phenol in each layer agreed within 5% or less of the original amount. Thereafter, analyses were usually confined to the cyclohexane phase, except in those instances where the partition coefficient was very high or low.

Despite the fact that no close temperature control was maintained during distribution, duplicate partition coefficients, determined at intervals of several days, agreed within 5% of each other in the intermediate range and within 10% in the very high or low range. Since a 30% change in partition coefficient would affect the calculated *pK* value by only a 0.1 unit, it is believed that the calculated values are within about ±0.1 *pK* unit of the exact values.

Summary

The partition coefficients of 14 phenols have been measured in systems composed of cyclohexane–water and cyclohexane–phosphate buffer. A relationship was observed between partition coefficient and *p*H which permitted calculation of approximate ionization constants. Partition coefficients and ionization constants were correlated with molecular configuration of phenols.

PITTSBURGH, PA. RECEIVED JANUARY 19, 1949

[CONTRIBUTION FROM THE RESEARCH AND DEVELOPMENT BRANCH, OFFICE OF SYNTHETIC LIQUID FUELS, BUREAU OF MINES]

Partition Studies on Phenols. II. Separation of Isomeric Phenols by Countercurrent Distribution[1]

BY CALVIN GOLUMBIC[2]

Phenolic compounds of wide variety are potentially available in huge amounts from coal-hydrogenation oils.[3] It is therefore of considerable interest to determine the composition of the complex tar-acid fractions in which these phenols are found. Such mixtures are composed largely of homologous and isomeric phenols, and separation by the usual procedures of precise fractional distillation and fractional crystallization is difficult. Since the method of countercurrent distribution[4] has successfully resolved mixtures of isomeric and homologous acidic and basic compounds,[5,6] it appeared to be applicable in isolating individual phenols and testing their purity. Previous partition studies[7] on phenol homologs showed significant relative differences among such compounds, particularly in organic solvent–buffer systems. Alkaline buffer when used as one of the immiscible phases allows adjustment of the partition coefficient to any desired value and frequently increases the partition ratios among groups of closely related phenols. Separation of some isomeric phenols and proof of their homogeneity are described in this manuscript.

Experimental

Materials.—The source and purity of the phenols used in this work have been mentioned previously.[7]

Apparatus.—The countercurrent distribution machine was a stainless steel (8–18), 54-tube instrument manufactured by Otto Post, Maspeth, N. Y. This apparatus differs from Craig's original model[4] principally in the manner of sealing the bottom of the lower drum and top of the upper drum. Plate glass is used instead of steel plates, to permit observation of phase separation. The lower tubes of the instrument hold 10 ml. of solvent; the upper tubes, up to 15 ml.

Procedure.—Before the experiment was started, the organic solvent (cyclohexane or benzene) and 0.5 *M* phosphate buffer (Na_2HPO_4 + Na_3PO_4) were mutually saturated at room temperature by shaking in separatory funnels. Sufficient buffer layer was introduced into the lower drum of the countercurrent instrument to fill each tube to its brim; this was followed by addition of 10 ml. of the organic solvent to each upper tube. After inverting the instrument several times to test whether the glass-metal and metal–metal seals were leakproof, the upper phase of tube 0 was replaced with a solution of the entire material to be distributed. Stepwise countercurrent distribution was then conducted as described by Craig.[4] Upon completion of the run, the contents of each tube of the instrument were pipetted into glass-stoppered centrifuge tubes and acidified with 2 ml. of HCl (1:1). These tubes were shaken and centrifuged to clarify the layers. The organic phase was separated and analyzed by means of the Beckman Model DU quartz spectrophotometer. In cases where the analysis indicated the presence of a crystalline component in nearly pure form, a crystalline residue was always obtained by evaporation of the solvent. The amounts, however, were too small for determination of capillary melting points.

Results

Homogeneity Studies.—For this purpose, 53-plate distributions were run in a 54-tube Craig distribution machine[8] employing cyclohexane and 0.5 *M* phosphate buffer as the immiscible phases. Preliminary measurements of the partition coefficient at different *p*H values were made, to select a buffer that would provide a partition coefficient close to one.[7] After distribution, the total amount of phenol in each tube of the distribution machine was determined by ultraviolet measurement at the wave length of maximum absorption.

A typical example of the results is shown in Fig. 1, which represents the distribution of a 50-mg. sample of 3-methyl-5-ethylphenol in cyclohexane–phosphate buffer of *p*H 11.26. Because this compound had been isolated from a tar-acid fraction of a coal-hydrogenation product[3] containing

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(1) Not subject to copyright.

(2) Organic Chemist, Research and Development Branch, Office of Synthetic Liquid Fuels, Bureau of Mines, Bruceton, Pa.

(3) Golumbic, Bureau of Mines Rept. Investig., 4467, 56 pp. (1949).

(4) Craig, *J. Biol. Chem.*, **155**, 519 (1944).

(5) Craig, Golumbic, Mighton and Titus, *J. Biol. Chem.*, **161**, 321 (1945).

(6) Warshowsky and Schantz, *Anal. Chem.*, **20**, 951 (1948).

(7) Golumbic, Orchin and Weller, THIS JOURNAL, in press.

(8) Barry, Sato and Craig, *J. Biol. Chem.*, **174**, 221 (1948).

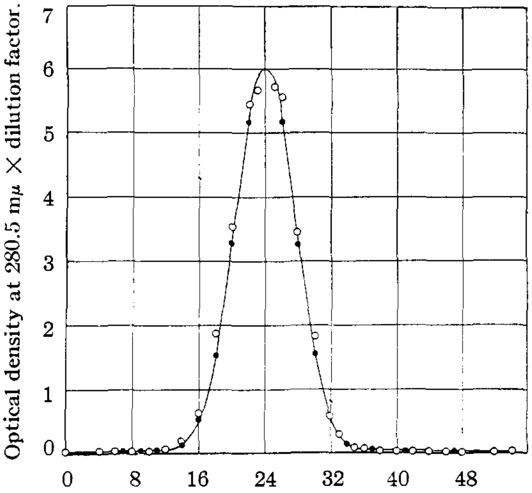

Fig. 1.—Distribution of 3-methyl-5-ethylphenol: ○, experimental; ●, theoretical.

several homologs and isomers, it was considered advisable to obtain this independent check on its homogeneity. From the position of the maximum of the distribution curve, the partition coefficient, k', was calculated by the equation

$$N = nk'/(k' + 1)$$

where N is the position of the tube of maximum concentration and n is the total number of plates applied.[4] A theoretical distribution curve for a compound with this partition coefficient (0.8) was calculated according to the method of Williamson and Craig.[9] The theoretical and experimental curves agreed very closely. Such data provide very strong evidence for the purity of a compound.[10]

Separation of Isomers.—Mixtures of *m*- and *p*-cresols, xylenols and ethylphenols were subjected to 53-plate countercurrent distributions; *m*- and *p*-cresols form a mixture that cannot be separated by distillation because they boil within a degree of each other. By countercurrent distribution a partial separation was effected.

A mixture of 100 mg. each of *m*- and *p*-cresols was distributed in the system benzene–phosphate buffer of *p*H 11.08. Ultraviolet measurements were made at wave lengths of 280 and 286 mμ. At both wave lengths, *p*-cresol exhibits a maximum in its ultraviolet curve, whereas *m*-cresol has a strong band only at 280 mμ. Therefore, when separation of these isomers occurs during the distribution, the optical density values at 280 mμ should be much higher than at 286 mμ for those tubes relatively rich in *m*-cresol and about the same in tubes in which the *p*-cresol is concentrated. The experimental results represented graphically in Fig. 2 show that *m*-cresol predominates in tubes 0–26 and that *p*-cresol is the major component in tubes 28–54.

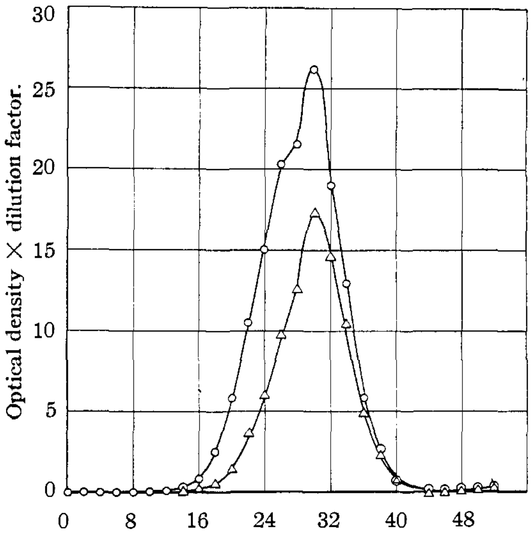

Fig. 2.—Distribution of cresol mixture: ○, 280 mμ; △, 286 mμ.

The amount of each isomer in each tube of the distribution machine was determined by solution of two simultaneous equations derived in the usual way from ultraviolet measurement at two wave lengths.[11] When plotted, the calculated amounts give curves (curves 1 and 2 of Fig. 3) that clearly show the degree of separation attained. Complete separation could be achieved by application of more plates by the technique of "alternate withdrawal" or "single withdrawal."[8] Comparison of the experimental curves with theoretical curves (indicated by dotted lines) shows that the *m*- and *p*-cresol used were not entirely pure. The

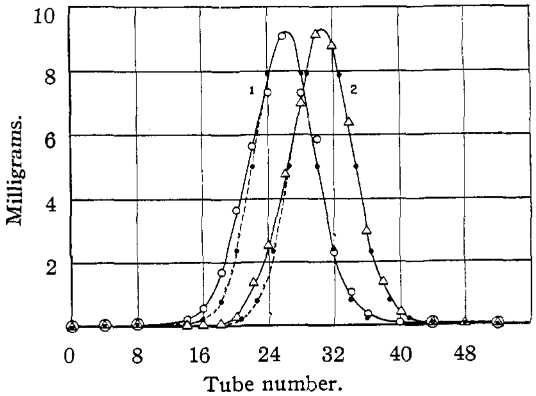

Fig. 3.—Separation of cresols by 53-plate distribution: ○, curve 1; *m*-cresol; △, curve 2, *p*-cresol; ●, theoretical.

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[9] Williamson and Craig, *J. Biol. Chem.*, **168**, 687 (1947).

[10] Craig, Mighton, Titus and Golumbic, *Anal. Chem.*, **20**, 134 (1948).

[11] These equations were developed by Dr. R. A. Friedel and are considered to measure total amounts of isomers in each tube with an accuracy in the order of 2% for the cresols and 5% for the xylenols and ethylphenols.

inhomogeneity present, determined as previously described,[10] amounts to about 5% for each isomer.

A 53-plate distribution was run on a mixture of 10 mg. each of 2,4-, 2,5- and 3,5-xylenol in the system cyclohexane–phosphate buffer of *p*H 11.08. Optical densities were measured at wave lengths of 275, 281 and 285 mμ. From these measurements, three simultaneous equations were derived for the determination of the quantity of each isomer in each tube.[11]

The calculated amounts are plotted in Fig. 4 (curves 1, 2 and 3). As can be seen, 3,5-xylenol separates readily from 2,4- and 2,5-xylenol. From the shape and appearance of each curve, it is evident that each compound distributes itself essentially independently of the others in accordance with the distribution law. Since the ratios of the partition coefficients of the un-ionized forms of these isomers are similar,[7] their separation in this distribution results mainly from their different acid strengths.

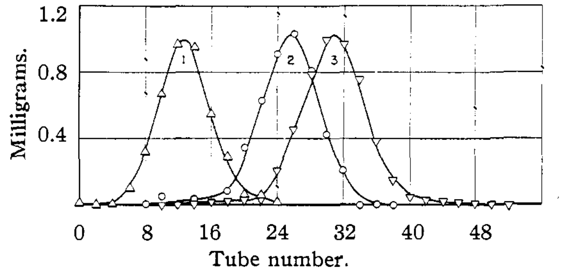

Fig. 4.—Separation of xylenols by 53-plate distribution: Δ, curve 1, 3,5-xylenol; O, curve 2, 2,5-xylenol; ∇, curve 3, 2,4-xylenol.

An experiment using similar quantities and solvent pair was conducted with a mixture of *o*-, *m*- and *p*-ethylphenols. For determination of each compound, optical densities were measured at wave lengths of 272, 279 and 285 mμ.[11]

A plot of these calculated amounts is given in Fig. 5. It may be observed that the *o*-ethylphenol separates easily from the *meta* and *para* isomer. This is so because the ortho compound has a higher partition coefficient and greater *pK* than the other isomers.[7]

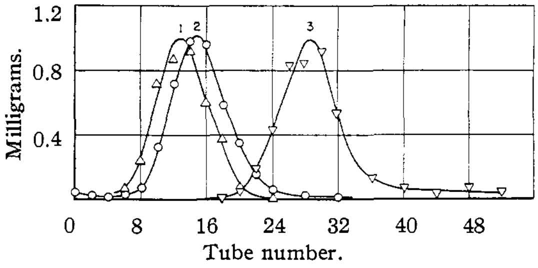

Fig. 5.—Separation of ethylphenols by 53-plate distribution: Δ, curve 1, *m*-ethylphenol; O, curve 2, *p*-ethylphenol; ∇, curve 3, *o*-ethylphenol.

For both xylenols and ethylphenols, the isomers separate in order of decreasing acid strength. When dealing with a material suspected of containing a mixture of isomeric phenols, important clues concerning molecular orientation could be obtained from the countercurrent distribution curves by identifying one component and then applying previously observed correlations between molecular structure on the one hand and partition coefficients and ionization constants on the other.

Acknowledgment.—The author is indebted to George Goldbach for technical assistance.

Summary

Application of the method of countercurrent distribution to the separation of isomeric phenols is described. The method was also used successfully for establishing purity of phenols.

CENTRAL EXPERIMENT STATION
DEPARTMENT OF INTERIOR
U. S. BUREAU OF MINES
PITTSBURGH, PA.
RECEIVED FEBRUARY 12, 1949

[CONTRIBUTION FROM THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS]

Pyridines. IV. A Study of the Chichibabin Synthesis[1]

BY ROBERT L. FRANK AND RAYMOND P. SEVEN

The condensation of aldehydes, ketones, α,β-unsaturated carbonyl compounds or various derivatives of such compounds with ammonia or its derivatives to form substituted pyridines is one of the oldest of organic reactions.[2] The transformations involved, as studied extensively by Chichibabin and his co-workers,[3] can be regarded as aldol condensations, generally in conjunction with Michael-type reactions, and ring closures involving ammonia.

Because of the formation of mixtures of pyridines and various by-products, the method has had a poor reputation for synthetic purposes.[4] Indeed, in all of Chichibabin's reports, there are but two instances[3a,b] of yields of single products higher than 20%. Some notable exceptions,[5,6]

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[1] For the previous communication on pyridine chemistry, see Frank and Weatherbee, THIS JOURNAL, **70**, 3482 (1948).

[2] Hübner and Geuther, *Ann.*, **114**, 45 (1860).

[3] (a) Chichibabin, *Bull. soc. chim.*, [5] **4**, 1826 (1937); (b) Chichibabin, *J. Russ. Phys.-Chem. Soc.*, **37**, 1229 (1905); *Chem. Centr.*, **77**, I, 1438 (1906); (c) Chichibabin and co-workers, *J. prakt. Chem.*, **107**, 109, 122, 129, 132, 138, 145, 154 (1924).

[4] See Sidgwick, "Organic Chemistry of Nitrogen," Oxford University Press, 1937, p. 518.

[5] (a) Farbenwerke vorm. Meister Lucius u. Brüning, British Pat. 146,869 (July 5, 1920); (b) Frank, Blegen, Dearborn, Myers and Woodward, THIS JOURNAL, **68**, 1368 (1946).

[6] Stitz, *Oesterr. Chem. Z.*, **45**, 159 (1942); *C. A.*, **38**, 2040 (1944).