

compromise between fraction purity and analysis time and could be readjusted either way. The specified conditions are optimum for samples with carboxylic acid concentrations of 1% or higher; a more gradual gradient slope and/or a lower concentration of tetramethylammonium hydroxide in the mobile phase may be desirable for samples containing $\leq 0.2\%$ acids (15). Also, optimization of the method could potentially be sample dependent. For example, as pointed out in the Results and Discussion section, other acidic compound classes represent the most difficult types to resolve from carboxylic acids. Thus, samples containing high levels of phenols, for example, might require a slightly modified solvent program—assuming that the presence of phenols would interfere with the ultimate use of the carboxylic acid concentrate.

Rapidization of the Method, Future Work. In this work, relatively large quantities of oil were separated; then tetramethylammonium hydroxide was extracted from acid concentrates to enable gravimetric analysis; after which, portions of each concentrate were esterified separately and ultimately analyzed by GC and other methods.

On the other hand, rapid screening of large numbers of oils would require taking a number of shortcuts. Thus, a much more rapid system would be comprised of an analytical scale HPLC, a fraction collector, and a GC or GC/MS. First, relatively small amounts of filtered oil (≤ 1 g) would be separated by HPLC under conditions analogous to those used here. During HPLC separation, the majority of the oil could be directed to waste and only the carboxylic acids would need to be collected. Solvent removal (a few milliliters) could be accomplished by any of several methods and the resulting concentrate injected directly into the GC. Tetramethylammonium hydroxide pyrolyzes in GC injection ports and methylates carboxylic acids (16); hence, a separate derivatization step may not be necessary. Such a system would take study of carboxylic acids in petroleum out of the realm of a strictly research and development type project. As stated in the Introduction, that is the ultimate goal of this work.

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LITERATURE CITED

- (1) Lochte, H. L.; Littman, E. R. "The Petroleum Acids and Bases"; Chemical Publishing Co.: New York, 1955.
- (2) Selfert, W. K. In "Progress in the Chemistry of Organic Natural Products"; Herz, W., Grisebach, H., Kirby, G. W., Eds.; Springer-Verlag: New York, 1975; Vol. 32, pp 1-49.
- (3) Schmitter, J. M.; Arpino, P.; Guiochon, G. *J. Chromatogr.* **1978**, *167*, 149-158.
- (4) Amat, M.; Arpino, P.; Orritt, J.; Lattles, A.; Guiochon, G. *Analisis* **1980**, *8*, 179-184.
- (5) Stalling, D. L.; Petty, J. D.; Smith, L. M. *J. Chromatogr. Sci.* **1981**, *19*, 18-26.
- (6) Green, J. B.; Grizzle, P. L. In "Trace Analysis"; Lawrence, J. F., Ed.; Academic Press: New York, 1982; Vol. 2, pp 223-265.
- (7) Thenot, J. P.; Horning, E. C.; Stafford, M.; Horning, M. G. *Anal. Lett.* **1972**, *5*, 217-227.
- (8) McKay, J. F.; Cogswell, T. E.; Weber, J. H.; Latham, D. R. *Fuel* **1975**, *54*, 50-61.
- (9) Jewell, D. M.; Weber, J. H.; Bunger, J. W.; Plancher, H.; Latham, D. R. *Anal. Chem.* **1972**, *44*, 1391-1395.
- (10) Green, J. B.; Hoff, R. J.; Woodward, P. W.; Stevens, L. L. *Fuel* **1984**, *63*, 1290-1301.
- (11) Budzikiewicz, H.; Wilson, J. M.; Djerassi, C. *J. Am. Chem. Soc.* **1963**, *85*, 3688-3700.
- (12) Cyr, T. D.; Strausz, O. P. *J. Chem. Soc., Chem. Commun.* **1983**, 1028-1030.
- (13) Chong, S.-L.; McKay, J. F. *Fuel* **1984**, *63*, 303-309.
- (14) Dooley, J. E.; Thompson, C. J.; Hirsch, D. E.; Ward, C. C. *Hydrocarbon Process.* **1974**, *53* (4), 93-100.
- (15) Green, J. B., submitted to *J. Chromatogr.*
- (16) Darbre, A. In "Handbook of Derivatives for Chromatography"; Blau, K., King, G. S., Eds.; Heyden: Philadelphia, PA, 1978; pp 57-59.

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Isolation and Determination of Hydroxylated Nitrogen Heterocycles in a Coal Liquid

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Polycyclic aromatic compounds containing both oxygen and nitrogen heteroatoms (specifically the hydroxylated nitrogen heterocycles) in a solvent-refined coal liquid were separated by use of a two-step column adsorption chromatographic method on neutral alumina and silicic acid and were identified and quantified by capillary column gas chromatography and gas chromatography/mass spectrometry. Among the numerous isomers detected, a number of compounds were positively identified for the first time in coal liquids by comparison of the retention data with available and newly synthesized standard compounds. Hydroxylated indoles, quinolines, carbazoles, and acridines predominated among the compound types found in this sample.

Numerous studies have been undertaken in the past to identify the heterocyclic compounds in coal-derived products. In most cases, compounds containing only one heteroatom were considered. However, there is evidence for the existence of polycyclic aromatic compounds (PAC) containing two heteroatoms in coal-derived products (1-5); compounds containing combinations of sulfur, nitrogen, and oxygen have been only tentatively identified by high-resolution mass spectrometry.

In our previous work (6, 7), nitrogen/sulfur-containing PAC, and oxygen/sulfur-containing PAC were identified for the first time in a solvent-refined coal liquid by capillary column gas chromatography and gas chromatography/mass spectrometry

(GC/MS). The major nitrogen/sulfur-containing PAC in the coal liquid were aminodibenzothiophenes and alkylated aminodibenzothiophenes. All isomers of the aminodibenzothiophenes were detected. The 2- and 3-aminodibenzothiophenes demonstrated 10 to 100 times greater mutagenicity in the Ames test than benzo[*a*]pyrene (6). The major oxygen/sulfur-containing PAC in this sample were hydroxybenzothiophenes, hydroxyphenylthiophenes, hydroxydibenzothiophenes, and hydroxyphenylbenzothiophenes (7).

The PAC containing two heteroatoms are usually less abundant in coal liquids than are those compounds with one heteroatom. Nevertheless, their characterization is important for the study of coal structure, coal processing, and the health and environmental aspects of coal utilization.

In order to identify compounds such as the PAC containing two heteroatoms, it was necessary to isolate or concentrate these compounds in select fractions or to use selective detection. Since it was difficult to separate the nitrogen/sulfur-containing PAC, and oxygen/sulfur-containing PAC fractions, a sulfur-selective detector was applied to the nitrogen- and hydroxy-PAC fractions in our previous work. Selective stationary phases and derivatization methods were also used. Complementary information for identification purposes was provided by GC/MS.

Oxygen is known to be present in coal in several structural forms including ether, furan, carbonyl, carboxylic acid, and hydroxyl groups, but the hydroxyl group is the major form (8). Previous studies have indicated the presence of hydroxylated nitrogen heterocycles in coal-derived products (9–11) and petroleum products (12–15). Bodzek and Marzec (11) extracted a high-volatile bituminous coal with various organic solvents, and reported high-resolution MS evidence for possible structures in the extract including hydroxyquinolines, hydroxycarbazoles, hydroxyacridines, and hydroxyazapyrenes. Jewell and Hartung tentatively identified hydroxyhexahydrocarbazoles and hydroxyindolines in hydrogenated furnace oil by gas-liquid chromatography, UV-absorbance spectrometry, and mass spectrometry (12), and hydroxybenzoquinolines in heavy gas oil by paper chromatography, infrared spectrometry, and titrimetry (13). However, no positive identification and quantification of individual species have been reported.

In this study, capillary column gas chromatography with nitrogen-selective detection was applied to the hydroxylated nitrogen-PAC fraction, and a number of compounds were positively identified for the first time by comparison with the retention times of available and newly synthesized standard compounds. A selective stationary phase, a biphenyl polysiloxane (16), and GC/MS were also used.

EXPERIMENTAL SECTION

A solvent-refined coal heavy distillate (SRC II HD, 260–450 °C boiling point range) was collected during the processing of a West Virginia coal from the Pittsburgh Seam, and obtained from the Fort Lewis, Washington, pilot plant which was operated by the Pittsburgh and Midway Coal Mining Co. This material was of pilot plant origin and should not necessarily be considered as representative of products that may eventually be produced on a commercial scale. The standard reference compounds used in this study were purchased (Aldrich Chemical Co.) or synthesized in our laboratories (17).

A schematic diagram of the separation method is shown in Figure 1. The SRC II HD was first fractionated into chemical classes by column adsorption chromatography on neutral alumina as previously described (18). Approximately 50 mg of the fourth fraction (hydroxylated PAC) was adsorbed onto 0.5 g of silicic acid (Mallinckrodt No. 2847, 100-mesh standardized to 5% H₂O), and the solvent was removed by stirring the mixture under dry nitrogen gas. A 22 mm i.d. glass column was packed using an *n*-hexane slurry of 2 g of silicic acid. The silicic acid on which the sample was adsorbed was then added to the top of the 2-g

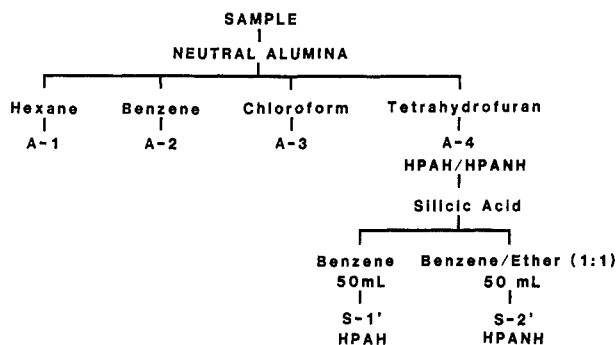


Figure 1. Scheme for the separation of hydroxylated nitrogen heterocycles (HPANH) from synthetic fuel products.

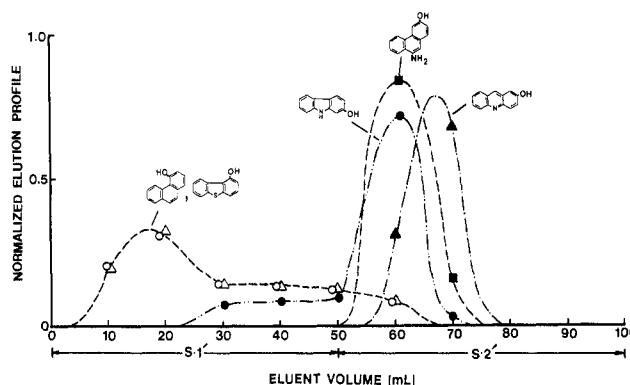


Figure 2. Elution profiles of selected hydroxylated PAC on silicic acid adsorption chromatography columns.

column and two fractions were obtained as follows: fraction S-1', hydroxy polycyclic aromatic hydrocarbons (HPAH) and hydroxy polycyclic aromatic sulfur heterocycles (HPASH) with 50 mL of benzene; fraction S-2', hydroxy polycyclic aromatic nitrogen heterocycles (HPANH) with 50 mL of 1:1 (v/v) benzene/ethyl ether.

A Hewlett-Packard Model 5880 gas chromatograph equipped with a flame ionization detector (FID) and nitrogen-selective thermoionic detector (NPD) was used for gas chromatographic analysis in this study. Sample injection was made in the splitless mode, the helium carrier gas was set at a linear velocity of 50 cm s⁻¹, and the selectivity of the NPD was 1:2000 for naphthalene/4-hydroxyindole.

Capillary columns were prepared by statically coating fused silica capillary tubing (20 m × 0.32 mm i.d., Hewlett-Packard, Avondale, PA) with a 25% biphenyl methylpolysiloxane (0.25 μm film thickness). This phase (16) was cross-linked using azo-*t*-butane (19), and conditioned overnight at 280 °C under nitrogen gas flow.

Compounds were identified by comparison of retention times of the pure standards with the retention times of components in the coal liquid S-2' fraction and GC/MS. A Hewlett-Packard 5982A GC/MS system was operated in the electron impact mode at 70 eV. Spectra were acquired and processed with an HP 5934A data system. Semiquantitation of the HPANH was accomplished by comparing sample component peak areas with those of the standard references, 4-hydroxyindole and 2-hydroxycarbazole.

RESULTS AND DISCUSSION

Prefractionation of coal-derived products is generally required for detailed characterization of individual components. The separation and identification of the nitrogen-containing PAC (N-PAC) in synfuels has been recently reviewed by Later (20). Current methods for isolating the N-PAC fraction employ liquid-liquid or liquid-solid chromatography, or a combination of both separation techniques. The N-PAC fraction was recently isolated on neutral alumina and further separated into secondary nitrogen heterocycles (2°-PANH), amino polycyclic aromatic hydrocarbons (APAH), and tertiary nitrogen heterocycles (3°-PANH) by silicic acid adsorption chromatography (18, 21, 22). The separation primarily followed

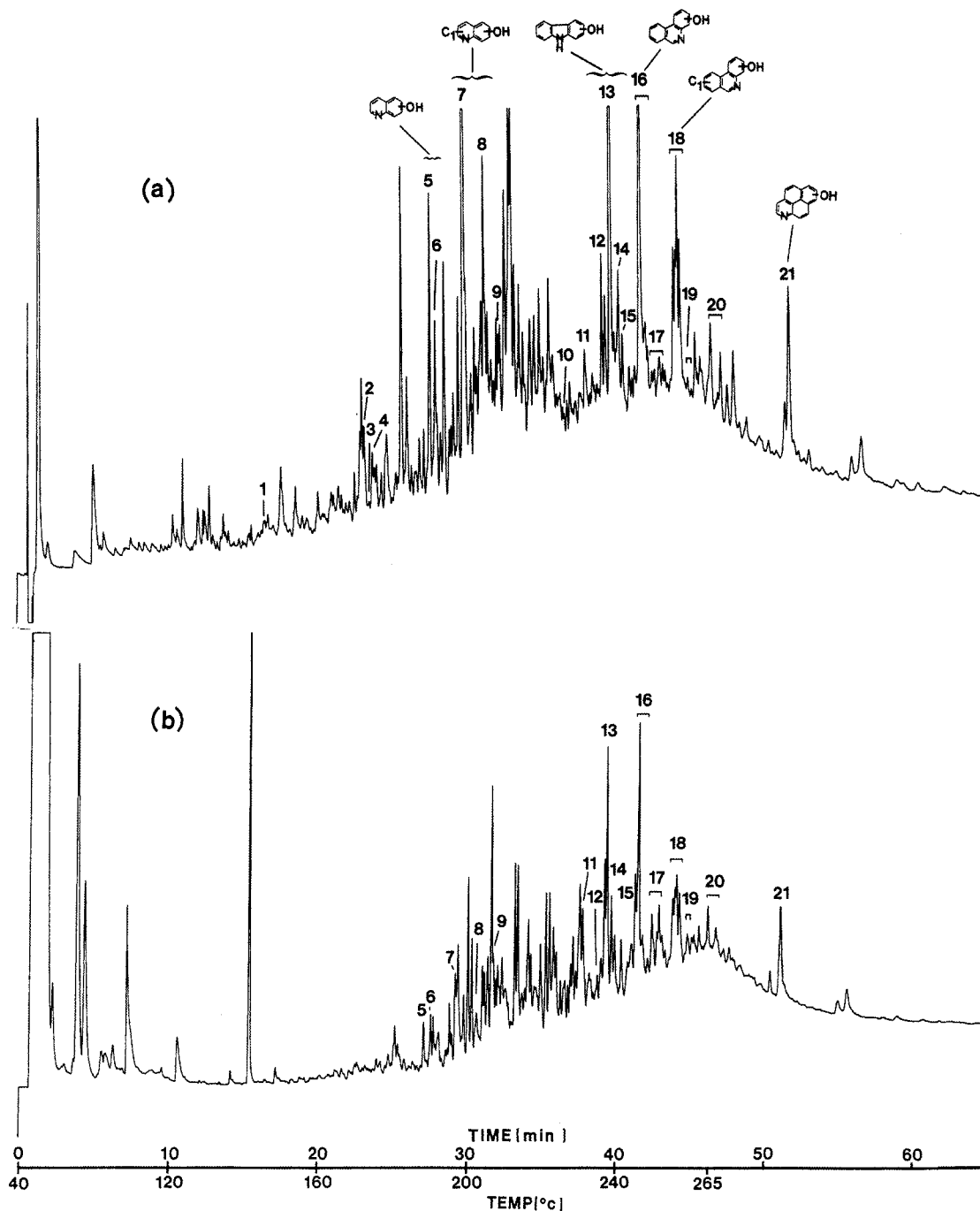


Figure 3. Gas chromatograms of the HPANH fraction of an SRC II HD using (a) NPD and (b) FID detection: column, 20 m \times 0.32 mm i.d. fused silica column coated with a 25% biphenyl polysiloxane; temperature programmed from 40 $^{\circ}$ C to 265 $^{\circ}$ C at 4 $^{\circ}$ C min^{-1} after an initial 2-min isothermal period; helium carrier gas at 50 cm^{-1} . Peak numbers refer to compounds listed in Table I.

compound basicity, with the more basic compounds demonstrating greater retention.

Since it was thought that the HPANH could also be separated and classified according to the basicity of the nitrogen functionality after the hydroxyl group was blocked, the isolation of the HPANH was first attempted by column adsorption chromatography on neutral alumina and silicic acid in the same way as the method of Later et al. (18) after forming the trimethylsilyl derivatives. These derivatives were, however, hydrolyzed during the chromatographic fractionation steps and could not be recovered quantitatively.

However, the HPANH could be separated from the HPAH and HPASH on silicic acid alone when the proper eluent was used. The application of the method reported here to a selection of standard compounds is shown in Figure 2. A small portion of the secondary nitrogen-containing HPAH eluted in the S-1' fraction, but nearly all of the HPANH eluted later

Table I. Recoveries of Selected Hydroxylated PAC by Column Adsorption Chromatography on Silicic Acid

compound	% recovered	
	S-1'	S-2'
4-hydroxyphenanthrene	70-80	0-5
1-hydroxydibenzothiophene	70-80	0-5
2-hydroxycarbazole	10-15	65-75
3-hydroxy-9-aminophenanthrene	0	75-85
2-hydroxyacridine	0	75-85

in the S-2' fraction with a more polar solvent. The elution order of these HPANH is in accordance with the basicity of the nitrogen in the HPANH. The secondary nitrogen heterocycles are slightly acidic, while the amino nitrogens and the tertiary heterocyclic nitrogens are basic due to their lone pairs of electrons. However, the basicity of the tertiary ni-

Table II. Compounds Identified and Quantified in an SRC II Heavy Distillate Coal Liquid

peak no.	compound	rel retention ^a	method of identification ^b	concn, ^c $\mu\text{g/g}$
1	8-hydroxyquinoline	0.554	GC	0.03
	5-hydroxyindole	0.622		
2	4-hydroxyindole	0.840	GC	0.37
3	5-hydroxyisoquinoline	0.858	GC	0.02
4	2-methyl-8-hydroxyquinoline	0.863	GC	0.19
5	1-hydroxyisoquinoline	1.018	GC, GC/MS	1.4
6	2-hydroxyquinoline	1.042	GC, GC/MS	0.85
7	methylhydroxyquinolines and/or aminohydroxynaphthalenes		GC/MS	5.4
8	methylhydroxyquinolines and/or aminohydroxynaphthalenes		GC/MS	0.99
9	2-hydroxy-4-methylquinoline	1.240	GC	0.23
10	4-hydroxyquinoline	1.451	GC	0.03
11	1-hydroxycarbazole	1.497	GC	0.63
	2-methyl-4-hydroxyquinoline	1.500		
12	4-hydroxycarbazole	1.575	GC, GC/MS	0.76
	4-hydroxyacridine	1.599		
	2-hydroxyacridine	1.599		
13	hydroxycarbazoles		GC/MS	13
14	3-hydroxycarbazole	1.618	GC, GC/MS	0.89
15	2-hydroxycarbazole	1.637	GC, GC/MS	0.51
16	hydroxybenzoquinolines		GC/MS	
17	C ₁ -hydroxycarbazoles		GC/MS	
18	C ₁ -hydroxybenzoquinolines and/or hydroxyaminophenanthrenes and/or hydroxyaminoanthracenes		GC/MS	
19	C ₂ -hydroxycarbazoles		GC/MS	
20	C ₂ -hydroxybenzoquinolines and/or C ₁ -hydroxyaminophenanthrenes and/or C ₁ -hydroxyaminoanthracenes		GC/MS	
21	hydroxyazapyrenes and/or hydroxyazafluoranthenes		GC/MS	1.4

^aRetention time relative to phenanthrene. These values are the average of two measurements. Both measurements agreed to within ± 0.004 . ^bGC, capillary column gas chromatography; GC/MS, capillary column gas chromatography/mass spectrometry. ^cApproximate concentration in $\mu\text{g/g}$ in the crude SRC II heavy distillate.

trogen is greater than the amino group, and the former are more strongly retained. The recoveries of each compound by this fractionation method are given in Table I. Recovery losses seem to stem mainly from irreversible adsorption onto glassware and column packings.

The application of this method to a coal liquid, the SRC II HD, produced for the first time an isolated chemical class fraction of HPANH. Capillary column gas chromatograms of the S-2' fraction of the SRC II HD using both nitrogen-selective and universal detection are shown in Figure 3. Comparison of these two chromatograms indicates the quality of the HPANH enrichment scheme developed in this study.

The compounds labeled as peaks in the chromatograms of the HPANH fraction (Figure 3) were identified by GC/MS and by comparison of retention data with those of available standard samples. Table II lists the compounds identified as well as other available standard compounds which were not found to be present. The relative retention and semiquantitative data for these compounds are also included. A number of compounds were positively identified for the first time, including all hydroxycarbazole isomers. Mass spectral evidence for the presence of hydroxyazapyrenes or hydroxyazafluoranthenes in this sample was also found. Since the aminohydroxynaphthalenes have the same molecular weight as the methylhydroxyquinolines, mass spectral data alone are insufficient for more than tentative identification of these compounds. This situation occurs again with the corresponding three-ring HPANH. Hydroxylated 3°-PANH such as the hydroxyquinolines, hydroxyacridines, and hydroxyazapyrenes were found to be major components of this sample. Nitrogen-containing compounds in coal-derived liquids were found to be largely aza heterocycles (18, 23). Carbazole, benzoquinoline, and their alkylated derivatives were the major N-PAC in the SRC II HD. Likewise, hydroxycarbazoles, hydroxybenzoquinolines, and their alkylated derivatives were the predominant HPANH in this coal liquid. As mentioned

above, hydroxyquinolines and hydroxycarbazoles were tentatively identified in a high-volatile bituminous coal; but in this work, several hydroxyquinoline and hydroxyisoquinoline isomers, and all of the hydroxycarbazole isomers were positively identified by comparison with the retention times of standard compounds.

The HPANH were present at the 1–10 ppm level in this coal liquid, which was comparable to the observed levels of other PAC which contain two heteroatoms, such as the aminodibenzothiophenes and the hydroxylated thiophenic compounds (6, 7). In comparison, the corresponding parent N-PAC were found to be present at the approximate 100–1000 ppm level (18, 21).

LITERATURE CITED

- (1) McKay, J. F.; Weber, J. H.; Latham, D. R. *Anal. Chem.* **1976**, *48*, 891–898.
- (2) Paudler, W. W.; Cheplen, M. *Fuel* **1979**, *58*, 775–778.
- (3) Hunt, D. F.; Shabanowitz, J. *Anal. Chem.* **1982**, *54*, 574–578.
- (4) Burchill, P.; Herod, A. A.; Prichard, E. *J. Chromatogr.* **1982**, *242*, 65–76.
- (5) Burchill, P.; Herod, A. A.; Prichard, E. *Fuel* **1983**, *62*, 20–29.
- (6) Nishioka, M.; Campbell, R. M.; West, W. R.; Smith, P. A.; Booth, G. M.; Lee, M. L.; Kudo, H.; Castle, R. N. *Anal. Chem.* **1985**, *57*, 1868–1871.
- (7) Nishioka, M.; Lee, M. L.; Kudo, H.; Muchiri, D. R.; Baldwin, L. J.; Pakray, S.; Stuart, J. G.; Castle, R. N. *Anal. Chem.* **1985**, *57*, 1327–1330.
- (8) Speight, J. G. In "The Chemistry and Technology of Coal"; Marcel Dekker: New York, 1983; pp 313–315.
- (9) Pichler, V. H.; Herlan, A. *Erdol Kohle, Erdgas, Petrochem.* **1973**, *26*, 401–407.
- (10) Bodzek, D.; Krzyzanowska, T.; Marzec, A. *Fuel* **1979**, *58*, 196–202.
- (11) Bodzek, D.; Marzec, A. *Fuel* **1981**, *60*, 47–51.
- (12) Jewell, D. M.; Hartung, G. K. *Proc. Am. Pet. Inst.* **1962**, *42*, 92–94.
- (13) Jewell, D. M.; Hartung, G. K. *J. Chem. Eng. Data* **1964**, *9*, 297–304.
- (14) Snyder, L. R. *Anal. Chem.* **1969**, *41*, 314–323.
- (15) Peter, A. W.; Bendoraitis, J. G. *Anal. Chem.* **1976**, *48*, 968–973.
- (16) Kuei, J. C.; Shelton, J. I.; Castle, L. W.; Kong, R. C.; Richter, B. E.; Bradshaw, J. S.; Lee, M. L. *HRC CC, J. High Resolut. Chromatogr. Chromatogr. Commun.* **1984**, *7*, 13–18.
- (17) Castle, R. N., unpublished results.
- (18) Later, D. W.; Lee, M. L.; Bartle, K. D.; Kong, R. C.; Vassilaros, D. L. *Anal. Chem.* **1981**, *53*, 1612–1620.

- (19) Wright, B. W.; Peaden, P. A.; Lee, M. L.; Stark, T. J. *Chromatogr.* **1982**, *248*, 17-34.
- (20) Later, D. W. In "Handbook of Polycyclic Aromatic Hydrocarbons"; Bjorseth, A., Ramdahl, T. Eds.; Marcel Dekker: New York, Vol. 2, in press.
- (21) Later, D. W.; Lee, M. L.; Wilson, B. W. *Anal. Chem.* **1982**, *54*, 117-123.
- (22) Later, D. W.; Andros, T. G.; Lee, M. L. *Anal. Chem.* **1983**, *55*, 2126-2132.
- (23) Burchill, P.; Herod, A. A.; Prichard, E. J. *Chromatogr.* **1982**, *246*, 271-295.

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Resolution of Ortho, Meta, and Para Isomers of Some Disubstituted Benzene Derivatives via α - and β -Cyclodextrin Inclusion Complexes, Using Reversed-Phase High-Performance Liquid Chromatography

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Separation of various disubstituted benzene derivatives on a LiChrosorb RP18 column, with mobile phases containing α - or β -cyclodextrin, was systematically studied. The model compounds tested were cresols, fluoronitrobenzenes, chloronitrobenzenes, bromonitrobenzenes, iodinitrobenzenes, nitroanilines, nitrophenols, and dinitrobenzenes. Ethanol, 20 vol %, was used as an additional component of the aqueous mobile-phase solution. The course of the relationship of the apparent capacity factor vs. cyclodextrin (CD) concentration was consistent with that resulting from simple theoretical considerations only in the case of β -CD. This permitted evaluation of the capacity factors of β -CD complexes, as well as of their stability constants. It was found that only β -CD imposes a distinct selectivity toward ortho, meta, and para isomers on RP systems, thus enabling their complete separation in all cases studied.

Cyclodextrins (CDs) are torus-shaped cyclic oligosaccharides made up of six or more α -1,4-linked D-glucopyranose units. The nonpolar central cavity of CDs can selectively include various inorganic and/or organic species of neutral or ionic nature (1). These inclusion processes are influenced mainly by hydrophobicity and shape of guest molecules, i.e., by the fit of the complexed molecule to the CD cavity. Hence CD complexation is a procedure of choice for separation of isomers. It has been used to advantage in classical liquid chromatography (2, 3). However, the columns usually containing polymers with incorporated CD molecules are of very low efficiency, owing to the complex mechanism of sorption involving both gel permeation and molecular inclusion. CDs dissolved in the mobile phase have also been used in thin-layer chromatography with polyamide stationary phase (4, 5).

The CD inclusion phenomena have recently been utilized for separation of disubstituted benzene isomers by high-performance liquid chromatography (HPLC) in two ways: by using chemically bonded α - and/or β -CD-silica stationary phases (6-10) and by applying CDs as the mobile phase components in reversed-phase systems (11, 12).

Studies dealing with the latter method have concerned only three nitrobenzoic (11) and three nitrocinnamic acids (12). The present paper reports the results of further systematic

research on resolution of various disubstituted benzene derivatives of acidic, basic, or neutral nature. The work was aimed at establishing the general rules similar to those valid in clathrate chromatography (13, 14) which relate the shape and size of molecules to their chromatographic behavior in reversed-phase systems containing α - or β -CD in the mobile-phase solution.

EXPERIMENTAL SECTION

Reagents. α - and β -CD were supplied by Chinoin (Budapest, Hungary) and were purified by recrystallization from water. Methanol (MeOH), ethanol (EtOH), 1-propanol (1-PrOH), 2-propanol (2-PrOH), and tetrahydrofuran (THF) were of p.a. grade. Water was purified by double distillation and ion exchange treatment.

The following ortho-, meta-, and para-disubstituted benzene derivatives were used as solutes: cresols, fluoronitrobenzenes, chloronitrobenzenes, bromonitrobenzenes, iodinitrobenzenes, nitroanilines, nitrophenols, and dinitrobenzenes. All aromatic compounds purchased from various suppliers were of analytical or reagent grade and were used without further purification.

Apparatus and Procedure. Chromatographic measurements were performed with a Type 302 apparatus (Institute of Physical Chemistry, Polish Academy of Sciences, Warsaw, Poland) equipped with a 5- μ L high-pressure injection valve and with a UV detector (254 nm) with Z-shaped passage (volume 8 μ L).

Stainless steel columns (100 \times 4.6 mm i.d. and 150 \times 4.6 mm i.d.) were packed with 10- μ m LiChrosorb RP18 (E. Merck, Darmstadt, FRG) by the viscosity technique. All chromatographic experiments were carried out at constant flow rate of 2.4 mL/min and constant temperature of 25 \pm 0.1 $^{\circ}$ C (using a water jacket), unless specified otherwise.

The mobile phases consisted of aqueous solutions containing 20 vol % organic solvents and α -CD at concentrations of 0.2 \times 10⁻², 0.5 \times 10⁻², 1.0 \times 10⁻², 1.5 \times 10⁻², and 3.0 \times 10⁻² M or β -CD at concentrations of 0.2 \times 10⁻², 0.5 \times 10⁻², 1.0 \times 10⁻², 1.5 \times 10⁻², 2.0 \times 10⁻², and 2.5 \times 10⁻² M. The ethanolic solutions of solutes (5.0 \times 10⁻³ M) were injected onto the columns both separately and as mixtures.

k' values were measured on a column (100 \times 4.6 mm i.d.) whose dead volume, determined from NaNO₂ retention (15), was 0.72 mL.

EQUILIBRIA AND EQUATIONS

RP systems containing CD in mobile-phase solutions may involve many species of the solute: neutral, ionic, free, or bound to one or more CD molecules. Consequently the ad-