

CHROMSYMP. 1113

## ENTHALPY EFFECT IN THE RETENTION OF AROMATIC ACIDS ON AN OCTADECYL-BONDED SILICA GEL

Y. ARAI, J. YAMAGUCHI and T. HANAI\*

*Gasukuro Kogyo Inc., 237-2 Sayamagahara, Iruma 358 (Japan)*

---

### SUMMARY

The enthalpy of aromatic acids was measured on an octadecyl-bonded silica gel in acidic acetonitrile-water mixtures, and their retention mechanism was investigated using reversed-phase liquid chromatography. As their Van der Waals volume increased, their retention increased; however, polar acids with a large energy effect were less retained. The enthalpy of aromatic acids was about 2.7 kcal/mol and was fairly constant. The predominant force in the retention of aromatic acids may therefore be the energy effect, *i.e.*, the solubility of these acids in the eluent.

---

### INTRODUCTION

Octadecyl-bonded silica gels are the most popular packing materials in liquid chromatography, but the retention mechanism involved is not well understood. The retention of various compounds in reversed-phase liquid chromatography on octadecyl-bonded silica gels has been discussed in conjunction with physico-chemical parameters, namely Van der Waals volume, pi-energy and hydrogen-bonding energy effects. The pi-energy effect was originally introduced to explain the difference in the retention times of polyaromatic hydrocarbons. The difference in their retentions,  $\Delta \log k'$ , was related to the delocalization energy of polyaromatic hydrocarbons. Further, this approach was expanded to explain the retention of compounds containing hydroxy groups, and the difference was termed the hydrogen-bonding energy effect<sup>1-3</sup>. However, the observed retention times of larger molecular sized compounds were greater than the predicted retention times. An increase in the number of methylene units did not significantly alter the energy effect of hydrophobic compounds on the retention, but it did dramatically increase the enthalpy<sup>4</sup>. This means that larger molecular sized compounds can be directly adsorbed on octadecyl-bonded silica gels.

The retention of phenols has also been considered together with their Van der Waals volume, pi-energy and hydrogen-bonding energy effects<sup>1</sup> and enthalpy<sup>5</sup>. The enthalpies of alkylphenols and chlorophenols were about 1.9 and 2.2 kcal/mol, respectively, but the energy effect of phenols varied from 7.5 to 10.1 units. The predominant force for the retention of phenols could therefore be the energy effect rather than direct adsorption.

TABLE I

## PHYSICAL PARAMETERS AND LOGARITHM OF CAPACITY FACTORS FOR STANDARD SOLUTES

Conditions: column, ERC 1000 (10 cm × 6.0 mm I.D.); eluent, 30% aqueous acetonitrile water containing 0.05 M phosphoric acid; flow-rate, 1 ml/min.

No.	Compound	$VWV^*$ ( $\text{cm}^3/\text{mol}$ )	$\text{Log } k'$							$\Delta E^{**}$	$-\Delta H$ ( $\text{kcal}/\text{mol}$ )
			Temperature, $1/T$ ( $\times 10^{-5}$ )								
			309.6	314.5	319.5	324.7	330.0	335.6	341.3		
1	Benzoic acid	65.36	0.4642	0.4915	0.5155	0.5450	0.5745	0.5999	0.6308	10.00	2.400
2	2-Methylbenzoic acid	76.51	0.7003	0.7335	0.7576	0.7926	0.8239	0.8503	0.8855	10.76	2.647
3	3-Methylbenzoic acid	76.51	0.7459	0.7740	0.8026	0.8336	0.8669	0.8935	0.9202	10.51	2.555
4	4-Methylbenzoic acid	76.51	0.7279	0.7539	0.7878	0.8141	0.8468	0.8736	0.9022	10.61	2.540
5	2,4-Dimethylbenzoic acid	87.66	0.9856	1.0248	1.0491	1.0810	1.1172	1.1458	1.1806	11.27	2.769
6	2,5-Dimethylbenzoic acid	87.66	0.9831	1.0151	1.0475	1.0825	1.1169	1.1445	1.1787	11.43	2.830
7	2,6-Dimethylbenzoic acid	87.66	0.7081	0.7354	0.7589	0.7878	0.8154	0.8396	0.8688	13.00	2.311
8	3,4-Dimethylbenzoic acid	87.66	0.9551	0.9870	1.0187	1.0484	1.0816	1.1105	1.1424	11.62	2.696
9	3,5-Dimethylbenzoic acid	87.66	1.0306	1.0591	1.0955	1.1241	1.1596	1.1885	1.2198	11.16	2.754
10	2,4,6-Trimethylbenzoic acid	98.81	0.9960	1.0284	1.0518	1.0848	1.1155	1.1395	1.1721	13.44	2.518
11	4-Ethylbenzoic acid	86.74	1.0257	1.0550	1.0923	1.1205	1.1571	1.1848	1.2163	10.85	2.771
12	2-Chlorobenzoic acid	74.84	0.5852	0.6185	0.6488	0.6796	0.7103	0.7378	0.7710	11.09	2.652
13	3-Chlorobenzoic acid	74.84	0.8564	0.8864	0.9245	0.9546	0.9927	1.0232	1.0566	9.715	2.915
14	4-Chlorobenzoic acid	74.84	0.8726	0.9027	0.9420	0.9720	1.0099	1.0408	1.0741	9.419	2.934
15	2,4-Dichlorobenzoic acid	84.32	1.0336	1.0696	1.1043	1.1405	1.1780	1.2120	1.2430	10.30	3.051
16	2,5-Dichlorobenzoic acid	84.32	0.9638	0.9990	1.0335	1.0710	1.1099	1.1415	1.1741	10.70	3.068
17	2,6-Dichlorobenzoic acid	84.32	0.6730	0.7065	0.7320	0.7673	0.7890	0.8169	0.8540	12.48	2.539

18	3,4-Dichlorobenzoic acid	84.32	1.2140	1.2537	1.2930	1.3293	1.3712	1.4060	1.4455	9.223	3.332
19	3,5-Dichlorobenzoic acid	84.32	1.2873	1.3268	1.3655	1.4008	1.4422	1.4770	1.5155	8.791	3.283
20	2-Bromobenzoic acid	77.96	0.6449	0.6730	0.7092	0.7388	0.7739	0.8043	0.8380	11.35	2.805
21	3-Bromobenzoic acid	77.96	0.9296	0.9665	0.9978	1.0350	1.0727	1.1040	1.443	9.641	3.075
22	4-Bromobenzoic acid	77.96	0.9516	0.9841	1.0248	1.0577	1.0956	1.1299	1.1653	9.522	3.104
23	Phenylacetic acid	79.55	0.4799	0.5122	0.5402	0.5689	0.5969	0.6218	0.6534	12.88	2.462
24	2-Chlorophenylacetic acid	85.07	0.7140	0.7452	0.7729	0.8073	0.8411	0.8689	0.9059	12.34	2.758
25	4-Chlorophenylacetic acid	85.07	0.8398	0.8710	0.9107	0.9423	0.9791	1.0119	1.0458	11.57	2.994
26	2-Tolylacetic acid	86.74	0.6884	0.7200	0.7494	0.7797	0.8093	0.8347	0.8662	12.83	2.544
27	3-Tolylacetic acid	86.74	0.7558	0.7846	0.8142	0.8447	0.8775	0.9063	0.9329	12.51	2.592
28	4-Phenyl- <i>n</i> -butyric acid	96.05	0.9601	0.9937	1.0243	1.0581	1.0935	1.1227	1.1565	13.05	2.835
29	L(+)-Mandelic acid	80.18	0.0036	0.0167	0.0385	0.0554	0.0758	0.0914	0.1097	16.03	1.562
30	<i>trans</i> -Cinnamic acid	82.32	0.7525	0.7845	0.8162	0.8495	0.8875	0.9196	0.9495	11.56	2.884
31	4-Methylcinnamic acid	93.47	1.0361	1.0718	1.1055	1.1425	1.1802	1.2139	1.2441	12.08	3.039
32	3-Phenyl- <i>n</i> -propionic acid	86.04	0.7215	0.7508	0.7821	0.8154	0.8492	0.8767	0.9133	12.49	2.767
33	Indole-3-acetic acid	91.65	0.4326	0.4710	0.5065	0.5440	0.5798	0.6139	0.6545	15.42	3.170
34	Indole-3-propionic acid	114.11	0.6661	0.7014	0.7467	0.7834	0.8266	0.8661	0.9097	18.19	3.528
35	Indole-3-butyric acid	121.34	0.8598	0.8985	0.9462	0.9848	1.0305	1.0714	1.1169	18.44	3.717
36	Hippuric acid	96.15	-0.0374	-0.0178	-0.0032	0.0156	0.0315	0.0441	0.0614	19.31	1.406

\* Van der Waals volume calculated by Bondi's method.

\*\* Energy effect from ref. 6.

In the same way, the enthalpy of aromatic acids was also measured in acidic acetonitrile-water mixtures, and their retention mechanism was investigated in reversed-phase liquid chromatography.

## EXPERIMENTAL

The liquid chromatograph consisted of an ERC Model 3510 degasser (Erma, Tokyo, Japan), a CCPD pump (Toyo Soda, Tokyo, Japan), a Rheodyne Model 7125 injector, an ERC Model 8710 UV detector and a Shimadzu Model C-R3A integrator (Shimadzu, Kyoto, Japan). An IBM AT computer was used for calculations.

The samples were 36 aromatic acids, mainly supplied by Tokyo Chemical Industries (Tokyo, Japan). The octadecyl-bonded silica gel column was an ERC 1000 (10 cm  $\times$  6 mm I.D.) column packed with 5  $\mu$ m Hypersil ODS, and was thermostated in a water-bath. The eluent was acetonitrile-0.05 M phosphoric acid. The acetonitrile was of HPLC grade from Kishidakagaku (Tokyo, Japan).

## RESULTS AND DISCUSSION

The capacity ratios measured in 30% aqueous acetonitrile containing 0.05 M phosphoric acid at different temperatures are listed in Table I.

*Ortho*-substituted acids, especially 2,6-disubstituted acids, were less retained than others, as can be seen in Fig. 1. Halogenated acids were more retained than alkyl-substituted acids, as with alkylbenzenes and halogenated benzenes<sup>2</sup>, where the retention times of hydrophobic compounds could be predicted from their Van der Waals volumes and pi-energy and hydrogen-bonding energy effects.

The correlation coefficient between Van der Waals volume and the value of

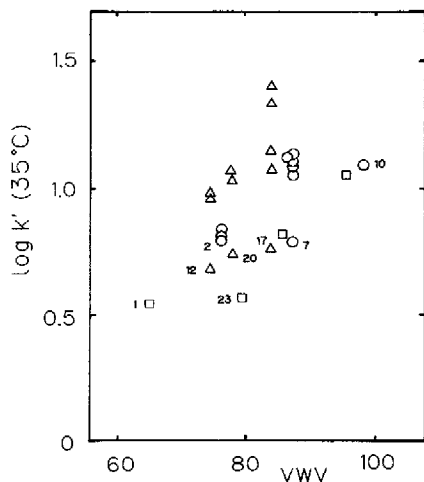


Fig. 1. Relationship between Van der Waals volumes (VWV) and  $\log k'$  values of benzoic acids, phenylacetic acid, 3-phenylpropionic acid and 4-phenylbutyric acid in reversed-phase liquid chromatography. Column, ERC 1000, 10 cm  $\times$  6.0 mm I.D., packed with 5  $\mu$ m Hypersil ODS; eluent, 30% aqueous acetonitrile containing 0.05 M phosphoric acid; column temperature, 35°C. Numbers beside symbols as in Table I.  $\circ$  = Alkylbenzoic acids;  $\triangle$  = halogenated benzoic acids;  $\square$  = others.

log  $k'$  for aromatic acids was 0.041 ( $n = 36$ ) and that for alkylbenzoic acids was 0.848 ( $n = 11$ ). However, the value was 0.989 ( $n = 9$ ) when 2,6-dimethyl- and 2,4,6-trimethylbenzoic acids were eliminated from the calculation. The correlation coefficient for halogenated benzoic acids was 0.712 ( $n = 12$ ) and 0.912 ( $n = 9$ ), except for 2-bromo-, 2-chloro- and 2,6-dichlorobenzoic acids.

Further, the correlation coefficient between log  $k'$ , measured in 40% acidic aqueous acetonitrile and the log  $P$  values of aromatic acids was 0.884 ( $n = 36$ ); this value improved to 0.966 ( $n = 28$ ) when 2-, 2,6-di- and 2,4,6-trisubstituted acids were eliminated from the calculation. This means that using the log  $P$  values was better than using Van der Waals volume only, but the log  $P$  values had limitations with respect to the optimization of the eluent.

The enthalpy of aromatic acids was calculated from the equation

$$\ln k' = -\Delta H/RT + \Delta S/R + \ln \varphi$$

where  $\Delta H$  is the enthalpy,  $T$  is the absolute temperature,  $\Delta S$  is the entropy,  $R$  is the gas constant,  $\varphi$  is the phase ratio and  $k'$  is the capacity ratio. The enthalpies are given in Table I, together with the Van der Waals volumes<sup>6</sup>.

The enthalpies of alkylbenzoic and halogenated benzoic acids were about 2.6 and 3.0 kcal/mol, respectively, in 30% acidic aqueous acetonitrile and 2.3 and 2.6 kcal/mol, respectively, in 40% acidic aqueous acetonitrile. There was no significant difference within these substituted groups.

The retention of phenylacetic acid was about the same as that of benzoic acid, even though their Van der Waals volumes are different. In addition, their enthalpies

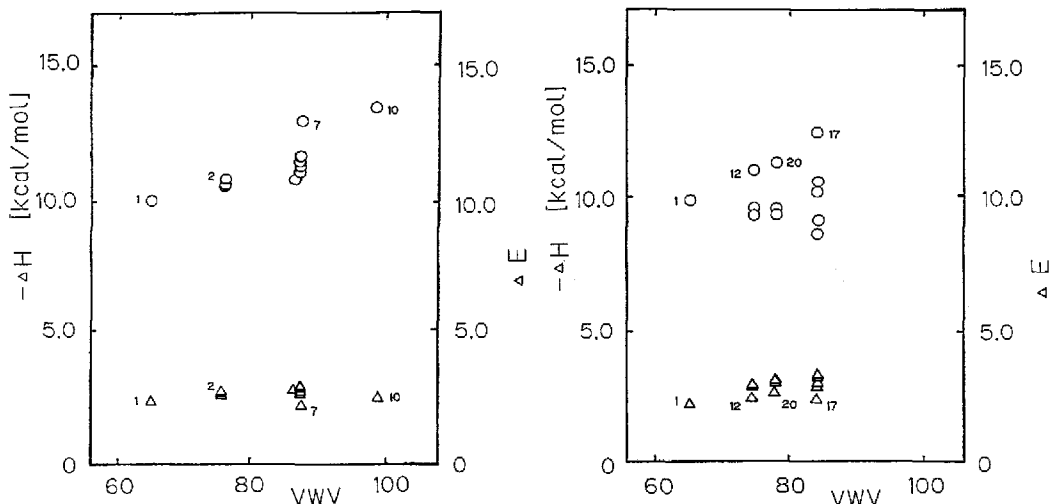


Fig. 2. Relationship between the enthalpy ( $-\Delta H$ ) and the energy effect ( $\Delta E$ ) and the Van der Waals volumes (VWV) of alkylbenzoic acids. Conditions as in Fig. 1. Numbers beside symbols as in Table I.  $\circ$  = Energy effect;  $\Delta$  = enthalpy.

Fig. 3. Relationship between the enthalpy ( $-\Delta H$ ) and the energy effect ( $\Delta E$ ) and the Van der Waals volumes (VWV) of halogenated benzoic acids. Conditions as in Fig. 1. Numbers beside symbols as in Table I.  $\circ$  = Energy effect;  $\Delta$  = enthalpy.

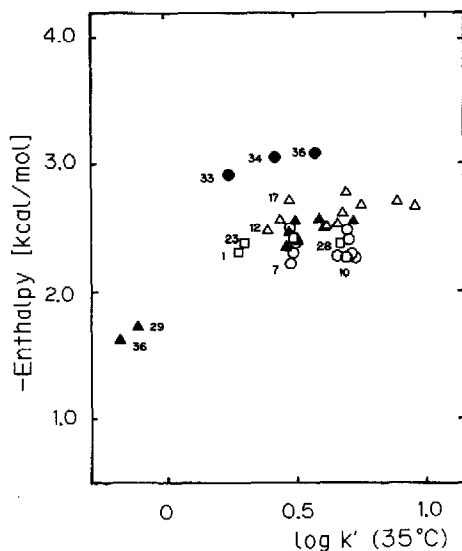


Fig. 4. Relationship between  $\log k'$  values and the enthalpy ( $-\Delta H$ ) for benzoic acids. Conditions as in Fig. 1, except for the eluent (40% aqueous acetonitrile containing 0.05 M phosphoric acid). Numbers beside symbols as in Table I.  $\circ$  = Alkylbenzoic acids;  $\triangle$  = halogenated benzoic acids;  $\square$  = benzoic, phenylacetic, 3-phenylpropionic and 4-phenylbutyric acids;  $\bullet$  = indole acids;  $\blacktriangle$  = others.

were almost identical, but their energy effects were different by 2.9 units. When the retentions of benzoic and phenylacetic acids was related to their hydrophobicities ( $\log P$ ), their  $\log P$  values were nearly identical and their  $\log k'$  values were also similar<sup>7</sup>. However, a comparison of enthalpy and energy effects for benzoic, phenylacetic, 3-phenylpropionic and 4-phenylbutyric acids indicated that the enthalpy of benzoic acid was slightly higher than that expected from its Van der Waals volume, and the energy effect of benzoic acid was lower than that expected from its Van der Waals volume. This means that the direct adsorption of benzoic acid may be responsible for its strong retention. The relationship between Van der Waals volume and the enthalpy and the energy effect of benzoic acids is shown in Figs. 2 and 3.

2,6-Disubstituted benzoic acids had a weak enthalpy and a larger energy effect; such a substituent effect was also found for the retention of phenols<sup>5</sup>. There was no good linear correlation between  $\log k'$  and enthalpy for benzoic acids (Fig. 4).

There was also no linear relationship between the enthalpy and the energy effect. This means that the predominant force in the retention of aromatic acids in the above system may be the energy effect and not the enthalpy.

#### REFERENCES

- 1 T. Hanai and J. Hubert, *J. Chromatogr.*, 302 (1984) 89.
- 2 T. Hanai and J. Hubert, *J. Chromatogr.*, 290 (1984) 197.
- 3 T. Hanai and J. Hubert, *J. Chromatogr.*, 291 (1984) 81.
- 4 T. Hanai, A. Jukurogi and J. Hubert, *Chromatographia*, 19 (1984) 266.
- 5 Y. Arai, M. Hirukawa and T. Hanai, *J. Chromatogr.*, 384 (1987) 279.
- 6 T. Hanai, *J. Chromatogr.*, 332 (1985) 189.
- 7 T. Hanai, K. C. Tran and J. Hubert, *J. Chromatogr.*, 239 (1982) 385.