Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 799 (2004) 179–183
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Short communication

Determination of the basicity of Mannich ketones by capillary electrophoresis

Zs. Dobos [a], T. Lóránd [b], F. Hollósy [a], B. Hallgas [a], D. Erös [a], Gy. Mészáros [a], Gy. Kéri [a], M. Idei [a,*]

[a] *Peptidbiochemical Research Group, Hungarian Academy of Sciences and Semmelweis University, Department of Medical Chemistry, Molecular Biology and Pathobiochemistry, Semmelweis Medical University, Puskin u. 9., H-1088 Budapest, Hungary*
[b] *Department of Biochemistry and Medical Chemistry, Faculty of Medicine, University Pécs, H-7624 Pécs, Szigeti út 12, Hungary*

Received 12 June 2003; received in revised form 17 October 2003; accepted 17 October 2003

Abstract

Capillary electrophoretic (CE) method to characterise Mannich ketones (MKs) containing morpholine moiety was developed. Basicity ($pK_{a,exp}$) of the MKs was characterised by measured data derived from the electrophoretic mobility values obtained in the CE separation. The MKs were found to be weaker bases than the parent morpholine molecule itself and the experimentally determined basicity values proved to be dependent on the chemical structure of the MKs. Since the basicity of the MKs has an influence on their reactivity and biological activity it seems to be useful to determine experimentally the $pK_{a,exp}$ values of the newly synthesised compounds to support rational drug design or screening of the molecule libraries.

Keywords: Basicity; Mannich ketones

1. Introduction

Determination of physico-chemical properties of the drug candidate molecules is essential at the early stage of drug research. The activity of several molecules of biological/pharmacological interest depends on the presence of charged groups on the molecule. It has been demonstrated that the passage of many drugs into the cells and across the membranes is function of the environmental pH and the pK_a of the drug [1]. Therefore, the dissociation constant may be a parameter of pivotal importance for understanding the biological activity. Furthermore, the knowledge of this constant of the drug candidate molecules is very important to provide information on biological uptake, biological transport, and environmental fate: absorption, distribution, metabolism, excretion (ADME). The pK_a may offer support to explain chemical phenomena, such as reactivity, reaction rates, and salt formation; or may support the development of formulation or separation methods [2,3]. The dissociation constant may be important from the point of view of receptor binding or the decomposition of the molecule within the cell [4]. Since the knowledge of the relationship between dissociation constants and structure may prove to be useful in rational drug design studies and in estimation of the expected biological (pharmaceutical) properties of molecules, it is worth to screen newly synthesised compounds for their pK_a values. A concern of the analysis process in the discovery phase is the small amount and great number of materials to be analysed. The new compounds usually exist in small quantities, therefore a step towards a significant improvement in discovery productivity is achieved if the pK_a of less than a milligram of material can be determined [4]. The low solubility of many pharmaceutical and agricultural compounds in water precludes convenient, classical pK_a determinations [2,4]. These difficulties and demands enhance the application of fast, automatisable analytical methods demanding small quantities for pK_a determination.

Capillary electrophoresis (CE) offers good possibility for separation of wide range of analytes [5–8]. Recently, capillary electrophoresis has been introduced as a method for convenient and precise determination of aqueous pK_a. Selectivity, small sample- and time demands and possibility for

* Corresponding author. Fax: +36-12667480.
E-mail address: miki@puskin.sote.hu (M. Idei).

1570-0232/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2003.10.038

unattended sample analysis in aqueous solutions are among the main reasons [4]. The CE method applied relies on the principle that an analyte exhibits an electrophoretic mobility continuum versus pH [2]. In its neutral state, the analyte has no electrophoretic mobility, while in its fully charged state it has the maximum electrophoretic mobility. Intermediate mobilities are the function of the dissociation equilibrium [4]. Therefore, electrophoretic mobility can be expressed as a function of the pK_a of the analyte and the pH of the electrophoresis buffer [9,10]. There are several experimental approaches, including linear and non-linear models (the non-linear one is considered to be the simplest and most precise one [7]) applicable for the experimental determination of p$K_{a,exp}$ values [3,4,7,9–17].

The family of the α,β-unsaturated ketones is known to possess antimicrobial (antibacterial, antifungal) effects. A number of Mannich ketones (MKs)—as a special type of this family—could show more selective toxicity toward microorganisms than the parent unsaturated ketones [18]. Beyond their antimicrobial effect, several MKs are described to have cytotoxic activity [19–22]. All these compounds are potential alkylating reagents. The fused and unsaturated MKs offer one or two sites for alkylation, respectively. The mode of their action is based on their reaction with thiol groups of the enzymes [18]. Their breakdown can afford in 1,2 elimination (deamination) reactive vinyl ketones, which can undergo addition reaction with cellular thiols [18]. The thiol alkylating action of these compounds may contribute to their biological activity, because the presence of free thiols is essential for the viability of the cells. The vinyl ketones produced during the breakdown of the MKs have much higher affinity toward thiols than hydroxyl- and amino groups present in the nucleic acids, therefore, they do not show the mutagenic side effect of some alkylating agents used in the therapy [23]. The rate of deamination is inversely proportional to the basicity of the amine side chain of the MKs: the weaker the basicity the higher the deamination rate [18,22].

Our aim was to determine the pK_a values of various MKs containing morpholinyl moiety in each molecule. Therefore, in this work the electrophoretic mobility values versus pH curves have been determined to obtain p$K_{a,exp}$ values—derived from CE measurements. Beyond the experimental values, predicted basicity (p$K_{a,ACD}$), based on the chemical structure of the MKs have been calculated too. Both the experimental and predicted basicity values have been compared with the pK_a of the parent morpholine molecule.

2. Materials and methods

2.1. Mannich ketones

MKs (Table 1) have been prepared in our laboratory by the classical Mannich reaction applying ethanol as solvent and HCl as catalyst [18]. The products were purified through recrystallisation, and Mannich bases were liberated and purified using very mild conditions. Then they were precipitated with methanolic HCl. NMR measurements were done on the Mannich bases to validate their structure [18]. Every compound were analysed by a Waters LC/MS system equipped with a Waters 996 DAD UV detector, and a Micromass ZMD MS detector. Purity of the compounds was better than 95% in each case [24,25].

2.2. Reagents

HPLC grade chemicals were applied. Triethylamine and phosphoric acid were purchased from Fluka (Buchs, Switzerland), acetonitrile (ACN) and acetone from Chemolab (Budapest, Hungary). Solutions were prepared of deionised, bacteria free water made by Elgastat UHP system (Elga Ltd., Bucks, England).

2.3. Capillary electrophoresis

Analysis of the samples were performed with Bio-Rad Biofocus 3000 capillary electrophoresis system; capillary: uncoated silica capillary (50/40 cm × 50 μm; Polymicro Technologies, Phoenix, AZ, USA); temperature, 22 °C; injection with pressure (20 psi s); voltage, 25 kV; polarity, positive polarity; λ = 200–320 nm with 5 nm steps. Running buffers, 0.083 M triethyl ammonium phosphate (TEAP) of equally spaced pH values (pH = 2.0, 3.0, 4.0, 5.0, 6.0 and 7.0) and of the same ionic strength were used. Acetone was used to determine mobility of the electroosmotic flow (μ_{EOF}); sample concentration, 1 mg/ml acetonitrile–water (1:1). Solutions were filtrated before injection through a 0.2 μm Millipore filter. Capillary was regenerated by sequential washing with 0.1N NaOH and water daily before the first analysis and it was rinsed with buffer between runs. Capillary was rinsed with 0.1N NaOH, water and running buffer between the different analytes. Four injections have been performed from each sample to control repeatability.

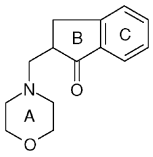
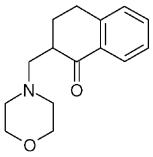
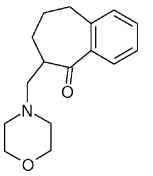
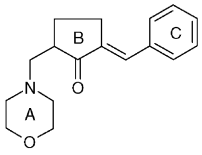
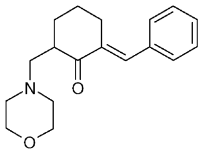
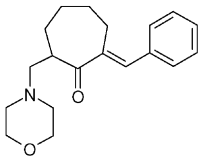
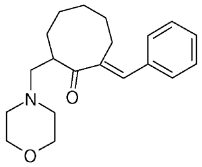
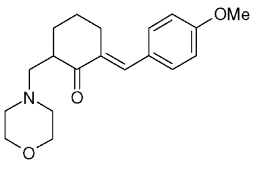
2.4. Determination of the p$K_{a,exp}$ values by capillary electrophoresis

There are several experimental approaches, linear and non-linear models applicable for the experimental determination of pK_a values [2,3,7,10,13–16]. In this work the pK_a values of the analytes were calculated with the well known and widely applied [2,3,7,13,14,26] non-linear regression method considered to be the most precise and simple one to describe the connection between the pK_a, pH and mobility. This relationship can be written for bases:

$$\mu_e = \frac{\mu_b}{10^{(pH-pK_a)} + 1} \quad (1)$$

where μ_e is the electrophoretic mobility at a given pH and μ_b is the mobility of the fully ionised species determined at low pH (pH = 2.0; it was controlled that all of the Mannich

Table 1
Electrophoretic mobilities, $pK_{a,exp}$ and $pK_{a,ACD}$ values of the Mannich ketones investigated

MKs investigated	Structures	Electrophoretic mobility (μ_e) ($\times 10^6$, $cm^2/(V\,s)$)[a]						$pK_{a,exp} \pm$ S.D.[b]	pK_a determined by ACD	Parameters of the fitting	
		pH = 2.0	pH = 3.0	pH = 4.0	pH = 5.0	pH = 6.0	pH = 7.0				
MK1		132.4	131.8	126.6	90.28	23.35	2.775	5.15 ± 0.081	6.94	S.D. F_{prob} R	0.0699 <0.0001 0.99930
MK2		152.5	150.0	129.1	53.97	7.913	0.8300	4.73 ± 0.016	6.89	S.D. F_{prob} R	0.0137 <0.0001 0.99997
MK3		124.7	124.1	118.4	81.39	19.72	2.299	5.15 ± 0.070	6.83	S.D. F_{prob} R	0.0607 <0.0001 0.99948
MK4		115.6	115.3	112.5	90.59	30.71	4.036	5.56 ± 0.023	6.83	S.D. F_{prob} R	0.0772 <0.0001 0.98235
MK5		112.1	110.9	100.1	50.52	8.489	0.9109	4.91 ± 0.022	6.85	S.D. F_{prob} R	0.0189 <0.0001 0.99995
MK6		115.4	112.9	92.93	33.55	4.541	0.4707	4.58 ± 0.282	6.88	S.D. F_{prob} R	0.0167 <0.0001 0.99020
MK7		106.6	104.8	89.27	36.00	5.167	0.5402	4.71 ± 0.020	6.88	S.D. F_{prob} R	0.0173 <0.0001 0.99996
MK8		93.76	93.55	91.44	74.59	26.24	3.507	5.63 ± 0.022	6.90	S.D. F_{prob} R	0.1867 <0.0001 0.99547

Parameters of the correlation (correlation coefficient, S.D. value of the regression function) are given in the last column of the table (B is not significantly different from 1.000).

[a] Average value of the mobility determinations is shown for each MK at each pH applied. Number of repetitions at each pH 4, R.S.D. of the mobility (μ_e) values <3% for each pH value. For experimental details see Section 2.3.

[b] $pK_{a,exp} \pm$ S.D. values were determined with fitting based on the linearized form of Eq. (1): $y = A + Bx$ where $y = \lg((\mu_b/\mu_e) - 1)$, $x =$ pH, and $A = -pK_a$.

ketones investigated here reached their maximum mobility at this pH value). The linearized form of Eq. (1) makes possible the safe determination of pK_a,exp based on mobility determination performed at limited number of pH values:

$$\lg\left(\frac{\mu_b}{\mu_e}-1\right)=\mathrm{pH}-\mathrm{p}K_a \tag{1b}$$

Electrophoretic mobility (μ_e) values were calculated according to the following equations:

$$\mu_e=\mu_{app}-\mu_{EOF} \tag{2}$$

$$\mu_{app}=\frac{L_C L_D}{t_{app} V} \tag{3}$$

where μ_{app} is the apparent mobility, μ_{EOF} the electroosmotic flow, L_C the total length of the capillary, L_D the distance between the injection point and the detector, t_{app} the migration time of the analyte and V is the applied voltage.

2.5. Determination of software calculated p$K_{a,ACD}$ values

The software-based calculation based on the chemical structure of the MKs was performed with the help of ACD/Labs (Ver. 6.00, 2002) (Advanced Chemistry Development Inc. Toronto, Ont., Canada).

3. Results and discussion

Electrophoretic mobilities (μ_e) of the MKs were measured at an equally spaced pH range between 2.0 and 7.0, mobility values of the MKs have been determined in their fully protonated, deprotonated and partially protonated forms. At each pH the number of repetition was 4, R.S.D. of the mobility values (μ_e) was less than 3% for each of the MKs investigated. Average value of the four mobility determination is shown for each MK and for each pH in the Table 1. Mobility versus pH curves determined in a given composition can be applicated to determine optimum pH for separation in that buffer.

The pK_a can be obtained from the mobility values (μ_e) determined at different pHs as intercept of the $\lg((\mu_b/\mu_e)-1)$ versus pH fitting (based on the linearized form of Eq. (1). The p$K_{a,exp}$ ± S.D. values obtained by the fitting, and parameters of the regression analysis (standard deviations and correlation coefficients of the fittings) are also given in Table 1. Cross validation of the [$\lg((\mu_b/\mu_e)-1)$] versus pH fitting performed with the "leave on out" method of Allen [27] has revealed the presence of outliner points in neither case of the MKs investigated.

Experimentally determined p$K_{a,exp}$ values can be applied in quantitative structure–activity relationship (QSAR) calculations [28] as p$K_{a,exp}$s may reveal how the chemical structure can influence the basicity of the molecule. A close correlation between the basicity and cytotoxic activity was demonstrated in the case of some unsaturated MKs by Loránd et al.: the weaker the basicity of the MKs (i.e. the lower its p$K_{a,exp}$), the higher the deamination rate and the greater the cytotoxic activity [18]. The comparison of the p$K_{a,exp}$ values of the MKs and the pK_a value of the parent morpholine base may provide important information for rational drug design, it may reveal how the building in of the morpholine base into a Mannich ketone molecule could change the basicity of the parent morpholine base. Based on these considerations p$K_{a,exp}$ values of the newly synthesised MKs were compared to that of the parent morpholine. This comparison proved that there was a decrease in the p$K_{a,exp}$ value comparing to the basicity of the parent morpholine base (p$K_{a,morpholine}$ = 8.30; [29]) both in the case of fused (Table 1, MK1–3.) and unsaturated (Table 1, MK4–8.) molecules. It means that the incorporation of the morpholinyl group into the molecules resulted in a decrease of its p$K_{a,exp}$ in each case. These values proved to be dependent on the chemical structure of the molecules. The decrease of the p$K_{a,exp}$ was 3 units in the case of the fused MK with five-membered B ring (analyte MK1), while it was greater for the MK with six-membered B ring (analyte MK2), and a small increase was observed in the case of the seven-membered B ring (analyte MK3). Similarly, decrease of the p$K_{a,exp}$ was found in the case of the unsaturated MKs, too. An increase in the carbon number of ring B (from C_5 to C_7) was followed by a decrease in the basicity, and only a small increase was found at the eight-membered B ring. The incorporation of a methoxy group into ring A increased the basicity (increased the p$K_{a,exp}$) of the molecule with six-membered B ring (analyte MK8), its p$K_{a,exp}$ slightly exceeded the basicity of the five-membered B ring (analyte MK4).

Software-based calculation of the basicity of MKs (Table 1, p$K_{a,ACD}$ data determined by the ACD software) confirmed the experimental results. The involvement of the morpholinyl moiety in the MK molecule resulted in the decrease of the basicity (compared to the parent morpholine). However, comparison of the experimentally determined and software calculated values (p$K_{a,exp}$ and p$K_{a,ACD}$, respectively) revealed discrepancies between the measured and calculated values. The calculated p$K_{a,ACD}$ values were higher than the experimental ones for each MK. Contrary to the experimental method applied, the software-based calculation method was not able to follow the pK_a changes caused by the structural differences (e.g. change of the carbon number of the ring B connected to the morpholinyl moiety, MK5–8). These differences between the experimental and predicted pK_a values suggest the importance of the determination of the experimental pK_a values by high through-put method.

4. Conclusions

A suitable CE method was developed to determine p$K_{a,exp}$ values of Mannich ketones. Experimental basicity (p$K_{a,exp}$)

values were obtained from mobilities determined at different pH values. Both the experimental and software calculation proved, that incorporation of the morpholine into the MK resulted in the decrease of basicity. In each cases, the MKs were found to be weaker bases than the parent morpholine molecule itself and the value of the $pK_{a,exp}$ proved to be dependent on the chemical structure (carbon number of the B ring). Considering the fact, that the basicity of the MKs had an influence on their reactivity and biological activity it seems to be useful to determine the basicity values of the newly produced molecules experimentally to support rational drug design or screening of molecule libraries.

Acknowledgements

This work was supported by the following grants: OTKA 37188, 32415, FKFP 386/2000, ETT 232/2001 and NKFP 1/047 (Medichem), NKFP 1/A/0020/2002 and OTKA T 30261 and Hungarian State Eötvös Scholarship.

References

[1] Y. Ishihama, Y. Oda, N. Asakawa, J. Pharm. Sci. 83 (1994) 1500.
[2] S.J. Glück, J.A. Cleveland Jr., J. Chromatogr. A. 680 (1994) 49.
[3] G.A. Caliaro, C.A. Herbots, J. Pharm. Biomed. Anal. 26 (2001) 427.
[4] J. Barbosa, D. Barrón, E. Jiménez-Lozano, V. Sanz-Nebot, Anal. Chim. Acta 437 (2001) 309.
[5] G.M. Khaledi, High Performance Capillary Electrophoresis, Wiley, New York, 1998.
[6] R. Weinberger, Practical Capillary Electrophoresis, Academic Press, New York, 2000.
[7] S.J. Gluck, K.P. Steele, M.H. Benkö, J. Chromtogr. A. 745 (1996) 117.
[8] C. Hansch, A. Leo, Exploring QSAR, vol. 1, American Chemical Society, Washington, DC, 1995.
[9] I. Canals, E. Bosch, M. Rosés, Anal. Chim. Acta 458 (2002) 355.
[10] J.A. Cleveland, M.H. Benko, S.J. Gluck, Y.M. Walbroehl, J. Chromatogr. A. 652 (1993) 301.
[11] D. Koval, V. Kasicka, J. Jiracek, M. Collinsova, T.A. Garrow, J. Chromatogr. B. 770 (2002) 145.
[12] S.F.Y. Li, Capillary electrophoresis, J. Chromatogr. Lib. 52 (1994) 547 (Elsevier, Amsterdam).
[13] J.M. Beckers, F.M. Everaerts, M.T. Ackermans, J. Chromtogr. 537 (1991) 407.
[14] J. Cai, J.T. Smith, Z.E. Rasi, J. High Resolut. Chromtogr. 15 (1992) 30.
[15] J.A. Cleveland, M.H. Benko, S.J. Gluck, Y.M. Wahlbroehl, J. Chromatogr. A 652 (1993) 301.
[16] S.J. Gluck, J.A. Cleveland, J. Chromatogr. A. 680 (1994) 43.
[17] A. Jouyban-Gharamaleki, M.G. Khaledi, B.J. Clark, J. Chromatogr. A. 868 (2000) 277.
[18] T. Lóránd, B. Kocsis, P. Sohár, G. Nagy, Gy. Kispál, H.G. Krane, H. Scmitt, E. Weckert, Eur. J. Med. Chem. 36 (2001) 705.
[19] J.R. Dimmock, A.M. Gureshi, L.M. Noble, P.J. Smith, H.A. Baker, J. Pharm. Sci. 65 (1976) 38.
[20] J.R. Dimmock, M. Chamankhah, T.M. Allen, S. Halleran, Pharmazie 50 (1995) 221.
[21] J.R. Dimmock, M. Chamankhah, A. Seniuk, T.M. Allen, G.Y. Kao, S. Halleran, Pharmazie 50 (1995) 668.
[22] J.R. Dimmock, K.K. Sidhu, M. Chen, R.S. Reid, T.M. Allen, G.Y. Kao, G.A. Truitt, Eur J. Med. Chem. 28 (1993) 313.
[23] J. Cairns, Nature 286 (1980) 176.
[24] F. Hollósy, T. Lóránd, L. Örfi, D. Erös, Gy. Kéri, M. Idei, J. Chromtogr. B. 768 (2002) 361.
[25] F. Hollósy, T. Lóránd, L. Örfi, D. Erös, Gy. Kéri, M. Idei, J. Liq. Chromtogr. Rel. Technol. 25 (7) (2002) 1129.
[26] S.C. Smith, M.G. Khaledi, Anal. Chem. 65 (1993) 193.
[27] D.M. Allen, Technometrics 16 (1974) 125.
[28] D. Erös, I. Kövesdi, L. Örfi, K. Takács-Novák, Gy. Acsády, Gy. Kéri, Curr. Med. Chem. 9 (2002) 1819.
[29] R. Stuart, The proton, applications to organic chemistry, in: Organic Chemistry, vol. 46, Academic Press, New York, 1985.