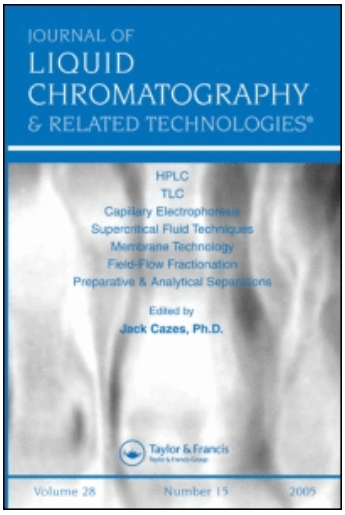

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M. R. Clark[a]; L. E. Garcia-roura[a]; C. R. Clark[a]
[a] Division of Medicinal Chemistry Department of Pharmacal, Sciences School of Pharmacy Auburn University, Auburn, Alabama

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INTRAMOLECULAR HYDROGEN BONDING EFFECTS ON THE REVERSED-PHASE RETENTION OF SUBSTITUTED ACETOPHENONES

MATTHEW R. CLARK, LAURA E. GARCIA-ROURA AND C. RANDALL CLARK*
Division of Medicinal Chemistry
Department of Pharmacal Sciences
School of Pharmacy
Auburn University
Auburn, Alabama 36849

INTRODUCTION

Reversed-phase liquid chromatography (RPLC) is widely recognized as a valuable technique for the separation of compounds of varying lipophilic/hydrophobic nature. RPLC on hydrocarbon stationary phases has been used for the separation of homologues of various compound classes with outstanding success (1-3). Excellent correlations have been obtained between RPLC capacity factors and various parameters of the solvophobic theory (4-6). This theory suggests that solute-solvent interactions assume the primary role in the RPLC retention process (7-9). The creation of a suitable cavity in the mobile phase is a key factor affecting the retention of the solute. The size and shape of the cavity formed in the solvent depends on solute molecular volume and hydrophobic surface area as well as the dielective constant and surface tension of the solvent. Relationships between RPLC capacity factors,

partition coefficients (10,11) and biological activities (12) have been reported. These high correlations suggest RPLC can be a very useful tool for the study of molecular phenomena in solution.

Intramolecular hydrogen bonding in various series of 1,2-disubstituted benzenes have been studies in the crystal state (13) and in solution by infrared (14) and nuclear magnetic resonance (15) techniques. These solution studies have been carried out using solvents such as carbon tetrachloride and carbon disulfide which are not capable of participating in hydrogen bond formation. Studies (15) using hydrogen bonding solvents such as deuterium oxide have failed to demonstrate intramolecular hydrogen bonding for similar solutes. The competition of the solvent in forming intermolecular associations appears to be the major force preventing intramolecular association. The decreased retention of 2-nitroanaline relative to the 4-isomer in normal phase chromatography has been attributed (16) to intramolecular hydrogen bonding. Normal and reversed-phase paper chromatographic techniques (17) have shown that chromatographic retention reflects the decreased polarity produced by intramolecular hydrogen bonding in some conformationally restrained anthroquinones.

In this study we have examined the RPLC structure-retention relationships for a series of substituted acetophenones. Both hydrogen-bond acceptor and donor substituent groups were examined for their ability to interact with the conformationally flexible acetophenone carbonyl-group in aqueous solvents.

EXPERIMENTAL

The liquid chromatograph consisted of a Waters model 6000A pump, U6K injector, 440 Ultraviolet absorbance detector and a Linear recorder. Hydro-organic mobile phases were prepared by mixing (V/V) acetonitrile and water or methanol and water. In some studies the aqueous component of the mobile phase was 4% aqueous acetic acid or 0.05M phosphate buffer (pH 4.0). All solvent mixtures were degassed and allowed to equilibrate for at least one hour before use. The mobile phase flow rate was 1.0 or 1.5 mL/min and the UV absorbance

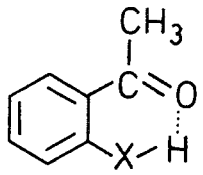
detector was operated at 254nm and 0.02 AUFS. Individual solutions of the compounds were prepared in acetonitrile and the chromatographic studies were done at ambient temperature.

The reversed-phase columns used in this study were packed with either C_{18} or C_8 hydrocarbon bonded to 5 um silica. Column 1 was 15 cm x 4.6 mm id packed with Hypersil 5 C_{18}; Column 2 was 15 cm x 4.6 mm id packed with Delta Bond 300 Octyl 5; Column 3 was 15 cm x 4.6 mm id packed with Nucleosil 5 C_{18}. The void volume of the chromatographic system was determined by injecting aqueous solutions of sodium nitrate and uracil. Capacity factors (k') were calculated in the usual manner and the reported values are the average of at least two determinations.

RESULTS AND DISCUSSION

The 2-, 3- and 4-substituted methyl-, methoxy-, hydroxy- and amino-acetophenones (Table 1) examined in this study were selected on the basis of appropriate acceptor/donor relationships for intramolecular hydrogen bond formation. A hydrogen bond is formed via the dipolar association of an electron deficient hydrogen atom of a donor group and an electron rich acceptor group. The donor group is composed of a hydrogen attached covalently to an electronegative atom such as oxygen or nitrogen (O-H or N-H) and the acceptor group is an electron rich element like oxygen, nitrogen or halogen. The association of acceptor and donor groups contained in the same molecule is an intramolecular hydrogen bond. The carbonyl-group of the acetophenones is capable of acting only as an acceptor group through the electron rich oxygen. The methyl-group in 2,3 and 4-methylacetophenone is not capable of acting as either acceptor or donor group for hydrogen bonding while the methoxy-group can act only as an acceptor group. The hydroxy- and amino- group can act as either acceptor or donor in the formation of hydrogen bonds. However, these groups must act as donors for intramolecular hydrogen bonding to occur in the acetophenones. Intramolecular hydrogen bonds involving N-H...O and O-H...O groups are most stable when the association results in a 6-membered ring. The

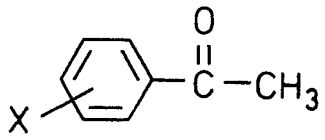
reversed-phase retention properties for the twelve substituted acetophenones examined in this study were determined on three hydrocarbon bonded phases at various strengths of aqueous acetonitrile and methanol. The aqueous portion of the mobile phase was varied among unbuffered, 4% acetic acid and 0.05 M phosphate buffer (pH4). The capacity factors reported in Table 1 are representative of the relative retention obtained in all the studies. Table 1 shows the capacity factors for the twelve acetophenones obtained on Hypersil C_{18} (column 1) in 20% acetonitrile-water. Although the capacity factors varied with different solvent systems and stationary phases, the relative elution order was constant for each set of positional isomers. These compounds are relatively polar and require only low concentrations of organic modifier to achieve capacity factors in the optimum range. The more polar substituents such as the amino- and hydroxy-groups show very low retention when at the 3- or 4-position even in 20% acetonitrile. However, the retention of the 2-amino- and 2-hydroxy-acetophenones is quite high relative to the corresponding 3- or 4-substituted compound. In terms of α-values, k' of the 2-substituent/k' of the 3- or 4-substituent, a minimum value of $\alpha = 3.0$ was obtained for the 2-/3-position relationship in the aminoacetophenones. The α-values for the hydroxyacetophenones show the same trend as the amino-isomers. The amino- and hydroxyacetophenones contain the appropriate acceptor/donor relationship for the formation of an intramolecular hydrogen bond in the 2-substituted isomers. The resulting hydrogen bond would be a 6-membered ring formed via the N-H or O-H donor groups and the O-acceptor oxygen of the carbonyl-group. Although these same substituents are present in the 3- and 4-isomers, geometric factors prevent the formation of intramolecular hydrogen bonds.



X=NH,O

TABLE 1

Capacity Factors and α-values for substituted acetophenones[1].



	Position of Substitution				
	2	3		4	
X	k′	k′	α^2	k′	α^2
$-CH_3$	18.0	18.0	1.0	17.0	1.1
$-OCH_3$	11.0	11.0	1.0	9.0	1.2
$-NH_2$	6.0	2.0	3.0	1.7	3.5
-OH	11.3	2.3	4.9	1.7	6.7

[1]Results obtained from Column 1 using 20% acetonitrile-water.

[2]α = k′ 2-position/k′ 3- or 4-position

In comparing the results for the hydroxy- and aminoacetophenones in Table 1 the α-values for the hydroxy-series are almost double those of the corresponding aminoacetophenones. The capacity factors is Table 1 for the 3- and 4 amino- and hydroxy-isomers are very similar and the almost double value of α in the hydroxy-isomers is due to the increased k′-value for 2-hydroxy-acetophenone. The 2-hydroxy-isomer shows retention almost double that of the 2-aminoacetophenone. One possible explanation for the enhanced retention of the 2-hydroxyacetophenone is the relative strength of the intramolecular hydrogen bond. The O-H...O association is reported (17, 18) to be stronger than the corresponding N-H...O association, thus, the 2-hydroxy-isomer may exist in the more completely intramolecularly hydrogen bonded state.

The enhanced reversed-phase retention of the intramolecularly associated 2-amino- and 2-hydroxyacetophenones may be due to a decrease in dipolar solute-solvent associations. The internal association of the polar groups would decrease the number of interaction sites available for association with the solvent. This decrease in solute-solvent association then should produce a decrease in solute polarity resulting in greater association with the hydrocarbon stationary phase. The corresponding 3- and 4-substituted acetophenones contain the same polar groups but are capable of only intermolecular association primarily with the water molecules of the solvent. The 2-isomers are capable of both inter- and intramolecular hydrogen bonding and these results indicate that considerable intramolecular association occurs even in strong hydrogen-bonding solvents such as water or aqueous organic.

The methoxy- and methyl-substituents were examined as examples of groups incapable of intramolecular hydrogen bonding with the carbonyl oxygen of the acetophenone. The methoxyacetophenones are examples of a donor/donor relationship while the methyl-substituent is capable of neither acceptor nor donor activity. The retention data in Table 1 shows these isomers to be essentially unresolved even though the capacity factors are quite high. Similar results were obtained in all organic solvents and on all stationary phases used in this study. The 2-substituent was not observed to show a higher capacity factor than the 3- or 4-isomer under any reversed-phase conditions examined. Thus, the enhanced retention for the 2-amino-and 2-hydroxyacetophenones appears to be the direct result of intramolecular association.

The replacement of the hydroxyl-group by the methoxy-group in the acetophenones can be viewed as a simple methylation of the hydroxyl oxygen and has the effect of converting an acceptor/donor relationship in the hydroxyacetophenones to an acceptor/acceptor relationship in the methoxyacetophenones. Figure 1 illustrates the reversed-phase chromatographic results of this simple structural alteration. The retention of the 2-hydroxy-isomer is quite similar

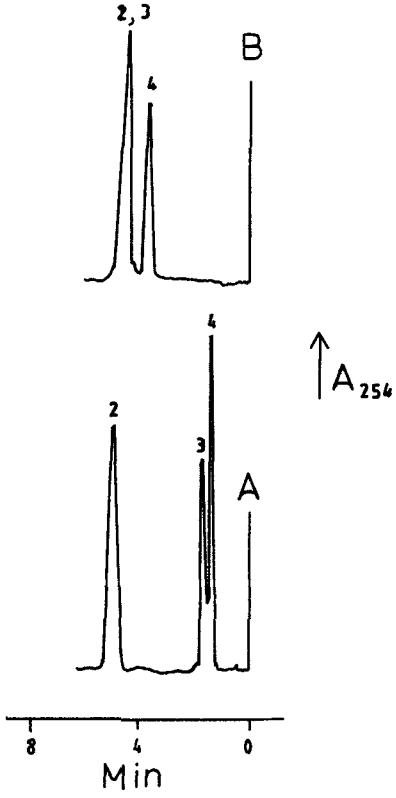


Figure 1

Liquid chromatographic separation of substituted acetophenones on Column 3 in 30% acetonitrile-water. A: 2-,3- and 4-hydroxyacetophenones; B: 2-,3-and 4-*methoxyacetophenones*.

to the poorly resolved isomers of methoxyacetophenone, again pointing out the masking of the hydroxyl-group polarity produced by the intramolecular hydrogen bond. The acceptor/acceptor relationship in the 2-methoxyacetophenone does not yield any intramolecular association, thus it does not display enhanced retention relative to the 3- and 4-isomers. The role of the methoxy-group as an intramolecular hydrogen bond acceptor was established in some previous studies on substituted benzamides (3) and benzoic acids (14).

Franc and Sechovec (17) have reported the effects of intramolecular hydrogen bonding in reversed-phase systems using paper chromatography. Their work showed that 1-amino- and 1-hydroxyanthroquinones always displayed lower R-values than the corresponding 2-isomers. The observed retention was attributed to the internal hydrogen-bonds formed in the 1-isomers of these conformationally locked anthroquinones. Our observations in the acetophenones show that these intramolecular associations can occur in nonplanar compounds capable of many nonassociating conformations. Additionally, these studies continue to illustrate the role of solute intramolecular hydrogen bonding in reversed-phase retention and perhaps more importantly the utility of reversed-phase liquid chromatography for observing these effects in aqueous solvents.

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