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INVESTIGATIONS OF THE RELATIONSHIP BETWEEN MOLECULAR STRUCTURE AND CHROMATOGRAPHIC PARAMETERS

IX. EXTRACTION OF PHENOLIC 2-BENZOYLBENZOIC ACIDS WITH CYCLOHEXANE SOLUTIONS OF *n*-HEXANOL AND OLEIC ACID

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SUMMARY

Fifteen hydroxyl derivatives of 2-benzoylbenzoic acid were chromatographed in a series of solvent systems of the type (cyclohexane + polar solvent)-buffer solution. *n*-Hexanol and oleic acid were used as polar associated solvents (S). From the slopes of the R_M versus $\log [S]$ plots, the probable molecular mechanism of liquid-liquid partition and especially solvation by associated solvents were established.

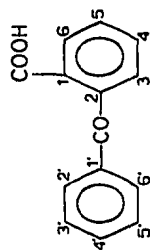
INTRODUCTION

In preceding papers in this series^{1,2}, the molecular mechanism of the partition of phenols and phenolic acids was investigated by the dilution method. These investigations have now been continued to include a series of derivatives of 2-benzoylbenzoic acid synthesized by Gronowska, Rumiński and Wazgird³. The differences in the molecular structures of this set of related compounds permit the effects of various substituents and their positions on the slopes of the R_M versus $\log [S]$ plots ($S =$ polar solvent), which provide information on the molecular interactions in the organic phase, to be elucidated.

The investigations, as in the previous work^{1,2}, had several purposes. Variation of solvent composition in order to optimize chromatographic separations is a standard technique, and a quantitative knowledge of solvent composition effects is essential particularly in gradient elution^{4,5}. Solvation effects are also important in other phenomena, such as adsorption from solution and crystallization, and the molecular interactions involved are especially complex when associated solvents are used^{6,7}. Partition coefficients in aqueous systems and the effect of molecular structure on them have gained additional significance in recent years owing to attempts to correlate liquid-liquid partition with the accessibility of organic solutes to receptors in living

* To whom the authors express their thanks for sending samples of the compounds.

TABLE I
 SLOPES (ABSOLUTE VALUES) OF R_M versus LOG [S] LINES FOR VARIOUS pH VALUES OF THE AQUEOUS PHASE
 Values are the means of three runs except for those in parentheses which are the means of two runs.



No.	Substituents						<i>n</i> -Hexanol			Oleic acid		
	2'	3'	4'	5'	Symbol		pH 4.03	pH 5.08	pH 6.01	pH 4.03	pH 5.08	pH 6.01
1	OH	-	-	-	2HB	-	-	1.1	0.8	0.5-0.8	0.5-0.8	0.6-1.4
2	OH	CH ₃	-	-	2H3MB	-	-	0.8	0.8	0.5-0.9	0.4-0.9	0.4-1.1
3	OH	-	CH ₃	-	2H4MB	-	-	0.9	0.85	0.5-0.8	0.4-0.8	0.5-1.2
4	OH	-	-	CH ₃	2H5MB	-	-	1.3	0.8	0.5-0.9	0.4-0.8	0.5-1.2
5	OH	C ₂ H ₅	-	-	2H3EB	-	-	-	0.8	0.6-0.8	0.4-0.8	0.6-0.7
6	OH	Cl	-	-	2H3CB	-	-	1.1	0.9	0.5-0.8	0.5-0.8	0.6-1.4
7	OH	-	-	Cl	2H5CB	-	-	1.2	0.9	0.5-0.9	0.4-0.9	0.4-1.1
8	OH	-	OCH ₃	-	2H4MOB	-	1.0	1.0	0.9	0.5-0.8	0.5-0.9	0.6-1.2
9	-	-	OH	-	4HB	-	1.8	1.6	1.8	-	-	-
10	-	CH ₃	OH	-	4H3MB	-	1.8	1.5	1.6	-	-	-
11	CH ₃	-	OH	-	4H2M5IPB	-	1.8	1.7	1.7	0.6-1.8	0.8-1.8	1.0-1.8
12	-	OCH ₃	OH	-	4H3MOB	-	-	1.5	1.2-1.8	-	-	-
13	-	Cl	OH	-	4H3CB	-	-	1.6	1.6	-	-	-
14	-	Br	OH	-	4H3BB	-	1.8	1.6	1.5	-	-	-
15	OH	-	OH	-	24HB	-	1.7	1.6	1.8	(1.6)	(1.5)	-

systems and thus with biological activity (quantitative structure-activity relationships, QSAR-Hansch analysis^{8,9}). In such studies, chromatographic methods have also been employed¹⁰ and the dilution technique may provide additional information on the structural effects involved.

EXPERIMENTAL

Whatman No. 41 paper strips were impregnated with McIlvaine's buffer solutions of pH 4.03, 5.08 or 6.01, blotted between two sheets of filter-paper and dried in air until the content of buffer solution decreased to 0.5 g per gram of dry paper. The strips were then transferred immediately to all-glass chromatographic tanks for descending development¹¹. Under these conditions, the ratio of the volumes of the two liquid phases, V_{org}/V_w , is *ca.* 2.0.

The spots were detected with bis-diazotized benzidine reagent after spraying with a saturated solution of sodium hydrogen carbonate.

RESULTS AND DISCUSSION

The R_F values and the formulae of the solutes are given in Tables I and II. The results are presented as R_M versus $\log [S]$ plots, the R_M axis being directed downwards so that R_F and $\log D$ (D = distribution ratio) increase upwards (see parallel ordinates in Fig. 1a; $\log D$ was calculated assuming that $\log D = -R_M - 0.3$). The solutes are denoted by appropriate symbols, the last letter B indicating 2-benzoylbenzoic acid and the preceding letters and symbols the substituents and their positions on the second ring (*cf.*, Table I).

For partition systems with *n*-hexanol as the polar component of the mobile phase (Figs. 1a and 2a), linear R_M versus $\log [HxOH]$ graphs were obtained; the lines form two separate groups, the first containing the more hydrophilic 4'-hydroxy derivatives, for which the slope was *ca.* -1.6, and the second the 2'-hydroxy derivatives, for which higher R_F values and less steep R_M versus $\log [HxOH]$ lines were obtained (slope *ca.* -0.8). The parallel behaviour within the two groups of isomers is striking. For instance, the 4'-hydroxy-2'-methyl-5'-isopropyl derivative, which is more hydrophobic owing to substitution with two aliphatic radicals, is clearly distinguishable from the 2'-hydroxy derivatives.

In order to secure the optimum range of R_F values, buffer solutions of different pH were used; an increase in pH decreased the R_F values¹², permitting the R_M versus $\log [S]$ relationships of more hydrophobic compounds to be determined more accurately, shifting the curve downwards without any noticeable changes in slope and sequence within the two groups of compounds.

The differences in behaviour of 2'-hydroxy and 4'-hydroxy derivatives are probably due to the formation of an internal hydrogen bond in the former compounds. As *n*-hexanol has the properties of solvents of class B^{1,2,13,14}, it is able to solvate only proton-donor groups such as -OH or -COOH; comparison with 4'-hydroxy derivatives seems to indicate that it is the hydroxyl group in the 2'-position that can form the internal hydrogen bond with the carbonyl group, resulting in more hydrophobic properties of the 2'-hydroxy derivatives and less steep R_M versus $\log [HxOH]$ relationships. With 4'-hydroxy derivatives, both proton-donor groups can interact with *n*-

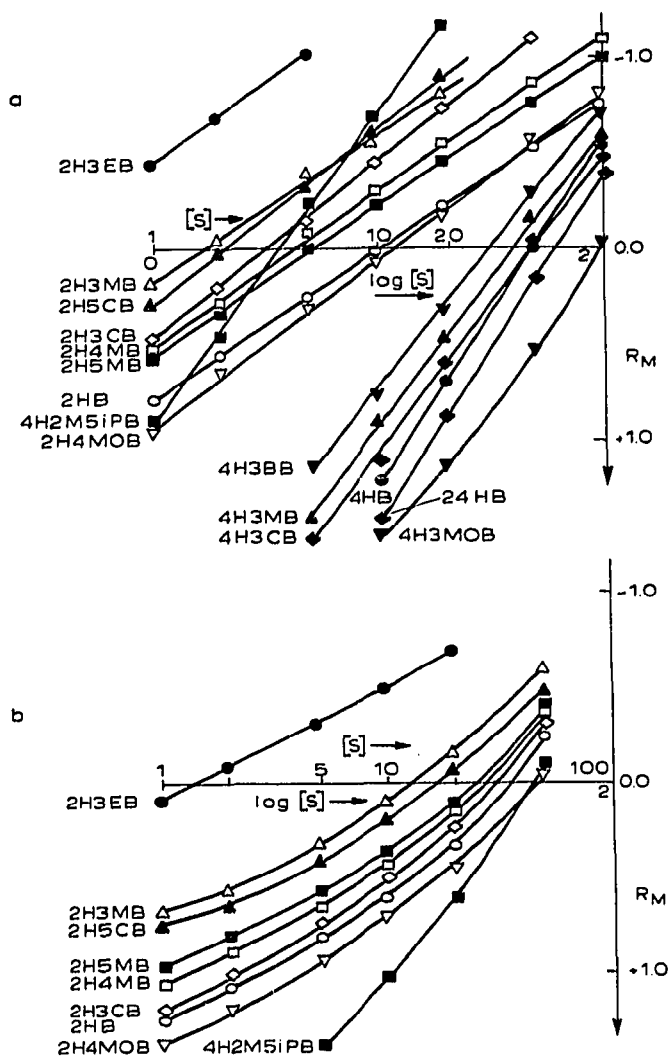


Fig. 2. As Fig. 1; pH = 6.01.

In Figs. 1b and 2b, analogous relationships are presented for oleic acid as the polar component of the developing solvent. Lower R_F values were observed for this solvent, indicating its lower extraction strength relative to phenolic acids, which is due, among other causes, to the lower molarity of the polar group¹. For most 4'-hydroxy compounds (except the 4'-methyl-5'-isopropyl derivative), low R_F values were obtained and for 2'-hydroxy derivatives the lines became less steep at higher dilutions of oleic acid. The slope of the lines was ≈ -0.5 at lower concentrations of oleic acid (Fig. 1b) and increased to -1.0 and above at higher concentrations (Fig. 2b). As with *n*-hexanol, the lines of the 4'-hydroxy derivatives are steeper than those of the 2'-hydroxy compounds.

As Jusiak¹⁵ demonstrated in his systematic investigations, solutions of oleic

acid in organic solvents are very efficient extractants of alkaloids, owing to the formation of ion pairs or more complex adducts (see also ref. 16). The present results indicate that for solutions in non-polar solvents, interactions of oleic acid with non-basic proton donor groups ($-\text{OH}$, $-\text{COOH}$) can contribute to the extraction ability of the mixed solvent.

The results reported in this and earlier papers in this series seem to indicate that for polyfunctional solutes the slopes are, to a first approximation, additive for a given system (*i.e.*, -1.0 for monofunctional solutes, -2.0 for bifunctional solutes, etc.), account being taken of steric shielding and especially internal hydrogen bonding. Thus, the presence of proton-donor groups can be reflected by increased slopes for extractants that contain *n*-hexanol, oleic acid and similar solvents.

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