was placed in the column in the form of a slurry, and tamped with a wooden pestle between each addition of 10 ml. portions. The adsorbent was thoroughly moistened with the solvent used to dissolve the phenols; then approximately 10 ml. of the moistened

adsorbent was placed in a stemless funnel at the top of the column. 5 ml. of the solvent was poured over the adsorbent and the two ran into the column as a slurry. After all the adsorbent was added and tamped, a filter paper disk was placed on top of the adsorbent to prevent disturbance of the adsorbent by the addition of the solvents. Once the adsorbent was wetted, it was never allowed to become dry. The solution of phenols was added to the column and allowed to pass onto the column by gravity flow. As soon as the last of the solution passed onto the column, the first eluent was added. Each succeeding eluent was added as soon as the last of the preceeding one passed onto the column. As the last eluent passed onto the column, the column was allowed to drain freely except for cases noted, when compressed air was applied to the top of the column to force the last liquid out. The eluents were collected in various sized samples and treated as noted throughout the experimental part. In cases where the column was extruded, it was forced out by pressure applied to a wooden pestle that was of the same diameter as the inside of the glass tubing.

III. EXPERIMENTAL

PRELIMINARY EXPERIMENTS. The first part of the problem was to develop the technique and discover some method of determining where the colorless phenols were located on the column. An attempt was made to separate phenol and resorcinol on an alumina column 19 cm. long. 0.1 g. of phenol and 0.01 g. of resorcinol were dissolved in 50 ml. of petroleum ether and passed onto the column. The column was then eluted successively with 50 ml. of petroleum ether, 50 ml. of a 1-1 mixture of petroleum ether and benzene, and 50 ml. of benzene. An earlier attempt to use ethyl alcohol as the eluent proved unsuccessful because both of the phenols were washed through the column.

The column was allowed to drain for 5 minutes and then suction was applied to the column for 15 seconds. The absorbent was then extruded from the column and tested for fluorescence with ultra-violet light. The ultra-violet showed no fluorescence on the column, so a strip of saturated ferric chloride solution was painted on the column of adsorbent (20,p.86). This colored the column where any phenols were present. There was no indication of any separation of phenol and resorcinol.

In order to see the development of the chromatogram while improving technique, a mixture of phenol and nitrophenols was used because of their yellow color. An attempt

was made to separate phenol from m-nitrophenol. 0.1 g. of phenol and 0.05 g. of m-nitrophenol were dissolved in 50 ml. of petroleum ether and placed on a 19 cm. long column of alumina. Upon the successive addition of 25 ml. of petroleum ether, 25 ml. of a 1-1 mixture of petroleum ether and benzene, 25 ml. of benzene, 25 ml. of a 1-1 mixture of benzene and chloroform, 15 ml. of chloroform, and 4 ml. of a 1-1 mixture of chloroform and alcohol, the two phenols were separated. The column was allowed to drain and then the adsorbent was extruded. By the use of ultra-violet, which intensified the color of the m-nitrophenol, and saturated ferric chloride, which colored the phenol, the m-nitrophenol was found on the top 5 cm. of the column, and the phenol band, covering 8 cm. of the column, started 3 cm. below the m-nitrophenol. Using the same procedure the separation was successfully repeated on a silica gel column.

The separation of phenol and p-nitrophenol was also accomplished by the same procedure on both alumina and silica gel columns. When 0.3 g. of phenol and 0.05 g. p-nitrophenol, on an alumina column, were eluted with 10 ml. of the eluents, the p-nitrophenol was not separated as completely from the phenol, as when 25 ml. of each of the eluents were used. On reducing the amount of phenol to 0.2 g. and using the same amount of p-nitrophenol and

eluents, a complete separation was accomplished.

To duplicate the work of Karrer (9), who separated o-, m-, and p-nitrophenols using calcium carbonate for the adsorbent and benzene as the eluent, a separation of these phenols was attempted. At the same time the effect of a longer column was determined. A column of alumina 102 cm. long was used. Due to the length of the column, a much larger volume of eluents was necessary; so the effect of various eluents was tested. Petroleum ether, benzene, chloroform, ethyl alcohol, and propionic acid were used in sequence. The first eluents separated the phenols, but as the more polar solvents were added, the phenols diffused into each other to form a single narrow band which traveled rapidly down the column. When silica gel was used as an adsorbent the o-phenol was carried out of the column, 107 cm. long, and the m- and p-nitrophenol were better separated but were still overlapping.

Because the adsorbents broke into many pieces when extruded, making the position of the phenols hard to determine, the use of a flowing chromatogram was attempted. 0.075 g. of pyrogallol and 0.15 g. of phenol were dissolved in 50 ml. of petroleum ether and passed onto the alumina column, 22 cm. long. 530 ml. of benzene were used as the eluent, the eluent from the column being collected in 5 ml. fractions. Each fraction was evaporated and the residue tested for phenols by the nitrous acid spot test

(Liebermann's nitroso reaction)(5,p.329). The amount of phenol in each fraction was so small that very few good tests were obtained; those obtained were not conclusive as to the separation of phenol and pyrogallol. This procedure was repeated with 0.3 g. of phenol and 0.075 g. of pyrogallol on a silica gel column 22 cm. long, using 400 ml. of a 1-1 mixture of benzene and chloroform as the eluent. Again the amount of phenol in each fraction was too small for the results to be conclusive.

Because of the loss of a considerable part of the phenols on evaporation in the above procedure, a ferric chloride test was used on liquid fractions as they came from the column. The sensitivity of the ferric chloride test for various phenols could not be found in the literature, so the sensitivity was determined for a group of phenols. This was done by dissolving the phenol in the solvent and shaking 25 ml. of the solution for two minutes with 10 ml. of saturated ferric chloride solution. Another portion of the solution was shaken for two minutes with 10 ml. of 1 N sodium hydroxide and then the water layer was neutralized with hydrochloric acid. 10 ml. of saturated ferric chloride was shaken with this neutral solution for two minutes. The limit of sensitivity of the ferric chloride test for various phenols in three solvents is recorded in Table I.

Table I

Sensitivity of Ferric Chloride Test for Phenols

Phenol	Sensitivity in parts of phenol per million parts of solvent				
	Benzene		Chloroform		Ethyl Alcohol
	solvent[1]	NaOH ext.[2]	solvent	NaOH ext.	solvent
p-Nitrophenol	1000	909	1667	1429	3333
Phenol	2500	2000	2500	2000	10,000
p-Cresol	1111	1000	2500	2000	2500
Catechol	100	50	25	20	17
Resorcinol	*		50	50	2000
Hydroquinone	*		*		1111
Pyrogallol	50	50	14	11	50
Phloroglucinol	*		*		2500

1 Phenol dissolved in solvent and shaken with ferric chloride.
2 Solution extracted with NaOH solution, extract neutralized with concentrated hydrochloric acid and shaken with ferric chloride.
* Phenol insoluble.

SEPARATION OF PHENOL, p-CRESOL, CATECHOL, AND PYROGALLOL. From the phenols in the table it was decided to attempt a separation of phenol, p-cresol, catechol, and pyrogallol. These four phenols were selected because of the large differences in sensitivity to the ferric chloride test which was assumed to result from widely different solubilities. In order to develop a procedure for the separation of each phenol from each of the others.

Separation of phenol and catechol. 0.3 g. of phenol and 0.3 g. of catechol were dissolved in 50 ml. of

benzene; the column was packed with alumina 25 cm. long and washed with 25 ml. of benzene; after the solution passed onto the column, 130 ml. of ethyl alcohol and the 20 ml. of propionic acid were added as eluents. The eluents issuing from the column were collected in nine 25 ml. fractions, starting as the solution was added to the column. 5 ml. of saturated ferric chloride was added to each fraction and the mixture was shaken. The third (benzene) and fourth (benzene and ethyl alcohol) fractions were colored dark brown indicating the presence of phenol, and the ninth (last) fraction was colored green indicating catechol.

Separation of phenol and pyrogallol. 0.3 g. of phenol and 0.3 g. of pyrogallol were dissolved in 50 ml. of benzene and passed onto a 25 cm. long, benzene washed, alumina column. 120 ml. of ethyl alcohol and 20 ml. of propionic acid were used as eluents. When the propionic acid was added, compressed air was applied to the top of the column to push the acid through at a fairly rapid rate, until no more liquid issued from the column. The eluents were collected in nine 25 ml. fractions, starting as the solution was added to the column. The third fraction (benzene) gave a good positive test for phenol. The ninth fraction (propionic acid) gave a positive test for pyrogallol, and all of the other fractions gave negative tests.

Because the last fraction gave a positive test for pyrogallol, 20 ml. of propionic acid, and after this passed onto the column, 30 ml. of water were forced through the column with compressed air. Each eluent was collected in a different fraction and tested with ferric chloride. Both fractions gave a positive test for pyrogallol. The adsorbent was extruded from the column and tested with ferric chloride. A positive test for pyrogallol was still obtained.

Separation of phenol and p-cresol. 0.3 g. of phenol and 0.3 g. of p-cresol were dissolved in 50 ml. of benzene and placed on a 25 cm. long washed alumina column. 50 ml. of a 1-4 mixture of ethyl alcohol and benzene, 50 ml. of a 1-1 mixture of ethyl alcohol and benzene, 90 ml. of ethyl alcohol, and 50 ml. of 12 N hydrochloric acid were used as eluents. Ten 25 ml. fractions were collected starting as the solution was added to the column. The third (benzene and the 1-4 mixture) and fourth (1-4 mixture) fractions contained the phenol. The tenth fraction (ethyl alcohol and hydrochloric acid) was the first fraction to give a positive test for p-cresol. After all of the acid passed out of the column by gravity flow, 20 ml. of water was passed down the column. This was collected in two 10 ml. and three 5 ml. fractions. The first three fractions gave a positive test, but the last two were negative.

Separation of catechol and pyrogallol. 0.2 g. of catechol and 0.2 g. of pyrogallol were dissolved in 50 ml. of benzene and placed on a 25 cm. long, washed alumina column. The series of eluents used was: 30 ml. of a 1-10 mixture of propionic acid and ethyl alcohol, 30 ml. of a 1-8 mixture, 30 ml. of a 1-5 mixture, 30 ml. of a 1-2 mixture, 30 ml. of a 1-1 mixture, 30 ml. of propionic acid, 30 ml. of a 1-1 mixture of propionic acid and water, 50 ml. of water. 5 ml. of saturated ferric chloride were added to each of the eleven 25 ml. fractions. There was not a good separation of the two phenols. Varying intensities of colors seemed to indicate a partial separation. By extrusion of the adsorbent and testing with ferric chloride some pyrogallol was still found on the column.

After running four different chromatograms, the amounts and types of mixtures being varied in each one, the fourth gave the results indicated in Table II. 0.3 g. of pyrogallol and 0.3 g. of catechol were dissolved in a mixture of 46 ml. of benzene and 4 ml. of ethyl alcohol and placed on a 25 cm. long, washed column. The eluents used were: 100 ml. of a 1-1 mixture of propionic acid and ethyl alcohol, 25 ml. of propionic acid, 150 ml. of a 1-1 mixture of propionic acid and water, 50 ml. of water. 5 ml. of saturated ferric chloride were added to the 25 ml. fractions and a proportional amount to the 10 ml. and 5 ml. fractions.

The colors obtained in the ferric chloride test

Table II

Separation of Pyrogallol and Catechol

Fraction	Volume, ml.	Solvent[1]	Color with $FeCl_3$[2]
1	25	B	neg.[3]
2	25	B	neg.
3	10	B	neg.
4	5	B	neg.
5	25	B + PA(1-1)	blk.
6	25	PA(1-1)	blk.
7	25	PA(1-1)	blk.
8	10	PA(1-1)	dk.br.
9	5	PA(1-1)	dk.br.
10	10	PA(1-1) + P	br.
11	10	P	lt.br.
12	5	P	lt.br
13	5	P + PW(1-1)	blk.
14	25	PW(1-1)	blk.
15	25	PW(1-1)	blk.
16	25	PW(1-1)	rd.blk.
17	25	PW(1-1)	rd.blk.
18	25	PW(1-1)	rd.blk.
19	25	PW(1-1) + W	red
20	10	W	lt.rd.
21	5	W	neg.
22	2	W	neg.

1 B,benzene; P, propionic acid; A,ethyl alcohol; W,water; PA(1-1),1-1 mixture of propionic acid and ethyl alcohol; PW(1-1),1-1 mixture of propionic acid and water.
2 Catechol in alcohol is green, in acid is green; pyrogallol in alcohol is brown, in acid is red, in water is red. Fairly concentrated solutions of both phenols appear black with $FeCl_3$ because of the very dark color of both the red and green; when the black solutions are diluted they become green for catechol and red for pyrogallol.
3 neg.,negative; dk.br.,dark brown; lt.br.,light brown; rd.blk.,reddish black; lt.rd.,light red.

indicate that the catechol is located in fractions numbered five through twelve and the pyrogallol in fractions numbered thirteen through twenty. Fraction thirteen probably contains

some catechol. The column gave a slight positive test for pyrogallol.

Separation of catechol and p-cresol. 0.3 g. of catechol and 0.3 g. of p-cresol were dissolved in 50 ml. of benzene and placed on a 25 cm. long, washed column of alumina. 100 ml. of a 1-30 mixture of propionic acid and alcohol and 75 ml. of a 1-1 mixture of propionic acid and water were used as eluents. The eluent was collected in thirteen various sized (10 and 25 ml.) fractions and tested with saturated ferric chloride. The p-cresol was found in the fourth fraction (benzene and 1-30 mixture). The catechol was found in the seventh (1-30 mixture) and all of the following fractions.

Separation of p-cresol and pyrogallol. Because of the different eluents used for separating p-cresol from catechol and pyrogallol from catechol, the separation of p-cresol and pyrogallol was not run. A 1-30 mixture of propionic acid to ethyl alcohol washed the p-cresol from the column, while propionic acid was necessary to wash the pyrogallol down the column, so a separation of these two phenols should be relatively simple.

Separation of phenol, p-cresol, catechol, and pyrogallol. Having separated the four phenols from each other, all four were dissolved in benzene and passed onto a column. After making four chromatograms, in which the amounts and

mixtures of eluents were varied the fourth gave the results as tabulated in Table III.

Table III

Separation of Phenol, p-Cresol, Catechol, and Pyrogallol

Fraction	Volume, ml.	Solvent[1]	Color with $FeCl_3$[2]
1	25	B	neg.[3]
2	25	B	neg.
3	10	B	lt.br.
4	10	B + BA(1-1)	br.
5	10	BA(1-1)	br.
6	10	BA(1-1)	lt.br.
7	10	BA(1-1)	neg.
8	10	BA(1-1)	neg.
9	10	BA(1-1)	neg.
10	10	BA(1-1) + AaA(1-30)	neg.
11	10	AaA(1-30)	gr.
12	10	AaA(1-30)	dk.gr.
13	10	AaA(1-30)	dk.gr.
14	25	AaA(1-30) + AaA(1-1)	dk.gr.br.
15	25	AaA(1-1)	dk.gr.br.
16	10	AaA(1-1)	dk.rd.br.
17	10	AaA(1-1)	dk.rd.br.
18	25	AaA(1-1) + AaW(1-1)	dk.rd.br.
19	25	AaW(1-1)	dk.rd.br.
20	25	AaW(1-1)	dk.rd.br.
21	5	AaW(1-1)	rd.br.
22	5	AaW(1-1)	rd.br.

1 B,benzene; A,ethyl alcohol; Aa,acetic acid; W,water; BA(1-1),1-1 mixture of benzene and ethyl alcohol; AaA(1-30), 1-30 mixture of acetic acid to alcohol; AaA(1-1),1-1 mixture of acetic acid and alcohol; AaW(1-1),1-1 mixture of acetic acid and water.
2 Phenol and p-cresol are brown in benzene and alcohol; catechol is green in alcohol and acid; pyrogallol is brown in alcohol, red in acid and water.
3 neg.,negative; lt.br.,light brown; dk.gr., dark green; dk.gr.br.,dark greenish brown; dk.rd.br.,dark reddish brown.

0.3 g. of each of the phenols, phenol, p-cresol, catechol, and pyrogallol, was dissolved in a mixture of 46 ml. of benzene and 4 ml. of ethyl alcohol and placed on a 25 cm. long column of alumina, that had been washed with 50 ml. of benzene. The eluents added were: 60 ml. of a 1-1 mixture of benzene and ethyl alcohol, 50 ml. of a 1-30 mixture of acetic acid and ethyl alcohol, 75 ml. of a 1-1 mixture of acetic acid and alcohol, 100 ml. of a 1-1 mixture of acetic acid and water. 5 ml. of saturated ferric chloride were added to the 25 ml. fractions with proportionate amounts being added to the 10 ml. fractions. The adsorbent was extruded and tested with saturated ferric chloride. A positive pyrogallol test was obtained.

It seemed that there was a fairly good separation of pyrogallol and catechol from the mixture of four. Fractions three, four, five, and six probably need to be run through a second column to separate the phenol from the p-cresol.

The separation of the mixture of phenol, p-cresol, catechol, and pyrogallol was repeated; the ferric chloride test was applied to one-half of the fractions, and derivatives were made from the remaining one-half of the fractions to confirm the identity of the phenols present in these fractions. The acyloxyacetic acid derivative (10) was made for phenol and p-cresol. The acetate derivative (3)

Table IV

Separation of Phenol, p-Cresol, Catechol, and Pyrogallol

Derivative[1]	Fraction	Volume, ml.	Solvent[2]	Color with[3] $FeCl_3$
I	1	25	B	
II	2	25	B	
III	3	10	B	neg.[4]
	4	10	B + AB(1-4)	br.
IV	5	10	AB(1-4)	br.
	6	10	AB(1-4)	lt.br.
V	7	10	AB(1-4)	lt.br.
	8	10	AB(1-4) + PA(1-30)	neg.
VI	9	10	PA(1-30)	neg.
	10	10	PA(1-30)	neg.
VII	11	10	PA(1-30)	gr.
	12	10	PA(1-30)	gr.
VIII	13	10	PA(1-30) + PA(1-1)	br.gr.
	14	10	PA(1-1)	br.gr.
	15	10	PA(1-1)	br.gr.
IX	16	10	PA(1-1)	blk.
	17	10	PA(1-1)	blk.rd.
X	18	10	PA(1-1) + PW(1-1)	blk.rd.
	19	10	PW(1-1)	blk.rd.
	20	10	PW(1-1)	blk.rd.
XI	21	10	PW(1-1)	red
	22	10	PW(1-1)	red
	23	5	PW(1-1)	red

1 In preparing derivatives I and II, the 25 ml. samples of eluent were used; in preparing derivatives III through XI, 5 ml. of each fraction indicated were used.
2 B,benzene; A,ethyl alcohol; P,propionic acid; W,water; AB(1-4),1-4 mixture of ethyl alcohol to benzene; PA(1-30), 1-30 mixture of propionic acid to ethyl alcohol; PA(1-1), 1-1 mixture of propionic acid and ethyl alcohol; PW(1-1), 1-1 mixture of propionic acid and water.
3 Phenol and p-cresol are brown in benzene and alcohol; catechol is green in alcohol and acid; pyrogallol is brown in alcohol, red in acid and water. Fairly concentrated solutions of pyrogallol and catechol are black because of the very dark color of the red and green; when these black solutions are diluted, the green and red are apparent.
4 neg.,negative; lt.br.,light brown; blk.gr.,blackish green; br.gr.,brownish green; blk.rd.,blackish red.

was made for catechol and pyrogallol.

0.3 g. of phenol, 0.3 g. of p-cresol, 0.3 g. of catechol, and 0.3 g. of pyrogallol were dissolved in a mixture of 47 ml. of benzene and 3 ml. of ethyl alcohol. The solution was placed on a column of Alcoa activated alumina, which was 25 cm. long and had been washed with 25 ml. of benzene. As the last of the solution passed onto the column the first eluent was added; as the last of each eluent passed onto the column the succeeding one was added. The eluents were: 30 ml. of a 1-4 mixture of ethyl alcohol and benzene, 50 ml. of a 1-30 mixture of propionic acid and alcohol, 50 ml. of a 1-1 mixture of propionic acid and alcohol, 70 ml. of a 1-1 mixture of propionic acid and water. The results of the ferric chloride test are indicated in Table IV.

The samples were evaporated to less than 1 ml. by use of a hot air fan. The derivatives were then made according to directions given in the references cited and melting points were determined for the dry derivatives. The results obtained are recorded in Table V.

Upon the addition of sodium hydroxide in the procedure for derivatives ten and eleven, alumina precipitated; therefore no derivatives were obtained.

Table V

Melting Point of Derivatives from Separation of Phenol, Pyrogallol, p-Cresol and Catechol

Derivative	Melting Point	Phenol Indicated
I	no product	none
II	no product	none
III	132°-134° C.	p-cresol (134°-136° C. (10))
IV	89°- 92° C.	inconclusive[1]
V	no product	none
VI	no product	none
VII	63°- 66° C.	catechol(64°- 65° C.(3))
VIII	106°-110° C.	inconclusive
IX	170°-172° C.	pyrogallol(172°-173° C.(3))
X	alumina	
XI	alumina	

1 The melting point of phenol is 98°-99° C.(10). It is possible that fractions 5 and 6 from which derivative IV was made contained largely phenol.

SEPARATION OF o-CRESOL, m-CRESOL, AND p-CRESOL. The problem of the separation of the three cresols was next undertaken. The method was the same as in the previous separations, that of the separation of pairs of phenols and then the separation of the three.

Separation of o-cresol and p-cresol. o-Cresol and p-cresol were dissolved in benzene and placed on a 25 cm.

long, washed column. After running five chromatograms, varying the amount and mixture of eluents, the following procedure was developed. 0.3 ml. of o-cresol and 0.3 g. of p-cresol were dissolved in 25 ml. of benzene and placed on the column. 30 ml. of a 1-10 mixture of ethyl alcohol and benzene and 50 ml. of a 1-30 mixture of propionic acid and alcohol were used as eluents. The results are indicated in Table VI.

The odor of the fractions indicated that fractions

Table VI

Separation of o-Cresol and p-Cresol

Fraction	Volume, ml.	Solvent[1]	Color with $FeCl_3$[2]
1	25	B	neg.[3]
2	10	B	neg.
3	5	B + AB(1-10)	neg.
4	5	AB(1-10)	neg.
5	5	AB(1-10)	neg.
6	5	AB(1-10)	neg.
7	5	AB(1-10)	lt.br.
8	5	AB(1-10)	br.
9	5	AB(1-10)	br.
10	5	AB(1-10) + PA(1-30)	br.
11	5	PA(1-30)	br.
12	5	PA(1-30)	lt.br.
13	5	PA(1-30)	neg.
14	5	PA(1-30)	neg.
15	5	PA(1-30)	neg.
16	2	PA(1-30)	neg.

1 B,benzene; A,ethyl alcohol; P,propionic acid; AB(1-10), 1-10 mixture of ethyl alcohol to benzene; PA(1-30),1-30 mixture of propionic acid to ethyl alcohol.
2 p-Cresol and o-cresol are brown in all solutions used.
3 neg.,negative; lt.br.,light brown.

seven and eight contained o-cresol, and ten and eleven contained p-cresol. Fraction nine probably contained both o- and p-cresol.

Separation of m-cresol and p-cresol. 0.3 ml. of m-cresol and 0.3 g. of p-cresol were dissolved in 25 ml. of benzene, and placed on a 25 cm. long column of alumina. 30 ml. of a 1-10 mixture of ethyl alcohol and benzene, and 50 ml. of a 1-30 mixture of propionic acid and alcohol were used as eluents. The results obtained are tabulated in Table VII.

Table VII

Separation of m-Cresol and p-Cresol

Fraction	Volume, ml.	Solvent[1]	Color with $FeCl_3$[2]
1	25	B	neg.[3]
2	10	B	neg.
3	5	B + AB(1-10)	neg.
4	5	AB(1-10)	neg.
5	5	AB(1-10)	neg.
6	5	AB(1-10)	lt.br.
7	5	AB(1-10)	br.
8	5	AB(1-10)	br.
9	5	AB(1-10)	lt.br.
10	5	AB(1-10) + PA(1-30)	br.
11	10	PA(1-30)	br.
12	5	PA(1-30)	lt.br.
13	10	PA(1-30)	neg.
14	5	PA(1-30)	neg.
15	6	PA(1-30)	neg.

1 B,benzene; A,ethyl alcohol; P,propionic acid; AB(1-10), 1-10 mixture of ethyl alcohol to benzene; PA(1-30),1-30 mixture of propionic acid to ethyl alcohol.
2 m-Cresol and p-cresol are brown in all solutions used.
3 neg.,negative; lt.br.,light brown.

The odor of the fractions indicated that fractions six, seven, and eight contained m-cresol and that ten and eleven contained p-cresol. Fraction nine probably contained both m- and p-cresol.

Separation of o-cresol and m-cresol. 0.3 ml. of o-cresol and 0.3 ml. of m-cresol were dissolved in 25 ml.

Table VIII

Separation of o-Cresol and m-Cresol

Fraction	Volume, ml.	Solvent[1]	Color with $FeCl_3$[2]
1	25	B	neg.[3]
2	10	B	neg.
3	5	B + BC(1-1)	neg.
4	5	BC(1-1)	neg.
5	5	BC(1-1)	neg.
6	5	BC(1-1)	neg.
7	5	BC(1-1)	neg.
8	5	BC(1-1)	neg.
9	5	BC(1-1)	neg.
10	5	BC(1-1)	neg.
11	5	BC(1-1)	neg.
12	5	BC(1-1)	lt.br.
13	5	BC(1-1) + AB(1-10)	br.
14	5	AB(1-10)	br.
15	5	AB(1-10)	br.
16	5	AB(1-10)	br.
17	5	AB(1-10)	br.
18	10	AB(1-10)	br.
19	10	AB(1-10)	br.
20	5	AB(1-10) + PA(1-30)	lt.br.
21	10	PA(1-30)	neg.

1 B,benzene; C,chloroform; A,ethyl alcohol; P,propionic acid; BC(1-1),1-1 mixture of benzene and chloroform; AB(1-10),1-10 mixture of ethyl alcohol to benzene; PA(1-30), 1-30 mixture of propionic acid to ethyl alcohol.
2 o-Cresol and m-cresol are brown in all solutions used.
3 neg.,negative; lt.br., light brown.

of benzene. The solution was placed on a 25 cm. long, washed column of alumina and eluted with 50 ml. of a 1-1 mixture of chloroform and benzene, 50 ml. of a 1-10 mixture of ethyl alcohol and benzene, and 20 ml. of a 1-30 mixture of propionic acid and alcohol. The results obtained are indicated in Table VIII.

The odor of the fractions indicated that fractions twelve, thirteen, and fourteen contained o-cresol and fractions sixteen, seventeen, and eighteen contained m-cresol. Fraction fifteen probably contained both o- and m-cresol.

Separation of o-cresol, m-cresol, and p-cresol. 0.3 ml. of o-cresol, 0.3 ml. of m-cresol, and 0.3 g. of p-cresol were dissolved in 25 ml. of benzene and passed onto a 25 cm. long, washed column of Alcoa alumina. The column was then eluted with 40 ml. of chloroform, 30 ml. of a 1-10 mixture of ethyl alcohol and chloroform, 20 ml. of ethyl alcohol, and 40 ml. of a 1-30 mixture of propionic acid and alcohol. The presence of the cresols was determined by the ferric chloride test and by odor. The results are tabulated in Table IX.

The odor of the fractions indicated the location of the various phenols. o-Cresol was found in fractions four through twelve; m-cresol was found in fraction fourteen; p-cresol was found in fractions sixteen and seventeen. The

fractions between the fractions enumerated probably contained mixtures of cresols.

The separation of o-cresol, m-cresol, and p-cresol was repeated and derivatives made from various fractions to confirm the identity of the cresols present in these fractions. The acyloxyacetic acid derivative (10) was made for the three cresols.

Table IX

Separation of o-Cresol, m-Cresol, and p-Cresol

Fraction	Volume, ml.	Solvent[1]	Color with $FeCl_3$[2]
1	25	B	neg.[3]
2	10	B	neg.
3	5	B + C	neg.
4	5	C	lt.br.
5	5	C	lt.br.
6	5	C	lt.br.
7	5	C	lt.br.
8	5	C	lt.br.
9	5	C	lt.br.
10	5	C	lt.br.
11	5	C	lt.br.
12	5	C	lt.br.
13	5	C + AC(1-10)	br.
14	10	AC(1-10)	br.
15	10	AC(1-10)	br.
16	10	AC(1-10) + A	br.
17	10	A	br.
18	10	A + PA(1-30)	neg.
19	10	PA(1-30)	neg.
20	10	PA(1-30)	neg.

1 B,benzene; C,chloroform; A,ethyl alcohol; P,propionic acid; AC(1-10),1-10 mixture of ethyl alcohol to chloroform; PA(1-30),1-30 mixture of propionic acid to ethyl alcohol.
2 o-, m-, and p-Cresol are brown in all solutions used.
3 neg.,negative; lt.br.,light brown.

0.3 ml. of o-cresol, 0.3 ml. of m-cresol, and 0.3 g. of p-cresol were dissolved in 25 ml. of benzene. The solution was placed on a column of Alcoa activated alumina,

Table X

Separation of o-Cresol, m-Cresol, and p-Cresol

Derivative	Fraction	Volume, ml.	Solvent[1]
I	1	25	B
	2	10	B
	3	5	B + C
II	4	5	C
	5	5	C
	6	5	C
	7	5	C
	8	5	C
III	9	5	C
	10	5	C
IV	11	5	C
	12	5	C
	13	5	C + AC(1-10)
V	14	10	AC(1-10)
	15	10	AC(1-10)
VI	16	10	AC(1-10) + A
	17	10	A
VII	18	10	A + PA(1-30)
VIII	19	10	PA(1-30)
IX	20	10	PA(1-30)

1 B,benzene; C,chloroform; A,ethyl alcohol; P,propionic acid; AC(1-10), 1-10 mixture of ethyl alcohol to chloroform; PA(1-30),1-30 mixture of propionic acid to ethyl alcohol.

which was 25 cm. long and had been washed with 25 ml. of benzene. As the last of the solution passed onto the column the succeeding one was added. The eluents used were:

40 ml. of chloroform, 30 ml. of a 1-10 mixture of ethyl alcohol and chloroform, 20 ml. of 95% ethyl alcohol, 50 ml. of a 1-30 mixture of propionic acid and alcohol. No ferric chloride tests were run. The fractions were separated into samples according to Table X.

The samples were evaporated to 1 ml. by a hot air fan and the derivatives made according to directions of the references cited. The results obtained from the melting points of the derivatives are tabulated in Table XI.

Samples three and four produced no product because of an error made in procedure.

Table XI

Melting Points of Cresol Derivatives

Derivative	Melting Point	Phenol Indicated
I	no product	none
II	150°-151° C.	o-cresol(151°-152° C.(10))
III	no product	none
IV	no product	none
V	103°-105° C.	m-cresol(102°-103° C.(10))
VI	133°-135° C.	p-cresol(134°-136° C.(10))
VII	no product	none
VIII	no product	none
IX	no product	none

IV. DISCUSSION AND CONCLUSION

The separation of phenol, p-cresol, catechol, and pyrogallol was not as successful as the separation of the cresols. It is apparent that phenol and p-cresol were not separated from one another when these four phenols were chromatographed. Unless a modification of the procedure used can be found that will bring about this separation, on one column, it appears that the fractions containing the phenol and p-cresol should be passed onto a second column and there separated. Although some of the fractions contained pure pyrogallol and catechol, the intermediate fractions containing a mixture of pyrogallol and catechol could be rechromatographed and the two phenols further separated.

The procedure, as outlined previously, seems to be successful for a separation of the three cresols from each other. More work will be necessary to determine the maximum concentration of cresols that can be used in this method. For practical large scale separation of phenols, columns of larger diameter and greater length will probably be necessary, using larger volumes of eluent. To avoid loss of this large volume of solvents, a method could be developed to recover them.

Attempts have been made to separate some of the simple phenols by paper partition chromatography (4,7,14).

Hossfeld (7) succeeded in the resolution of a mixture of phenol, o-, m-, and p-cresols except that o- and m-cresol were not separated from each other. Before chromatographing, he converted the phenols to the phenyl azo dyes coupling the phenols with diazotized sulfanilic acid. A 1-1 mixture of methyl ethyl ketone and water was used as the irrigant. This method, converted to column chromatography, would not be applicable to this problem as the phenols are desired in the pure unreacted form.

Riley (14) separated resorcinol, catechol, and pyrogallol from other more complicated phenols and each other by use of a 1-1 mixture of amyl alcohol and water or a 1-19-20 mixture of butyl alcohol-benzene-water. The resolution of a mixture of these phenols and phenol was proved unsuccessful when a 1-1-2 mixture of butanol-pyridine-saturated solution of sodium chloride was used as the irrigant (4). Using this irrigant, the R_F values for the four phenols differed by only 0.03 and therefore a separation by this irrigant would be unsuccessful.

A serious limitation of the use of paper partition chromatography to determine the effectiveness of various eluents is that the cresols and some other phenols evaporate and are lost during the determination. Paper chromatography is not suitable for practical large scale separation of phenols.

V. SUMMARY

The separation of o-, m-, and p-nitrophenol by Karrer (9) was duplicated using alumina as the adsorbent instead of calcium carbonate.

When the adsorbent was extruded from the column and painted with a strip of ferric chloride solution to determine the position of the phenols, the column broke into many pieces. To overcome this difficulty a flowing chromatogram was utilized. Various fractions were collected and tested with ferric chloride solution for the presence of the phenols. The sensitivity of the ferric chloride test for various phenols could not be found in the literature and was therefore determined.

A mixture of phenol, p-cresol, pyrogallol, and catechol dissolved in benzene was chromatographed on a 25 cm. long, 16 mm. diameter, packed alumina column. 30 ml. of a 1-4 mixture of ethyl alcohol and benzene, 50 ml. of a 1-30 mixture of propionic acid and alcohol, 50 ml. of a 1-1 mixture of propionic acid and alcohol, and 70 ml. of a 1-1 mixture of propionic acid and water were added successively as eluents. 10 ml. fractions were collected and tested with saturated ferric chloride solution. The phenol and p-cresol were found together in two of the first fractions. The pyrogallol and catechol were found separated from the other two phenols and from each other, the catechol

coming off the column after the phenol and p-cresol and before the pyrogallol. An intermediate fraction contained a mixture of these two latter phenols. The acyloxyacetic acid derivative for phenol and p-cresol and the acetate derivative for catechol and pyrogallol were made and melting points determined to prove the presence of the phenols in various fractions.

o-, m-, and p-Cresol, dissolved in benzene, were passed onto a 25 cm. long, 16 mm. diameter, packed alumina column. 40 ml. of chloroform, 30 ml. of a 1-10 mixture of ethyl alcohol and chloroform, 20 ml. ethyl alcohol, 50 ml. of a 1-30 mixture of propionic acid and alcohol were added successively as eluents. Various size fractions were collected and tested by odor and ferric chloride solution for the presence of the cresols. The three cresols were found to be separated from each other with intermediate fractions containing mixtures of two cresols. The o-cresol came off the column first, the m-cresol second, and the p-cresol last. The acyloxyacetic acid derivative was made for each of the fractions and the melting points determined to prove the presence of the cresols in the various fractions.

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