

ASSOCIATION OF BENFLURONE IN AQUEOUS SOLUTIONS STUDIED BY MEASURING THE ACTIVITY OF CHLORIDE IONS

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Received September 9th, 1985

The conventional activity of chloride ions was measured by an ion-selective electrode at 25°C in aqueous solutions of benflurone, *i.e.* 5-(2-(N,N-dimethylamino)ethoxy)-7-oxo-7H-benzo(c)fluorene hydrochloride, without or with additions of KCl. The results suggest a gradual association of benflurone; the formation of a hetero-associate, $(\text{BH}^+)_2\text{Cl}^-$, followed by higher associates was evaluated in the measured range up to 0.1 mol l^{-1} .

Benflurone was prepared and tested by Křepelka *et al.*^{1,2} as antineoplastically efficient substance. Some anomalies were found in the properties of its aqueous solutions, partition equilibria, and adsorption parameters³. Thus, we were led to the investigation of its association in dilute aqueous solutions, which form the subject of the present work. Since benflurone is hydrochloride of an organic base (aminium chloride) of the type BHCl , we followed its association by measuring the conventional activity, a_{Cl^-} , of Cl^- ions in its solutions with a commercial chloride-selective electrode, although we were interested primarily in the behaviour of the organic cation BH^+ . Such a procedure is not unusual: measurement of the activity of oppositely charged ions with an ion-selective electrode was, *e.g.*, employed in studying the micellization of various ionogenic surface active substances⁴⁻⁶, and our preceding work⁷ was devoted to the study of association of hydrochlorides of basic carbamates by measuring the activity of Cl^- anions.

EXPERIMENTAL

Chemicals

Benflurone, *i.e.* 5-(2-(N,N-dimethylamino)ethoxy)-7-oxo-7H-benzo(c)-fluorene hydrochloride (compound V in ref.²) was of the same origin and purity as in ref.³; KCl was of reagent grade and water was redistilled.

Measurement of activity of Cl^- ion in benflurone solutions at 25°C
We employed a chloride-ion selective electrode of the type Crytur 17-27 with a solid membrane

in combination with a saturated calomel reference electrode. Provision was made to prevent contamination of the measured solution with Cl^- ions. Electrodes with a liquid membrane did not prove well⁷. The electromotive force of the cell consisting of the two electrodes immersed in the measured solution of benflurone or in a KCl calibration solution was measured with an accurate digital pH meter Radelkis OP-208 (to ± 0.1 mV). Mean molal activity coefficients, γ_{\pm} , of KCl calibration solutions were taken from the literature⁸. Although the concentrations in our work are expressed in mol l^{-1} units rather than mol kg^{-1} , the difference is negligible when concentrations up to 0.1 mol l^{-1} are considered. The mean activity of KCl, a_{KCl} , is given as $c_{\text{KCl}}\gamma_{\pm}$; practically linear calibration functions $E = f(\log a_{\text{KCl}})$ were obtained from electromotive force (E) data for the calibration solutions.

Measurements of the dark orange solutions of benflurone were complicated by visible gradual adsorption on the membrane of the chloride electrode and on the diaphragm of the calomel electrode, resulting in changes of the electromotive force. Therefore, constant experimental conditions were observed⁷ and the measurements were repeated many times. Calibration was carried out always twice, *i.e.* immediately before the measurement of the benflurone solution and after it, and results differing by more than 1–2 mV were discarded. Correct functioning of the electrode couple was also checked by evaluating the slopes $\Delta E/\Delta \log a_{\text{KCl}}$ of the calibration straight lines. In cases where the slope differed from the Nernstian -59.2 mV by more than ± 2 mV, the electrode couple was discarded or regenerated by prolonged washing in dilute KCl solution. The concentration of the benflurone solutions was 0.005 – 0.1 mol l^{-1} . More concentrated solutions precipitate after some time, while more dilute solutions may give rise to errors caused by the liquid junction potential. It was not necessary to change their pH value to prevent acid-base hydrolysis (as in the preceding work⁷).

RESULTS AND DISCUSSION

From the E values measured in benflurone solutions and from the calibration diagrams $E = f(\log a_{\text{KCl}})$ the activities of Cl^- ions in these solutions were determined on the common assumption that a_{Cl^-} is equal to the mean molal activity of KCl in the calibration solution. Thus, the values of a_{Cl^-} are conventional in character. The values from a large number of measurements were graphically smoothed out to obtained data for rounded concentrations of benflurone c_{BHCl} at 25°C as follows (the estimated error in $\log a_{\text{Cl}^-}$ is about ± 0.02):

c_{BHCl} , mol/l	0.005	0.01	0.015	0.02	0.03	0.04	0.05	0.06	0.07
a_{Cl^-}	0.0044	0.0075	0.0105	0.0135	0.019	0.0245	0.0295	0.035	0.040
c_{BHCl}	0.08	0.09	0.10						
a_{Cl^-}	0.0445	0.0495	0.054						

Conventional activity coefficients of Cl^- ions in benflurone solutions were calculated from the measured activities as

$$\gamma_{\text{Cl}^-} = a_{\text{Cl}^-}/c_{\text{BHCl}} \quad (1)$$

The dependence of $\log \gamma_{\text{Cl}^-}$ on the square root of c_{BHCl} and the scatter of the experimental data is shown in Fig. 1.

To further study the association of benflurone, we also determined the values of a_{Cl^-} and γ_{Cl^-} in solutions of benflurone containing KCl of constant total concentration $c_{\text{BHCl}} + c_{\text{KCl}}$. The values of γ_{Cl^-} were in this case calculated as

$$\gamma_{\text{Cl}^-} = a_{\text{Cl}^-} / (c_{\text{BHCl}} + c_{\text{KCl}}). \quad (2)$$

The resulting plot of $\log \gamma_{\text{Cl}^-}$ on c_{BHCl} is shown in Fig. 2 for two series of solutions, for the first of which $c_{\text{BHCl}} + c_{\text{KCl}} = 0.05 \text{ mol l}^{-1}$ and for the second 0.08 mol l^{-1} . Here, the scatter of the experimental data is smaller, indicating a better reproducibility at constant concentration of chlorides.

Since c_{BHCl} in equations (1) and (2) is the total concentration of benflurone, the values of γ_{Cl^-} reflect possible association and may perhaps be considered as apparent.

Formally, benflurone is an electrolyte, BH^+Cl^- , of the same valency type as KCl used in calibration. However, Fig. 1 indicates different behaviour of the two electrolytes as compared with the Debye-Hückel limiting law. The mean activity coefficient of KCl shows with increasing concentration a positive deviation from the limiting law, as is the case with other nonassociated electrolytes. In contrast, the activity coefficient of Cl^- ions measured in benflurone solutions assumes markedly

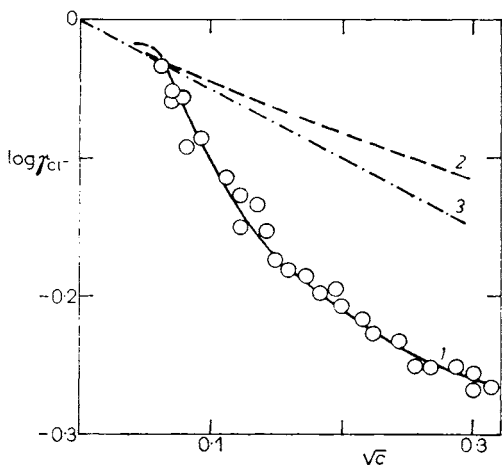


FIG. 1

Concentration dependence of conventional activity coefficient of Cl^- ions in 1 solutions of benflurone (BHCl) compared with 2 the mean activity coefficient⁸ of KCl and with 3 the Debye-Hückel limiting law

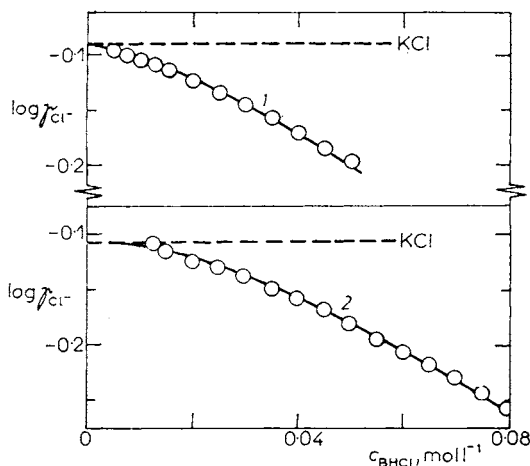


FIG. 2

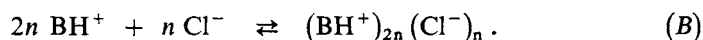
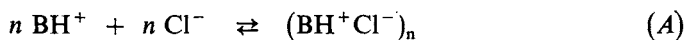
Conventional activity coefficient of Cl^- ions in mixed solutions of benflurone and KCl at constant total concentration $c_{\text{BHCl}} + c_{\text{KCl}}$: 1 0.05 mol l^{-1} , 2 0.08 mol l^{-1}

lower values than corresponding to the limiting law. This is obviously caused by association of benflurone at higher concentrations. At low concentrations (below 0.005 mol l^{-1}), $\log \gamma_{\text{Cl}^-}$ shows another anomaly: it does not coincide with the straight line of the limiting law, but intersects it as shown in Fig. 1. A rather similar concentration dependence was also found for the mean activity coefficients of some associated aminium bromides⁹.

The data for solutions of benflurone with KCl at constant total concentration (Fig. 2) can be judged on the basis of the so-called Harned rule^{10,11}. This refers to mean activity coefficients, but can be, in view of their definition, applied to our $\log \gamma_{\text{Cl}^-}$ data as well. Accordingly, the dependences in Fig. 2 should be linear if the electrolytes were nonassociated. In reality, however, they are nonlinear even at low total concentration $c_{\text{BHCl}} + c_{\text{KCl}}$, as evidence for association. Since KCl does not associate, the effect must be attributed to benflurone or its cation BH^+ . The curves in Figs 1 and 2 do not show a sharp break which could be interpreted unambiguously as critical micellization concentration¹²; especially the smoothness of the curves in Fig. 2 suggests a gradual association in the studied concentration range.

We therefore attempted to evaluate at least approximately the stoichiometry and equilibrium parameters of the associate(s). The first possibility considered is a simple self-association of BH^+ cations without participation of Cl^- anions. This would be manifested by a lowering of the activity of Cl^- caused by an increase in the ionic strength of the solution. Assuming complete association of BH^+ cations to $(\text{BH})_n^+$ polycations in the presence of free Cl^- ions, we obtain for the ionic strength $I = c_{\text{BHCl}}(n + 1)/2$. If we use the common equation $\log \gamma_{\text{Cl}^-} = -0.51 \sqrt{I}/(1 + \sqrt{I})$, we have to set at least $n = 20$ to obtain agreement between calculated $\log \gamma_{\text{Cl}^-}$ values and those following from the measured activities given above. In the case of such a big associate, however, the common concept of ionic strength could hardly be used and the attraction of oppositely charged ions would be considerable even if the ionic associates were much smaller^{4,5}. Therefore, the mentioned result is at variance with simple self-association and suggests the formation of heteroassociates of BH^+ cations with Cl^- anions.

The formation of heteroassociates was evaluated on the basis of an essentially opposite simplifying assumption. Use was made of the measured conventional activities a_{Cl^-} in benflurone solutions; the concentration of free Cl^- ions was set approximately equal to a_{Cl^-} . Even with regard to this simplification, it can be seen from the found values of a_{Cl^-} that at higher concentrations of benflurone more than one third of Cl^- ions is bound. It is therefore necessary to consider the formation of heteroassociates containing one Cl^- ion per less than three BH^+ ions according to the following equilibria



Equilibrium (A) leads to the equation

$$c_{\text{BHCl}} - [\text{Cl}^-] = nK[\text{Cl}^-]^{2n}, \quad (3)$$

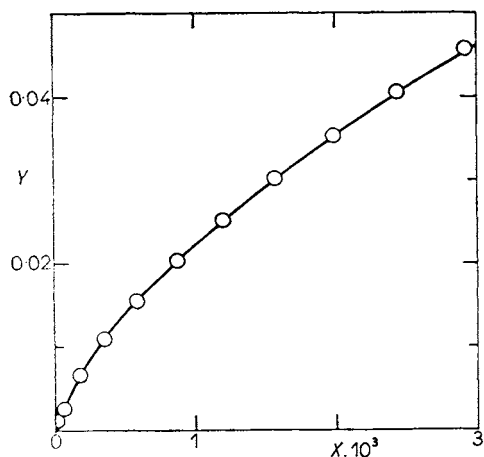


FIG. 3

Equation (3) as a plot of $c_{\text{BHCl}} - [\text{Cl}^-]$ versus $[\text{Cl}^-]^{2n}$ ($n = 2$)

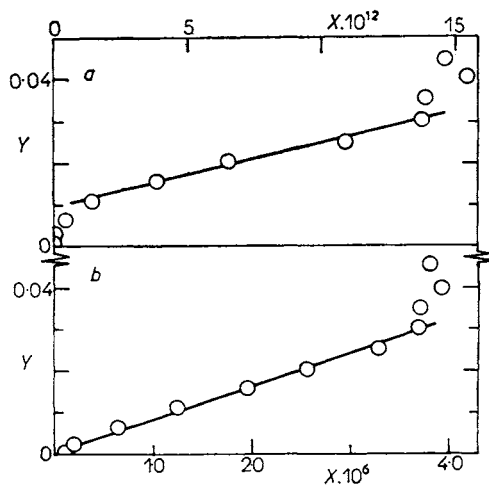
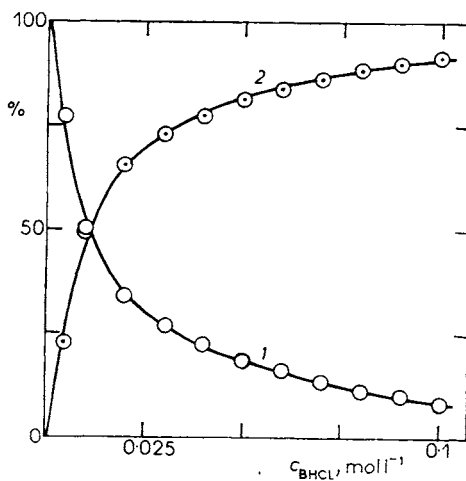


FIG. 4

Equation (4) as a plot of $c_{\text{BHCl}} - [\text{Cl}^-]$ versus $(2[\text{Cl}^-] - c_{\text{BHCl}})^{2n} [\text{Cl}^-]^n$, $a n = 2$, $b n = 1$

FIG. 5
Percentage of benflurone as free BH^+ cations and as associates according to equilibrium (B), 1 BH^+ , 2 $(\text{BH}^+)_{2n} (\text{Cl}^-)_n$



whereas equilibrium (B) implies that

$$c_{\text{BHCl}} - [\text{Cl}^-] = nK(2[\text{Cl}^-] - c_{\text{BHCl}})^{2n} [\text{Cl}^-]^n, \quad (4)$$

where K is the equilibrium constant of formation of the heteroassociate, corresponding to either of the two reactions.

If the association of benflurone proceeded according to reaction (A), a plot of $c_{\text{BHCl}} - [\text{Cl}^-]$ against $[\text{Cl}^-]^{2n}$ would give a straight line according to equation (3). The plot for $n = 2$ shown in Fig. 3, however, deviates from linearity, and this still more for $n > 2$. Hence, the equilibrium (A) does not describe well the association of benflurone, *i.e.* the formation of uncharged associates appears improbable. Since the measured distribution coefficient³ of benflurone at $\text{pH} > 7$ is very high, a possible uncharged associate should also be well extractable into the organic phase even from an acidic solution. We attempted to extract the orange benflurone from aqueous 0.01M HCl solution; it passed into octanol only to a small extent and was practically not extracted at all with chloroform. Since the latter is known to dissolve ion pairs, our finding is at variance with possible formation of heteroassociates bearing no charge.

The last possibility is the formation of charged heteroassociates of benflurone according to equilibrium (B). This should be manifested by a linear course of the function (4) in the coordinates used in Fig. 4. Its lower part indeed shows a linear course for $n = 1$ except for points corresponding to higher concentrations ($c_{\text{BHCl}} > > 0.07 \text{ mol l}^{-1}$). Thus, the most probable possibility is the formation of the heteroassociate $(\text{BH}^+)_2\text{Cl}^-$, which at higher concentrations undergoes gradual dimerization (Fig. 4, upper part) and possibly forms higher associates. The equilibrium constant of formation of $(\text{BH}^+)_2\text{Cl}^-$ was evaluated approximately from the slope of the lower linear portion of the curve in Fig. 4 as $K = 8000 \text{ mol}^{-2} \text{ l}^2$. Assuming the validity of equilibrium (B), we obtain the concentration of free benflurone cations $[\text{BH}^+] = 2[\text{Cl}^-] - c_{\text{BHCl}}$ regardless of the size of the associates. The percentage of free and associate-bound cations of benflurone thus calculated at various total concentrations c_{BHCl} is illustrated in Fig. 5. This shows the high degree of association, which necessarily influence the physico-chemical properties and biological accessibility of benflurone even in rather dilute aqueous solutions.

The authors are indebted to Dr J. Křepelka, Research Institute of Pharmacy and Biochemistry, for furnishing benflurone.

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Translated by K. Micka.