THE USE OF SOLUBILIZING AGENTS IN PARTITION PAPER CHROMATOGRAPHY

III. THE SEPARATION OF AROMATIC HYDROCARBONS BY SOLUBILIZERS IN MOBILE PHASE*

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INTRODUCTION

The aromatic hydrocarbons are present in molecular complexes as donors of π-electrons[1,2]. The solubilizing properties of some compounds in relation to aromatic hydrocarbons are also ascribed to the formation of these complexes. BROCK *et al.*[3] found that aqueous solutions of purines dissolve the aromatic hydrocarbons to a high degree. WEIL-MALHERBE[4] and BOYLAND AND GREEN[5] have studied the quantitative solubilizing properties of purines, concentrating on cancerogenous hydrocarbons. The ability to solubilize aromatic hydrocarbons is not only a specific characteristic of purines. The formation of aromatic hydrocarbon complexes with bile acids has been described by FIESER AND NEWMANN[6] and the solubilizing effects of sodium deoxycholate have been confirmed by WEIL-MALHERBE[4]. As the solubility of aromatic hydrocarbons in water is very low (not over 10^{-4} g/1000 ml of water at 27°) direct determination of their solubility in water and in aqueous solutions of solubilizing agents is experimentally very demanding and is liable to have a considerable average error[4,5,7,8]. Partition chromatography could offer a rapid method of evaluating solubilizing properties in relation to aromatic hydrocarbons. In the case of chromatography with reversed phases the magnitude of R_F values of aromatic hydrocarbons is proportional to the solubilizing effect of aqueous solutions of solubilizers which represent the mobile phase. This arrangement is useful also for the practical separation of some aromatic hydrocarbons. The ability of aromatic hydrocarbons to form donor–acceptor complexes with polynitro-compounds is also used by some authors[9,10] in thin-layer chromatography of aromatic hydrocarbons. When solubilizers are used, some opium[11] and purine[12] alkaloids can be separated.

In this paper, the solubilizing properties of some N-methylated cyclic ureides, purine derivatives and bile acid salts were studied namely:

(a) N-Methylated cyclic ureides: 1,3-dimethyluracil (DMU); 1,3-dimethylbarbituric acid (DMBA); 1,3,7,9-tetramethyluric acid (TMUA).

(b) Purine derivatives: 7-(2-hydroxyethyl)-theophylline (HETheoph.); 7-(2,3-dihydroxypropyl)-theophylline (DHPTheoph.); 1-(2,3-dihydroxypropyl)-theobromine (DHPTheobr.); caffeine (Caff.).

* For parts I and II, see refs 11 and 12.

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TABLE I

DEPENDENCE OF R_F VALUES OF AROMATIC HYDROCARBONS ON THE STATIONARY PHASES USED

	Di-n-butyl ether		*Decahydronaphthalene*		*s-Tetrachlorethane*		*Tetrachlorethylene*	
	DHPTheobr. 0.5 M	*TMUA 0.05 M*	*DHPTheobr. 0.5 M*	*TMUA 0.05 M*	*DHPTheobr. 0.5 M*	*TMUA* 0.05 M*	*DHPTheobr. 0.5 M*	*TMUA 0.05 M*
Anthracene	0.30	0.21	0.22	0.16	0.09	—	0.12	0.05
Fluoranthene	0.38	0.26	0.36	0.24	0.11	—	0.16	0.09
2:3-Benzofluorene	0.17	0.06	0.05	start	0.07	—	0.02	0.02
Pyrene	0.45	0.31	0.37	0.28	0.11	—	0.19	0.11
Chrysene	start	start	start	start	0.08	—	0.08	0.04

* TMUA is soluble in *s*-tetrachlorethane.

TABLE II

DEPENDENCE OF R_F VALUES OF AROMATIC HYDROCARBONS ON THE CONCENTRATION OF THE SOLUBILIZING AGENT IN THE MOBILE PHASE

Solubilizing agent: sodium salt of deoxycholic acid.

	Di-n-butyl ether			*Decahydronaphthalene*			*s-Tetrachlorethane*			*Tetrachlorethylene*		
	0.10 M	*0.15 M*	*0.20 M*	*0.10 M*	*0.15 M*	*0.20 M*	*0.10 M*	*0.15 M*	*0.20 M*	*0.10 M*	*0.15 M*	*0.20 M*
Anthracene	0.22	0.28	0.27	0.18	0.23	0.31	0.13	0.20	0.27	0.19	0.20	0.30
Fluoranthene	0.26	0.30	0.31	0.18	0.25	0.31	0.14	0.20	0.30	0.20	0.21	0.31
2:3-Benzofluorene	0.19	0.28	0.28	0.19	0.27	—	0.10	0.16	0.28	0.17	0.19	0.44
Pyrene	0.27	0.32	0.29	0.24	0.25	0.28	0.14	0.18	0.27	0.19	0.21	0.32
Chrysene	0.19	0.22	0.22	0.19	0.22	0.19	0.13	0.17	0.26	0.17	0.21	0.42

TABLE III

R_F VALUES OF AROMATIC HYDROCARBONS WHEN USING AQUEOUS SOLUTIONS OF BILE ACID SALTS IN THE MOBILE PHASE

	Di-n-butyl ether				*s-Tetrachlo. ethane*			
	CH-Na 0.1 M	*DOCH-Na 0.1 M*	*DHCH-Na 0.1 M*	*NaCl 0.5 M*	*CH-Na 0.1 M*	*DOCH-Na 0.1 M*	*DHCH-Na 0.1 M*	*NaCl 0.5 M*
Anthracene	0.19	0.22	0.05	0.05	0.09	0.13	0.05	0.05
Fluoranthene	0.22	0.26	0.06	0.06	0.10	0.14	0.05	0.06
2:3-Benzofluorene	0.14	0.11	0.03	0.03	0.08	0.10	0.04	0.06
Pyrene	0.20	0.32	0.05	0.05	0.10	0.14	0.04	0.05
Chrysene	0.13	0.22	start	start	0.10	0.13	0.05	0.07

(c) Bile acid salts: sodium salt of cholic acid (CH-Na); sodium salt of deoxycholic acid (DOCH-Na); sodium salt of dehydrocholic acid (DHCH-Na).

MATERIALS AND METHODS

Chemicals

Stationary phases were formed by pure di-*n*-butyl ether, decahydronaphthalene, *s*-tetrachlorethane and tetrachlorethylene. The solubilizing agents were pure commercial products, with the exception of TMUA[13], DHPTheobr.[14], DHPTheoph.[15], DMBA[16] and DMU[17], which were prepared in the Synthesis Laboratory of our Institute. The aromatic hydrocarbons spotted on the chromatogram were preparations of highest purity from Koch-Light, Fluka and Schuchardt.

Methods

The aqueous solutions of solubilizing agents forming a mobile phase were saturated by the stationary phase. The paper, SS 2043 b Mgl, was impregnated by passing it through a mixture consisting of the stationary phase–benzene (2:1) and the excess was mopped up between sheets of filter paper. After the benzene had evaporated, the aromatic hydrocarbons were spotted on the paper in 0.5–2.0 μg amounts from benzene solutions in an atmosphere of pure nitrogen. The chromatograms were developed by an ascending technique for 16–18 h. Aromatic hydrocarbons were detected by observing their fluorescence under U.V. light. Chemical methods of detection of aromatic hydrocarbons[18] are in the presence of solubilizer less sensitive and not reliable. The R_F values presented in the tables are the averages from four chromatograms. Differences in R_F were not greater than 0.03 with the exception of those systems where the mobile phase consisted of bile acid salts.

RESULTS AND DISCUSSION

The solubility of aromatic hydrocarbons in stationary phases is of great importance for the selection of systems appropriate for separation by solubilizers. Chlorinated solvents dissolve aromatic hydrocarbons relatively well, so that influence of the partition coefficients of the hydrocarbons in favour of the mobile polar phase by the solubilizer is only small (Table I). As can be seen from Table II, the magnitude of the R_F values is influenced by the concentration of the solubilizer in the mobile phase. The appearance of the spots of the aromatic hydrocarbons when using bile acid salts is oblong, which is probably due to the slow stabilization of the partition equilibrium on the paper. Bile acids are able to form complexes of various molar ratios with aromatic hydrocarbons[6]. The solubilizing effect was not observed with the sodium salt of dehydrocholic acid (Table III), when the aromatic hydrocarbons have very low R_F values. These numerically correspond to R_F values when using an inert electrolyte in the mobile phase, by which it is possible to increase the solubility of aromatic hydrocarbons only as a result of a salting-in effect[19].

The solubilizing effects of the N-methylated cyclic ureide group are pronounced only in the case of TMUA, which displays a considerable solubilizing effect even at a concentration of 0.05 *M*. The expected effect of DMU and DMBA was not apparent see Table IV).

TABLE IV

R_F VALUES OF AROMATIC HYDROCARBONS WHEN USING N-METHYLATED CYCLIC UREIDES IN THE MOBILE PHASE

	Di-n-butyl ether			*Decahydronaphthalene*			*Tetrachlorethylene*		
	DMU 0.50 M	*DMBA 0.25 M*	*TMUA 0.05 M*	*DMU 0.50 M*	*DMBA 0.25 M*	*TMUA 0.05*	*DMU 0.50 M*	*DMBA 0.25 M*	*TMUA 0.05 M*
Anthracene	0.13	0.06	0.21	0.10	0.06	0.16	0.03	0.04	0.05
Fluoranthene	0.15	0.08	0.26	0.09	0.06	0.24	0.03	0.04	0.09
2:3-Benzofluorene	0.05	0.03	0.06	start	start	start	start	start	start
Pyrene	0.14	0.07	0.31	0.10	0.06	0.28	0.02	0.04	0.11
Chrysene	0.05	0.03	start	start	start	start	start	0.03	0.04

TABLE V

R_F VALUES OF AROMATIC HYDROCARBONS WHEN USING AQUEOUS SOLUTIONS OF PURINE DERIVATIVES IN THE MOBILE PHASE

	Di-n-butyl ether				*Tetrachlorethylene*			
	HETheoph. 0.10 M	*DHPTheoph. 0.50 M*	*DHPTheobr. 0.50 M*	*Caff. 0.05 M*	*HETheoph. 0.10 M*	*DHPTheoph. 0.50 M*	*DHPTheobr. 0.50 M*	*Caff. 0.05 M*
Anthracene	0.19	0.32	0.30	0.19	0.04	0.13	0.12	0.03
Fluoranthene	0.20	0.41	0.38	0.26	0.05	0.17	0.16	0.04
2:3-Benzofluorene	0.08	0.20	0.17	0.05	0.02	0.03	0.02	0.02
Pyrene	0.20	0.45	0.45	0.22	0.05	0.23	0.19	0.04
Chrysene	0.11	start	start	0.09	0.02	0.09	0.08	0.02

Due to the low solubility of caffeine in water more hydrophilic derivatives of purines were prepared. The solubilizing effect was decreased after introduction of a hydrophilic group into the purine molecule, however this decrease can be compensated by higher solubilizer concentration (Table V). Caffeine is partially dissolved in the stationary phases used, which influences unfavourably the form of the aromatic hydrocarbon spots. This shortcoming did not appear with hydrophilic derivatives of purines. The systems containing DHPTheobr. and TMUA in the mobile phase were found to be useful for the chromatographic separation of aromatic hydrocarbons (Table VI). Using a run-off technique, it is possible to separate the dibenzopyrenes which are usually only separated with difficulty.

TABLE VI

R_F VALUES OF SOME AROMATIC HYDROCARBONS

	Di-n-butyl ether		*Decahydronaphthalene*	
	DHPTheobr. 0.50M	*TMUA 0.05M*	*DHPTheobr. 0.50M*	*TMUA 0.05M*
Anthracene	0.30	0.21	0.22	0.16
Chrysene	start	start	start	start
1:2;5:6-Dibenzanthracene	0.12	0.05	0.12	0.15
Anthanthrene	0.31	0.16	0.30	0.17
2:3-Benzofluorene	0.17	0.06	0.05	start
Fluoranthene	0.38	0.26	0.36	0.24
20-Methylcholanthrene	0.11	0.08	0.10	0.06
Pyrene	0.45	0.31	0.37	0.28
3:4-Benzopyrene	0.26	0.14	0.23	0.15
1:2;3:4-Dibenzopyrene	0.19	0.09	0.17	0.11
1:2;4:5-Dibenzopyrene	0.20	0.11	0.20	0.12
1:2;6:7-Dibenzopyrene	start	start	start	start
3:4;9:10-Dibenzopyrene	0.17	0.07	0.14	0.09

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SUMMARY

The solubilizing effects of N-methylated cyclic ureides, purine derivatives and bile acid salts on aromatic hydrocarbons were studied by reversed-phase chromatography. Systems containing 1-(2,3-dihydroxypropyl)-theobromine and 1,3,7,9-tetramethyluric acid in the mobile phase and di-*n*-butyl ether or decahydronaphthalene in the stationary phase were suitable for the practical separation of aromatic hydrocarbons.

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