CHROM. 14,367

RETENTION BEHAVIOUR OF ACETYL-INDANDIONES IN REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

GYULA VIGH*, ZITA VARGA-PUCHONY and ÁKOS BARTHA

Institute for Analytical Chemistry, University of Chemical Engineering, P.O. Box 28, Veszprém (Hungary)
and
SÁNDOR BALOGH

Reanal Fine Chemical Works, Budapest (Hungary)

SUMMARY

The retention factors of several acetyl-indandiones as functions of the methanol concentration, pH and tetrabutylammonium ion concentration of the eluent have been determined in an octadecyl silica–methanol–aqueous phosphate buffer reversed-phase system. Results have been interpreted by assuming that the acetyl-indandiones behave as monoprotic acids, and the apparent pK_a values have been calculated. These indicate that acetyl indandiones are stronger acids than the simple aromatic carboxylic acids.
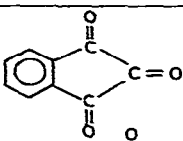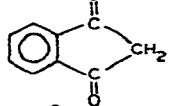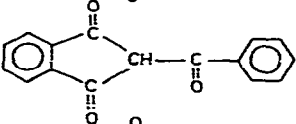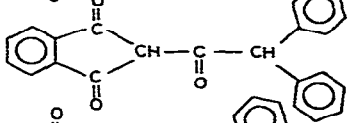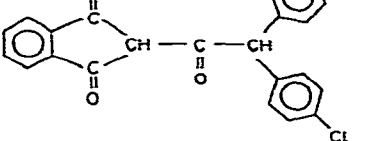
INTRODUCTION

Recently, a fast high-performance liquid chromatographic (HPLC) method had to be developed for the analysis of chlorophacinon, a member of the acetyl-indanedione family[1]. This work revealed that practically nothing was known of the liquid chromatographic behaviour of acetyl-indandiones. Also, the capacity factor, k' of chlorophacinon in a reversed-phase system was found to depend on the pH of the eluent. These observations prompted us to investigate the retention behaviour of a few acetyl-indandiones in more detail, and attempt the HPLC determination of the acid dissociation constants, pK_a, for these compounds.

The structures of the compounds investigated are shown in Table I.

EXPERIMENTAL

Experiments were carried out on a Varian LC 5020 liquid chromatograph (Varian, Walnut Creek, CA, U.S.A.) equipped with an LC 55 variable-wavelength detector (Perkin-Elmer, Norwalk, CT, U.S.A.) set at 285 nm. The eluents were prepared by weighing the required amount of methanol and the buffer components into a 1000-ml volumetric flask. The solution was brought up to mark at 25°C with double distilled water and its density was determined so that both w/w, w/v and v/v percentage compositions could be calculated. The apparent pH of the eluents was de-

TABLE I

STRUCTURE OF THE ACETYL-INDANDIONES TESTED

Name	Symbol	Structure
Ninhydrin (1,2,3-Indantrione)	NH	
Indandione (1,3-Indandione)	ID	
Benzoyl-indandione (2-Benzoyl-1,3-indandione)	BID	
Diphacinon (2-Diphenylacetyl-1,3-indandione)	DF	
Chlorophacinon {2-[(p-Chlorophenyl)-phenylacetyl]-1,3-indandione}	CF	

termined with a combined glass electrode (calibrated with pH 4 and 7 aqueous buffers) and a Type OH 208 precision digital pH meter (Radelkis, Budapest, Hungary).

Separations were carried out on a stainless-steel column (250 × 4.0 mm) (Labor-MIM, Budapest, Hungary), packed with 10-μm RP-18 (E. Merck, Darmstadt, G.F.R.). The column was jacketed[2] and thermostatted at 30°C using a Type U 10 circulating water-bath (MLW, Medingen, G.D.R.). The column dead volume at each methanol composition was determined by injecting saturated KI solution as described[3].

Except for tetrabutylammonium bromide (BDH, Poole, Great Britain), all chemicals were from Reanal (Budapest, Hungary) and were of the highest purity available.

RESULTS

k' vs. methanol concentration relationships

Aqueous methanol eluents, pH 6.8, were prepared in the 0–70% (w/v) range using various amounts of 0.1 M citric acid and 0.2 M disodium hydrogen phosphate (McIlvain buffer) solutions. The k' value of C_1–C_{10} n-nitroalkanes, benzoic acid, ninhydrin, indandione, benzoyl-indandione (BID), diphacinon (DF) and chlorophacinon (CF) were determined and are plotted in Fig. 1. As usual, slightly curved log k'
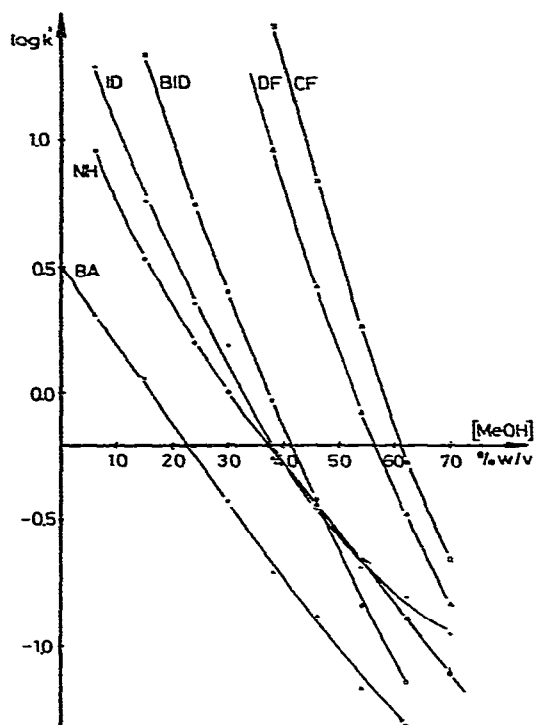
Fig. 1. Capacity factors of acetyl-indandiones as a function of the methanol concentration of the eluent. Column: RP-18. Eluent: pH 6.8 McIlvain buffer–methanol–water at 30°C. Abbreviations as in Table I: BA = benzoic acid: MeOH = methanol.

vs. [methanol] relationships were obtained over the relatively wide methanol concentration range. The curves of the three acetyl-indandione (BID, DF, CF) were parallel, and the elution order of benzoyl-indandione, indandione and ninhydrin is reversed at low and high methanol concentrations.

k' vs. pH relationships

Aqueous methanol eluents, 35, 50 and 60% (w/v) were prepared containing various amounts of 0.1 M citric acid and 0.2 M Na$_2$HPO$_4$ solutions, so that the pH could be varied between 2.4 and 6.9. The k' *vs.* pH curves of C$_2$ and C$_4$ nitroalkane, benzoic acid, ninhydrin, indandione and benzoyl indandione are shown in Fig. 2.

The k' *vs.* pH curves of benzoic acid, phenylacetic acid, cinnamic acid, benzilic acid and diphenylacetic acid in 50% (w/v) methanol eluents are shown in Fig. 3, while those of benzoyl-indandione, diphacinon and chlorophacinon in 60% (w/v) methanol eluents are in Fig. 4.

From Fig. 2 it is seen that at constant eluting strength of the successive eluents (constant k' values for the nitroalkanes) the retention of both benzoic acid and the indandiones changes with pH. The k' value of ninhydrin apparently does not depend on pH in the range tested. The k' value of indandione begins to decrease only at pH > 6, so the full curve could not be measured. The k' of benzoyl-indandione increases
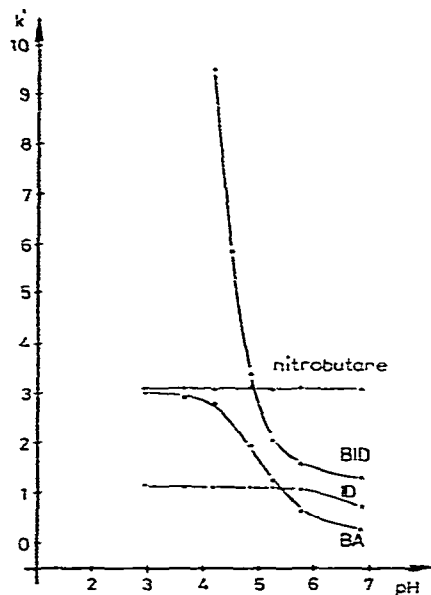
Fig. 2. Capacity factors of indandiones as a function of the pH in 35% (w/v) methanol eluents.
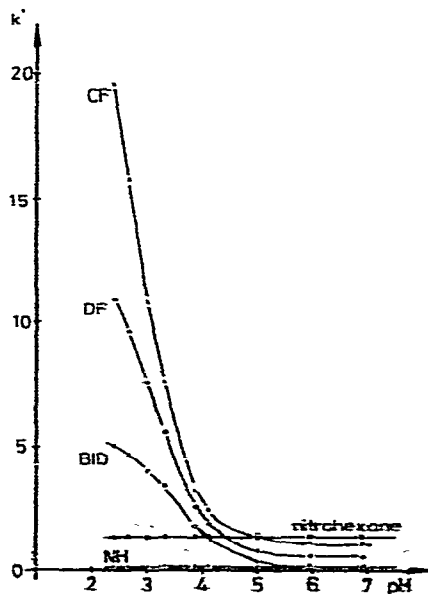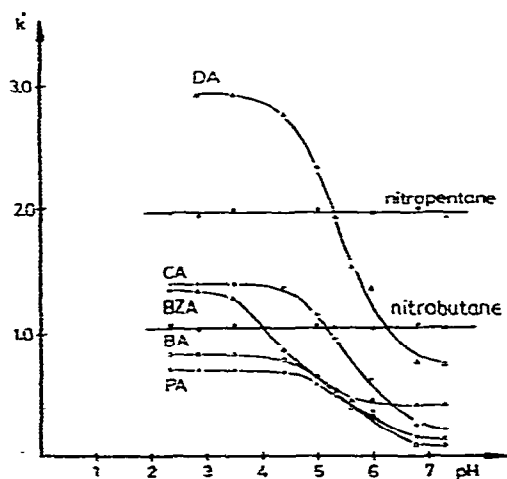
Fig. 3. Capacity factors of aromatic carboxylic acids as a function of pH in 50% (w/v) methanol eluents. BA = Benzoic acid, PA = phenylacetic acid; DA = diphenylacetic acid; BZA = benzilic acid; CA = cinnamic acid.

Fig. 4. Capacity factors of the acetyl-indandiones as a function of pH in 60% (w/v) methanol eluents.

rapidly at pH $<$ 4 and becomes so large that the plateau of the curve cannot be measured with sufficient precision.

However, the shape of both curves is similar to that of benzoic acid. In order to obtain the full curve the methanol content of the eluent was increased to 60 % (w/v) as shown in Fig. 4. Again, the k' value of ninhydrin does not depend on pH in the range tested, while the curves of the acetyl-indandiones show the characteristic acid behaviour. The final portion of the low pH plateau could not be measured, because the pH could not be decreased below 2.4 with the McIlvain buffer used.

In order to compare the acid strength of the acetyl-indandiones and a few more common organic acids, the pH dependence of a few aromatic carboxylic acids is shown in Fig. 3. The methanol content of the eluent was decreased to 50 % (w/v) in order to obtain suitable retention.

Horváth et al.[4], among others, showed that a close relationship existed between k' and the acid dissociation constant. Using an appropriately linearized form of their equation, the acid dissociation constant of a weak monoprotic acid can be determined from

$$k' = k'_{o} - K_{a} \cdot \frac{k' - k'_{-1}}{[H^{+}]}$$

where k'_{o} and k'_{-1} of the non-dissociated and dissociated forms of the acid, K_{a} is its acid dissociation constant and $[H^{+}]$ is the hydrogen ion concentration of the eluent.

Recently, Palalikit and Block[5] described a computer program for the determination of the acid dissociation constant using the second derivative of the k' vs. pH curve. They demonstrated that essentially identical pK_{a} values could be obtained by both the linearization and the second derivative method.

The apparent pK_{a} values obtained with the linearization method are listed in Table II. To allow for a visual comparison, the calculated pK_{a} values are plotted in

TABLE II

ACID DISSOCIATION CONSTANTS OF AROMATIC CARBOXYLIC ACIDS AND ACETYL-INDANDIONES DETERMINED BY THE LINEARIZATION METHOD[+]

pK_{a} values in pure water were obtained from refs. 6, 7.

Compound	pK_{a}			
	Pure water	35% (w/v) methanol	50% (w/v) methanol	60% (w/v) methanol
Benzilic acid	3.05		4.35	
Diphenylacetic acid	3.94		5.30	
Benzoic acid	4.20	4.95	5.45	
Phenylacetic acid	4.30		5.46	
Cinnamic acid	4.44		5.54	
Chlorophacinon				2.82
Diphacinon				3.21
Benzoyl-indandione		2.86		3.55
Indandione		>7		

Fig. 5 as a function of the methanol content of the eluent. The pK_a values of acetic acid as determined by Ong et al.[8] are also included in the figure to indicate the trend of the pK_a vs. [methanol] curves. It can be concluded that the acetyl-indandiones are fairly strong acids. The acid strength increases when an additional phenyl and p-chlorophenyl substituent is introduced into the molecule. It can be also seen that the apparent pK_a value increases with the methanol concentration of the eluent, and that this increase is approximately identical for all the aromatic carboxylic acids.
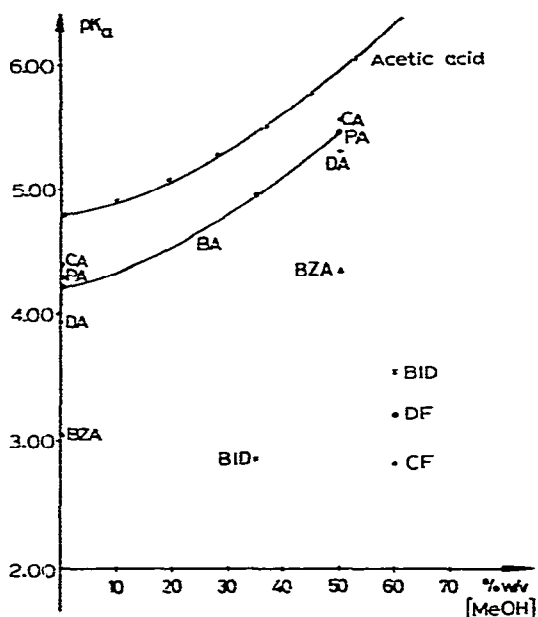

Fig. 5. Chromatographically determined pK_a values as a function of the methanol concentration of the eluent.

Effects of tetrabutylammonium bromide ion pair reagent

Since the acetyl-indandiones exhibited a pronounced acidic behaviour it seemed worthwhile to test the effects of tetrabutylammonium (TBA) cations upon their retention. 60% (w/v) methanol eluents pH 6.8, were prepared with $2.5 \cdot 10^{-4}$–$5 \cdot 10^{-2}$ M TBA$^+$. The k' vs. [TBA$^+$] curves of the acetyl-indandiones are shown in Fig. 6. For comparison, benzoic acid and xanthene-9-carboxylic acid were also included.

It can be seen that in the TBA$^+$ concentration range tested all curves are parallel, i.e., the acetyl-indandiones behave as "regular" aromatic carboxylic acids. At > 50 mM TBA$^+$, the k' vs. [TBA$^+$] curves tend to level off. It is interesting that the k' of the nitroalkanes (neutral components) begins to decrease at 50 mM TBA$^+$.

CONCLUSIONS

The k' vs. pH and k' vs. [TBA$^+$] relationships of acetyl-indandiones indicate that these compounds behave as monoprotic acids, and the chromatographically
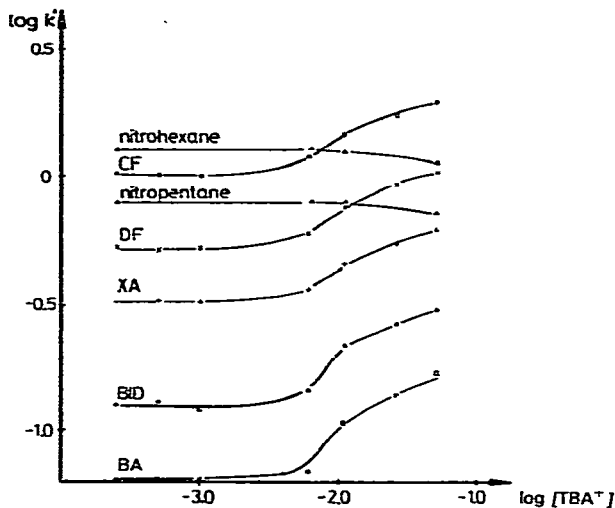
Fig. 6. Capacity factors of acetyl-indandiones as a function of the TBA$^+$ concentration (mol/l) in a 60% (w/v) methanol eluent pH 6.8. XA = Xanthene-9-carboxylic acid.
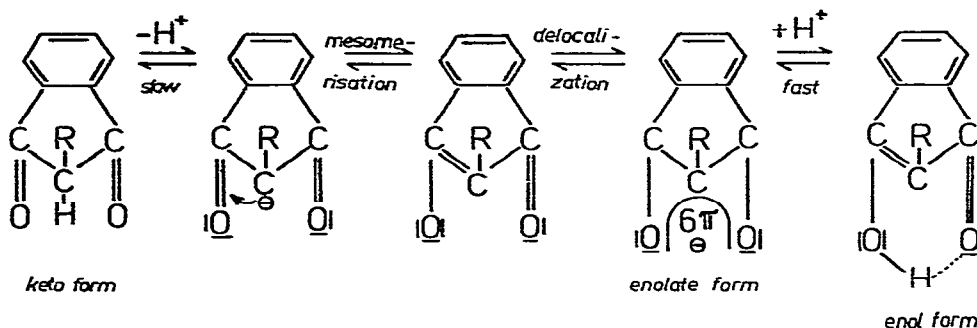

Fig. 7. The possible molecular structures of acetyl-indandiones at various pH values.

determined pK_a values prove that they are stronger acids than most of the common aromatic carboxylic acids. This behaviour can be rationalized as shown in Fig. 7.

At high pH the dissociated form is stabilized by the delocialized 6π/5 centrum bond-system as an enolate anion which readily participates in ion-pair formation with TBA$^+$. At low pH the anion is protonated and a neutral enol is obtained, which is stabilized by an enol-chelate ring. Some infrared spectroscopic studies support the existence of such an enol-chelate ring[9].

REFERENCES

1 Gy. Vigh, Z. Varga-Puchony, J. Hlavay and E. Papp-Hites, *J. Chromatogr.*, 214 (1981) 335.
2 Gy. Vigh, *J. Chromatogr.*, 117 (1976) 424.
3 G. Berendsen, P. Schoenmaker, L. de Galan, Gy. Vigh, Z. Varga-Puchony and J. Inczédy, *J. Liquid Chromatogr.*, 3 (1980) 1669.
4 Cs. Horváth, W.Melander and I. Molnár, *Anal. Chem.*, 49 (1977) 142.
5 D. Palalikit and J. H. Block, *Anal. Chem.*, 52 (1980) 624.

6 A. Albert and E. P. Serjeant, *The Determination of Ionization Constant. A Laboratory Manual*, Chapman and Hall, London. 1971.
7 G. Kortum, W. Vogel and K. Andrussow, *Dissociation Constants of Organic Acids in Aqueous Solutions*, Butterworths, London. 1961.
8 K. C. Ong, R. A. Robinson and R. G. Bates, *Anal. Chem.*, 36 (1964) 1971.
9 E. Grens, A. Grinvalde and J. Stradins, *Spectrochim. Acta Part A*, 31 (1975) 555.