

CHROMATOGRAPHIC ADSORPTION OF AZOBENZENES
AND AROMATIC NITROGEN HETEROCYCLES ON ALUMINA
STUDIES WITH ALUMINA-IMPREGNATED GLASS PAPER

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ZECHMEISTER¹ suggested that adsorption on a surface occurs through certain atomic groups, known as "anchoring groups" present in the adsorbate molecule. For chromatography of aromatic and conjugated unsaturated hydrocarbons on alumina or on polynitroarene-impregnated silicic acid KLEMM *et al.*^{2,3} have proposed that flatwise adsorption of the substrate is preferred. In such case the entire aromatic system would be considered the anchoring group, though it would seem preferable, rather, to speak of the general π -electronic system of the hydrocarbon as an anchoring site. The adsorption process is believed to involve π -complex formation between the hydrocarbon (electron donor or Lewis base) and the adsorbent (electron acceptor or Lewis acid). For alumina as adsorbent, adsorbability of the substrate was found to increase with increasing number of double bonds, approach to coplanarity, symmetry number, extent of conjugation, and number of sterically unhindered methyl or alkylene groups. Of special interest here are the observations that *trans*-stilbene is adsorbed more tenaciously than its stereoisomer *cis*-stilbene and that amongst groups of conjugated isomeric polyenes the all-*trans* isomers are adsorbed most tenaciously.

In contrast to the case of the stilbenes, it has been noted by COOK⁴ and by ZECHMEISTER *et al.*⁵ that *cis*-azobenzene and a number of its derivatives are adsorbed more strongly on alumina than are their respective *trans* isomers. Moreover, for the geometric isomers of 1,4-bis-(phenylazo)-benzene the order of adsorbability is reported to be *cis-trans* > *cis-cis* > *trans-trans*⁶. Since molecular models indicate that corresponding geometric isomers involving the azo -N=N- and the vinylene -CH=CH- linkages maintain closely similar spatial geometries, it would seem that the non-coplanar azo compounds contain an anchoring site which fosters adsorbability on alumina to a considerably greater extent than does that of the conjugated π -electronic system. We report here some additional studies between adsorbabilities in the azo-benzene-stilbene series and the arene-nitrogen heterocycle series, as well as an interpretation of differences observed for these analogous compounds.

EXPERIMENTAL

Most polynuclear aromatic hydrocarbons used were available in pure form from previous studies². Other substrates were obtained from commercial sources and, if solid, were recrystallized to constant m.p. Quinoline was purified by vacuum distillation. *cis*-Stilbene (Aldrich Chemical Co., Milwaukee, Wis., U.S.A.) was used without further purification. Chromatography on alumina columns was conducted essentially as in previous studies². In many cases, however, analysis of the residues from evaporation of the effluent fractions was performed spectrophotometrically rather than by m.p. determination. Data on these experiments are presented in Table I.

Chromatography on alumina-impregnated glass paper

A sheet (26 × 56 cm) of Schleicher and Schüll (Keene, N. H., U.S.A.) No. 26 pyrex fiberglass paper was clamped rigidly into a stainless steel frame and dipped into a 15% aqueous solution of reagent grade $\text{Al}_2(\text{SO}_4)_3$ where it was left for 1 h. It was withdrawn, allowed to drain for 10 min, dipped into 10% NH_4OH for 20 min, and drained for 10 min more. A second and a third dipping (10 min in each solution, 10 min for drainage between solutions) was also made in order to give a more nearly uniform layer of aluminum hydroxide of appropriate thickness. Immersion and withdrawal of the paper was made slowly so as to prevent tearing it. After impregnation of several sheets of paper, concentrations of the impregnant solutions were checked by titration⁷. The impregnated paper was washed by immersion in water for 15–30 min and then dried at 60° for at least 2 h (until dry to the touch). The paper (handled only with clean rubber surgical gloves) was removed from the frame and heated at 250–290° for at least 4 h. The hot paper was trimmed to about 20 × 48 cm and stored flat on a sheet of aluminum in a dry box (containing anhydrous CaCl_2) until cool. Just before use it was removed from the box, ruled off lengthwise in centimeters (by means of a lead pencil) and with the zero line about 15 cm from one end and reheated at 290° for 10 min. Small spots (2 μl) of $1-2 \cdot 10^{-2} M$ solutions of strongly fluorescent compounds in volatile solvents (ethanol, benzene, or ethyl acetate) were placed 2.5–4 cm apart on the zero line. The solvents were evaporated rapidly by means of a blast of hot air. The paper was transferred to a closed pyrex jar arranged for descending chromatography where it was conditioned in vapors of the developer (anhydrous benzene, reagent grade cyclohexane or a benzene–isooctane mixture) for 12–24 h. To prevent cracking of the brittle impregnated paper it was draped over a 3.5 cm (diameter) glass tube inserted adjacent to the solvent trough. Development of the chromatogram was determined as a function of time using direct observation against a daylight or incandescently lighted background for locating the solvent front and fluorescence in ultraviolet light (furnished by an external 100-W mercury arc source) against a dark background for locating the spots. Runs lasted 1–4 h. Selected linear plots of the centers of the spots (taken as the average of the positions of leading and following boundaries) *versus* the position of the solvent front for three different runs are shown in Figs. 1–3. It should be noted, however, that sets of curves obtained from repetitive

TABLE I
RELATIVE CHROMATOGRAPHIC ADSORBILITIES OF SELECTED COMPOUNDS ON ALUMINA COLUMNS

Run	Components used in mixture*		Eluent	Effluent fraction			Extent of separation of components	
	name	m.p. °C		No.**	mg of residue	m.p. °C		special analytical procedure
1	<i>cis</i> -Stilbene	< 25	petrol ether (30-60°)	1	82	< 25	IR (liq. between NaCl plates) visual observation	<i>ca.</i> pure <i>cis</i> -stilbene
	<i>trans</i> -Azobenzene	68-69		2	43	64-69		yellow
2	<i>trans</i> -Stilbene	123-125	spectral grade iso-octane	1	19	not determined	spectral absorption at 442 m μ of standard (w/v) solutions in iso-octane	pure <i>trans</i> -stilbene
				2	63			71% <i>trans</i> -stilbene
	<i>trans</i> -Azobenzene	68-69		3	32			63% <i>trans</i> -azobenzene
				6	15			pure <i>trans</i> -azobenzene
3	Anthracene	213-214	petrol ether	1	97	206-210	followed on column by fluorescence eluted with acetone	very good
	Acridine	106-110		last	26	96-100		
4	Anthracene	211-212	10% benzene in petrol ether	1	85	207-211		complete
	Phenanthridine	105-106		last	100	103-104.5		

5	Phenanthrene Phenanthridine	95-97 105-106	petrol ether	1 3	72 47	97-99 104-106	none	fair
6	Acridine Phenanthridine	108-110 105-106	benzene	1 2 3	64 40 59	not determined	IR (20 mg/0.5 ml CHBr ₃) ditto ditto	<i>ca.</i> pure acridine largely acridine largely phenanthridine good
7	Acridine 2-Methylacridine	108.5-110.5 134.5-135.5	benzene	1 3	47 65	93-95.5 103-114	compared m.p. of effluent fraction with standard m.p.-composition curve	<i>ca.</i> 60% acridine <i>ca.</i> 60% 2-methyl- acridine poor
8	Naphthalene Quinoline	78-80 < 25	petrol ether	1 last	86 94	78-80 < 25	eluted with acetone	odor of quinoline complete
9	Dibenzothiophene Phenanthrene	97-98 95-97	petrol ether	1 5	fair size ditto	86-95 90-95	IR (nujol mull) ditto, eluted with acetone	<i>ca.</i> pure dibenzothiophene <i>ca.</i> pure phenanthrene fair
10	Dibenzofuran Dibenzothiophene	83.5-84.5 98-99	petrol ether	1 middle last	fair size very small fair size	83.5-84.5 90-95 97-99	none	very good

* For each run the components are listed in order of appearance in the effluent. 100 mg of each component.

** Numbered in order of appearance.

runs were generally neither superimposable nor linear throughout, though each curve of the set had approximately the same position with respect to the other curves. Also curves from two different spots of the same compound run on one piece of paper were only approximately the same.

It is taken as a basic assumption that adsorbability decreases with increasing rate of movement of a spot on the paper. On this basis other adsorbability relationships found, but not shown in Figs. 1-3, are 2-methylacridine \geq acridine \geq benz(c)acridine.

DISCUSSION

In order to check the consistency of adsorbability data obtained from alumina columns and alumina-impregnated glass papers, some compounds were chromatographed by both methods. The results for a group of six hydrocarbons run on paper simultaneously are shown in Fig. 1. A total of six runs made with these same substrates all showed the adsorbability relationships 2-phenylanthracene \geq 1-phenylanthracene $>$ 9-phenylanthracene in agreement with results previously found on columns². However, in

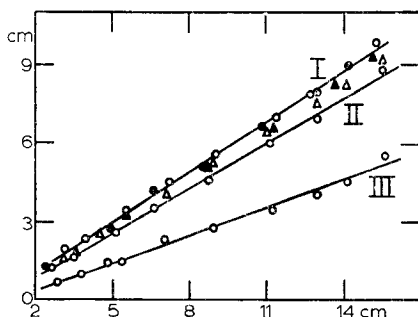


Fig. 1. Vertical axis: distance moved by substrate spot. Horizontal axis: distance moved by solvent front. Solvent: cyclohexane. I: 9-phenylanthracene; II: 1-phenylanthracene; III: 2-phenylanthracene; solid circles: anthracene; solid triangles: 9-methylanthracene; open triangles: pyrene.

non-linear plots the relative positions of anthracene, 9-methylanthracene and pyrene were variable with respect to one another and to 1- and 9-phenylanthracenes (but not to 2-phenylanthracene). It thus appears that the gross differences in adsorbability which were noted on columns were reproduced on paper, but some subtle differences noted in competitive runs on columns², *viz.* 9-methylanthracene $>$ anthracene (good separation), pyrene $>$ anthracene (fair separation) and 9-phenylanthracene $>$ anthracene (fair separation), are not consistently detected on the paper. Consistency was found for adsorbability of the substrate pair 2-methylacridine-acridine, former \geq latter on paper, poor separation on column (*cf.* run 7).

Cyclohexane or isooctane was found to be appropriate for development of the paper chromatogram with aromatic hydrocarbons. Use of benzene as developer was unsatisfactory for these compounds because the rate of movement of the substrates was too large to give sufficient differentiation among them. For the more strongly

adsorbed nitrogen heterocycles (Figs. 2 and 3), however, benzene, benzene–isooctane or benzene–cyclohexane was preferable. Many nitrogen heterocycles are so strongly adsorbed that it is difficult to elute them from columns with non-polar solvents. For these the use of paper appears to be more satisfactory.

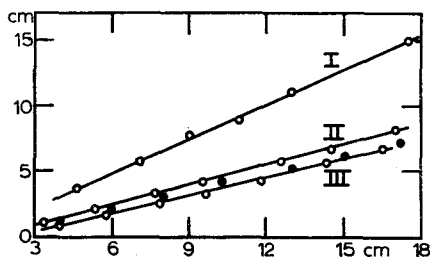


Fig. 2. Vertical axis: distance moved by substrate spot. Horizontal axis: distance moved by solvent front. Solvent: benzene. I: 5,6,11,12-tetraphenylnaphthacene (rubrene); II: acridine; III: benzo(*f*)quinoline; solid circles: benz(*a*)acridine.

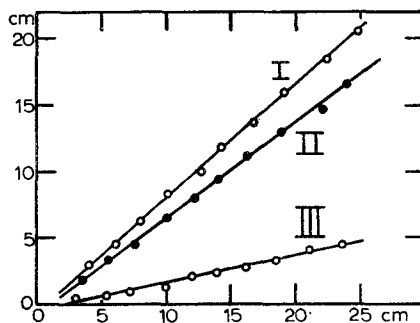


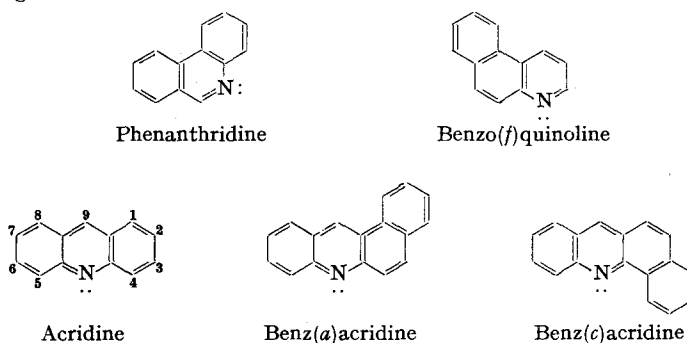
Fig. 3. Vertical axis: distance moved by substrate spot. Horizontal axis: distance moved by solvent front. Solvent: benzene–isooctane (1:1, v/v). I: benz(*a*)anthracene; II: benz(*c*)acridine; III: benz(*a*)acridine.

The amount of substrate used ($2 \cdot 10^{-8}$ moles, *ca.* 4 μ g) in these chromatograms was sufficient to allow observation of only very strongly fluorescent materials. Thus, attenuation caused the pyrene spot to become invisible after 160 min, while chrysenes could not be followed at all for it formed a spot which fluoresced on the dry paper but did not fluoresce as soon as the solvent front had passed through it. Within the limitations imposed by such characteristics of fluorescence of the adsorbates it is apparent that use of impregnated glass paper offers considerable saving in time and materials over use of columns for correlating adsorbability on alumina with structure of the adsorbate.

From results in Table I it is apparent that the Law of Inequalities² holds for chromatography on alumina columns of mixtures of =N— and =CH— analogs in the systems *trans*-azobenzene, *cis*- and *trans*-stilbenes (*cf.* runs 1, 2 and ref.⁸); phenanthridine, acridine, anthracene (*cf.* runs 3, 4 and 6); and phenanthridine, anthracene, phenanthrene (*cf.* runs 4, 5 and ref.⁸). Assuming this law is valid for other compounds

studied one finds the orders of adsorbability *cis*-azobenzene \gg *trans*-azobenzene $>$ *trans*-stilbene \gg *cis*-stilbene and phenanthridine $>$ acridine \gg anthracene $>$ phenanthrene $>$ dibenzothiophene $>$ dibenzofuran. Along with the results that quinoline \gg naphthalene (run 8), and benzo(*f*)quinoline = benz(*a*)acridine = 2-methylacridine \gg acridine \gg benz(*c*)acridine $>$ benz(*a*)anthracene, 5,6,11,12-tetraphenyl-naphthacene (Figs. 2 and 3, run 7) it is clear that replacement of the =CH— group by a =N— group serves to increase adsorbability and may drastically alter the rules (from those found for hydrocarbons) for the effects of structural features in the molecule on adsorbability. It is, moreover, noteworthy that dibenzothiophene and dibenzofuran, compounds containing the same total number of π -electrons as phenanthridine and acridine plus a similar non-bonding pair of electrons (*n*-electrons) on the heteroatom, do not exhibit enhancement of adsorbability (as compared to anthracene and phenanthrene).

It is here proposed that adsorption of azo compounds and aromatic nitrogen heterocycles on alumina may occur by (1) *n*-complex formation involving the alumina surface as electron acceptor and the *n*-electrons on the nitrogen atom as the anchoring site, (2) π -complex formation as described previously for polyenes and arenes, or (3) hybrid πn -complex formation involving both *n*-electrons and π -electrons from the substrate molecule. At least for the molecules considered in this paper it is believed that *n*-complex formation is energetically preferred so long as steric hindrance to the requisite edgewise or tilted orientation of the adsorbate molecule (with respect to the alumina surface—considered flat) is not too great. Thus, it is presumed that *cis*-azobenzene, quinoline, phenanthridine, acridine, 2-methylacridine, benz(*a*)acridine, and benzo(*f*)quinoline form *n*-complexes which are considerably more stable than the π -complexes formed by their hydrocarbon analogs, while *trans*-azobenzene, benz(*c*)acridine, and *trans-trans*-1,4-bis-(phenylazo)-benzene form π -complexes which are only slightly more stable than the corresponding complexes formed by their hydrocarbon analogs.



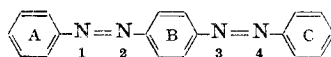
The *n*-electrons of these nitrogen compounds are the most readily polarizable ones in the molecule on the basis of the assignment of the long wavelength electronic absorption band to an *n* \rightarrow π transition⁹.

On the basis of only steric hindrance to *n*-complex formation one would expect

the order of adsorbability phenanthridine, benzo(*f*)quinoline, quinoline (one *peri* hydrogen) > acridine, benz(*a*)acridine (two *peri* hydrogens) \gg benz(*c*)acridine (one *peri* hydrogen and one *peri* CH). The observed order of adsorbability phenanthridine \geq benzo(*f*)quinoline = benz(*a*)acridine \geq acridine \geq benz(*c*)acridine is thus rationalizable largely on such basis. In fact the strong influence of a steric factor is apparent when one notes that phenanthridine is the weakest base of this group of five compounds toward the proton in 50% aqueous ethanol¹⁰ but perhaps the strongest base of the group toward the Lewis acid alumina. The slight enhancement of adsorbability brought about by the insertion of a methyl group into the 2-position of acridine (no added steric hindrance to *n*-complexation) might be ascribed to the inductive effect which would increase electron availability at the nitrogen atom. In this case, as expected, 2-methylacridine is also more basic than acridine toward the proton in 50% ethanol¹⁰.

If π -complexation were involved in adsorption of all of the preceding nitrogen heterocycles one would expect the adsorbability order benz(*a*)acridine \simeq benz(*c*)acridine > 2-methylacridine \geq acridine > phenanthridine \simeq benzo(*f*)quinoline > quinoline. The major difference found between the observed order and this hypothetical one indicates a lack of pertinence of the acene⁸ and unsaturation² rules in this series.

The proposals that benz(*c*)acridine and *trans*-azobenzene form π -complexes rather than weak *n*-complexes cannot be experimentally substantiated at this time. The fact that each is only slightly more strongly adsorbed than its hydrocarbon counterpart, benz(*a*)anthracene or *trans*-stilbene, respectively, would, however, be consistent with π -complexation enhanced to a slight extent by some weak interaction between the alumina surface and the *n*-electrons. Alternatively, *trans*-azobenzene could be adsorbed in a πn -hybrid complex involving the π -system of one benzene ring—held flatwise—and the *n*-electron pair of the adjacent nitrogen atom. Adsorption of *cis*-azobenzene is assumed to occur via *n*-complexation whereby the azobenzene molecule is held edgewise with both pairs of *n*-electrons directed toward the adsorbent surface and the phenyl rings protruding away from the surface. Invoking the preceding concepts one can also rationalize the order of adsorbability of the 1,4-bis-(phenylazo)-benzenes, though he cannot (without additional laboratory investigation) expound a unique stereochemical solution. One possibility is that the *trans-trans* isomer is adsorbed completely flatwise as a π -complex, the *cis-cis* isomer is adsorbed as a πn -hybrid involving the π -system of ring B and the *n*-electrons of nitrogen atoms



2 and 3, and the *cis-trans* isomer is adsorbed as a πn -hybrid involving the π -system of the *trans*-AN₁N₂B moiety and the *n*-electrons of nitrogen atom 3.

The reported differences in products from reaction of styrene¹¹, 2-vinylpyridine¹², and 4-vinylpyridine¹³ with hydrogen sulfide over an Al₂O₃-FeS catalyst at 600° may

be interpreted in terms of the formation of π - (from styrene) or n - (from the vinyl-pyridines) complexes of the corresponding $\text{ArCH}_2\text{CH}_2\text{SH}$ intermediates. When Ar is phenyl the flatwise adsorbed intermediate cyclizes readily to benzothiophene. When Ar is 2-pyridyl, there is mainly edgewise adsorption on the nitrogen atom with little resultant formation of thienopyridine but extensive concomitant splitting of the Ar-C bond. For Ar is 4-pyridyl, neither cyclization nor bond splitting is observed.

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SUMMARY

A semi-quantitative method is described for chromatography of fluorescent polynuclear aromatic hydrocarbons and aromatic nitrogen heterocycles on alumina-impregnated glass paper. Combining data obtained from such papers with those from alumina columns it is noted that the isomeric azobenzenes and nitrogen heterocycles are more strongly adsorbed than their hydrocarbon analogs (where CH replaces N). It is proposed that adsorption of such nitrogen compounds occurs preferentially by means of the electron pairs on the nitrogen atoms to form n -complexes. If steric hindrance to n -complexation is too great the substrate may form a π -complex or a πn -hybrid complex instead.

REFERENCES

- ¹ L. ZECHMEISTER, *Discussions Faraday Soc.*, 7 (1949) 54.
- ² L. H. KLEMM, D. REED, L. A. MILLER AND B. T. HO., *J. Org. Chem.*, 24 (1959) 1468.
- ³ L. H. KLEMM, D. REED AND C. D. LIND, *J. Org. Chem.*, 22 (1957) 739;
L. H. KLEMM AND D. REED, *J. Chromatog.*, 3 (1960) 364.
- ⁴ A. H. COOK, *J. Chem. Soc.*, (1938) 876.
- ⁵ L. ZECHMEISTER, O. FREHDEN AND P. F. JÖRGENSEN, *Naturwiss.*, 26 (1938) 495;
L. ZECHMEISTER, *Ann. N.Y. Acad. Sci.*, 49 (1948) 220.
- ⁶ A. H. COOK AND D. G. JONES, *J. Chem. Soc.*, (1939) 1309.
- ⁷ S. LACROIX, *Analyst*, 73 (1948) 353; *Anal. Chim. Acta*, 1 (1947) 3.
- ⁸ A. WINTERSTEIN AND K. SCHÖN, *Z. physiol. Chem.*, 230 (1934) 146.
- ⁹ M. KASHA, *Discussions Faraday Soc.*, 9 (1950) 14, 72;
A. BURAWOY, *Discussions Faraday Soc.*, 9 (1950) 70, 73;
F. A. MATSEN, *Chemical Applications of Spectroscopy*, Interscience, New York, 1956, pp. 642, 666-667;
R. N. BEALE, *Nature*, 178 (1956) 37;
J. SCHULZE, F. GERSON, J. N. MURRELL AND E. HEILBRONNER, *Helv. Chim. Acta*, 44 (1961) 428.
- ¹⁰ A. ALBERT, R. GOLDACRE AND J. PHILLIPS, *J. Chem. Soc.*, (1948) 2240;
A. ALBERT AND R. GOLDACRE, *J. Chem. Soc.*, (1946) 706.
- ¹¹ R. J. MOORE AND B. S. GREENSFELDER, *J. Am. Chem. Soc.*, 69 (1947) 2008.
- ¹² L. H. KLEMM AND D. REED, *J. Org. Chem.*, 25 (1960) 1816.
- ¹³ C. HANSCH AND W. CARPENTER, *J. Org. Chem.*, 22 (1957) 936.