Ionization constants of cholinesterase-reactivating bispyridinium aldoximes

SIR,—Since most pharmacologically active substances contain acidic or basic molecular groups, or both, which are ionized to a different degree at physiological pH, ionization constants are of particular biological importance.

In a number of substances with pK_a values within the physiological range, biological activity has been shown to depend on the pH of the medium, thus reflecting the actual concentrations of the active molecular species (for a review see Albert, 1960).

Quaternary pyridine aldoximes are reactivators of organophosphorus poisoned acetylcholinesterase. Wilson, Ginsburg & Quan (1958) suggested the oxime anion to be the "reactive species". Theoretically, the pK_a should be low enough to yield a sufficient concentration of anions at physiological pH. On the other hand changes in pK_a have been reported to affect reactivating potency to a small extent, giving rise to factors of only 2–3 for one pH unit. These findings have been reported to apply to most reactivations (Wilson & others, 1958).

The pK_a values of a number of mono-oximes have been determined by potentiometric titration (Wilson & others, 1958; Hobbiger, 1963). Wilson & Ginsburg (1958) titrated bisquaternary dibasic oximes for analytical reasons. In their titration curves they observed only one break. This led to the assumption that the pH at one-half neutralization should be equal to the "average" of the two supposed pK_a values. Although such an average value bears only analytical consequences, some authors (Hobbiger, 1963; Engelhard & Erdmann, 1964) refer to it as a true pK_a value.

Engelhard & Erdmann (1964) based their calculations of the percentage ionized on corresponding values. According to their figures the percentage ionization of TMB-4 is 19% and of obidoxime is 28% at pH 7·5. However,

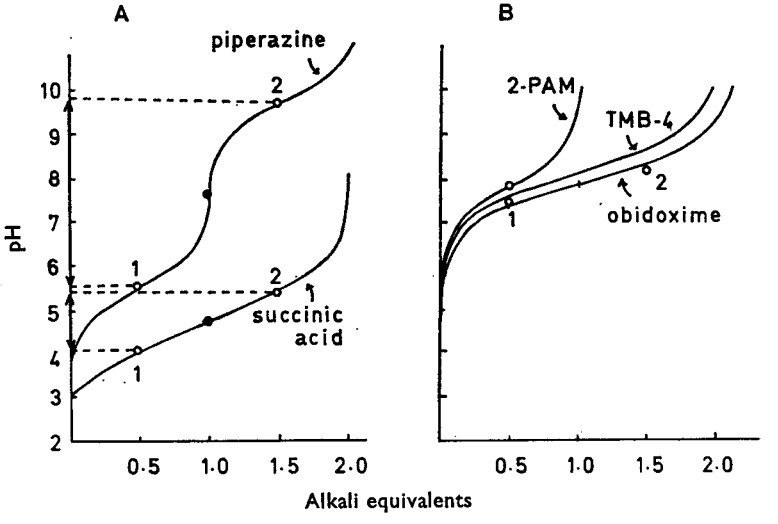


FIG. 1. Titration curves (0·01M, 0·1N KOH, 20° of (A) piperazine diperchlorate ($pK_{a_1} = 5{\cdot}54 \pm 0{\cdot}006$; $pK_{a_2} = 9{\cdot}79 \pm 0{\cdot}01$) and succinic acid ($pK_{a_1} = 4{\cdot}09 \pm 0{\cdot}03$; $pK_{a_2} = 5{\cdot}42 \pm 0{\cdot}03$) (Albert & Serjeant, 1962) and (B) 2-PAM, TMB-4 (1,1′-trimethylenebis(4-formylpyridinium bromide) dioxime and obidoxime (Toxogonin; bis(4-hydroxyiminomethyl pyridinium-(1)-methylether dichloride. (●) One-half neutralization.

the titration midpoint, i.e. the pH at one-half neutralization, may only be set as equal to the pK_a where there is only one ionizing group. In molecules with more than one ionizing group, the less basic may be completely ionized at "one-half neutralization", whereas the more basic group may remain entirely uncharged, as for example in piperazine diperchlorate. (Fig. 1A)

According to the ratio of the respective ionization constants the titration curve of a dibasic electrolyte will either have an inflexion where $K_1 > 16\ K_2$, or yield a straight line where $K_1 = 16\ K_2$, or resemble that of a monobasic compound where $K_1 < 16\ K_2$ (Auerbach & Smolczyk, cited by Britton, 1955) (Fig. 1). From titration curves of quaternary bispyridine aldoximes the presence of two adjacent ionization constants may be inferred. The endpoint of the first stage of ionization is not discernible, because at this instant titration of the second group has already begun (Fig. 1B). In polyvalent electrolytes pK_a values may only be calculated by means of the Henderson-Hasselbalch equation, if they are separated by more than 2·7 pH units ($K_1 > 500\ K_2$). Accurate separation of overlapping pK_a values, however, may be obtained by means of a method which is due to Britton (1955).

As this procedure involves extensive calculations, a programme has been developed for use on an electronic calculator (Bieger, Ehrich & Wassermann, 1967). By this means, ionization constants of a series of dioximes have been determined (Table 1). The two pK_a values of TMB-4, for instance have been found to be 7·78 and 8·61 respectively; for obidoxime these values are 7·54 and 8·24. Such figures permit the calculation of the true proportion of anions to molecules; e.g. in obidoxime the more acidic group is 50% and the less acidic group 17% ionized at pH 7·5. In TMB-4 the corresponding figures are 33% and 7% (Bieger & Wassermann, 1967).

These findings raise the question of the significance of the second oxime group. Comparison with related monoximes shows that the influence of an additional oxime group is consistent with an increase in acidity of the first group. On an

TABLE 1. IONIZATION CONSTANTS OF SOME BISPYRIDINIUM ALDOXIMES

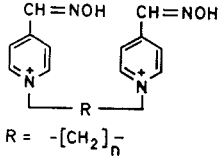
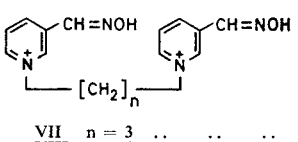
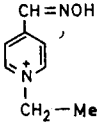
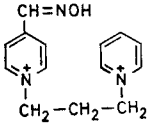
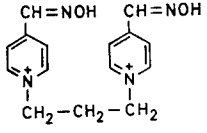
		pKa_1	pKa_2	Percentage ionized at pH 7·4	
				1	2
(a)  R = $-[CH_2]_n-$					
I	n = 2	7·58 ± 0·01	8·34 ± 0·01	39·83	10·33
II	n = 3	7·78 ± 0·01	8·61 ± 0·03	29·47	5·80
III	n = 4	7·93 ± 0·01	8·66 ± 0·01	22·78	5·17
IV	n = 5	7·93 ± 0·01	8·67 ± 0·01	22·69	5·12
V	n = 6	7·98 ± 0·01	8·69 ± 0·05	20·85	2·70
VI	R: $-CH_2-O-CH_2-$	7·54 ± 0·01	8·24 ± 0·02	41·79	12·73
(b) 					
VII	n = 3	8·59 ± 0·01	9·30 ± 0·03	6·08	1·24
VIII	n = 4	8·65 ± 0·01	9·46 ± 0·01	5·34	0·86
IX	n = 6	8·70 ± 0·01	9·50 ± 0·01	4·75	0·78

TABLE 2. REACTIVITY AND DEGREE OF IONIZATION OF A SERIES OF PYRIDINIUM-4-ALDOXIMES

Reactivator	Multiples of reactivation velocity of 2-PAM (diethylphosphoryl-AChE, acc. to Hobbiger & Sadler, 1959)	pKa	Percentage ionized (oxime anion) at pH 7·4
I 	$\frac{1}{33}$	8·2 (Hobbiger & others, 1960)	6%
II 	8	8·0 (Hobbiger, 1963)	20%
III  TMB-4	22	$pKa_1 = 7·78$ $pKa_2 = 8·61$	29·5% 5·8%

average, the 4-pyridine aldoximes—favoured by a possible quinonoid structure—are 0·7 pH units more acidic than the corresponding 3-aldoximes. Moreover, acidity may be promoted by a hydrogen bond between the ionized and the uncharged oxime group (Becker, 1965), since extension of the chain linking the two pyridinium nitrogens results in a decrease of the acidity of both oxime groups. For the above reasons, at physiological pH the proportion of active oxime anions will be higher in bispyridinium aldoximes than in corresponding mono-aldoximes.

The sequence of pyridinium aldoximes (I–III) shown in Table 2 is conspicuous for an increasing ability to reactivate acetylcholinesterase inhibited by tetraethyl pyrophosphate (TEPP) or paraoxon *in vitro*. The marked superiority of compound II to compound I (factor 120) may be due not only to an additional binding contribution at an optimal distance, but also to an increase in ionization. Introduction of a second oxime group again enhances the proportion of oxime anions and augments the rate of reactivation by a factor of 2·5. These data, however, do not permit a decisive conclusion, whether TMB-4 owes its superiority exclusively to the increased ionization of the first oxime group or to the presence of a second oxime group, possessing an additional reactivating function.

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Mathematics of three-phase *in vitro* absorption models

SIR,—The use of three-phase *in vitro* models and compartmental kinetics to study the drug absorption process would appear to be of great value to pharmacy. Recent reports by Perrin (1966, 1967), who used such a model, contain an error. Perrin pointed our that if the volumes of the compartments within a system differed, they must be taken into account in any kinetic analysis of data obtained from the system. The example used was for the following set of consecutive first order processes:

$$A \xrightarrow{k_1} B \xrightarrow{k_2} C$$

in which A, B and C represent the concentrations in three compartments with volumes V_A, V_B and V_C. The differential equations describing the kinetics were then given as:

$$V_A dA/dt = -V_A k_1 A \quad (1)$$

$$V_B dB/dt = V_A k_1 A - V_B k_2 B \quad (2)$$

$$V_C dC/dt = V_B k_2 B \quad (3)$$

Using the conditions that $V_A = V_C$, and that at time zero, $A = A_0$, $B = C = 0$, the integrated equations were given as:

$$A = A_0 \exp^{-k_1 t} \quad (4)$$

$$B = \frac{V_A k_1 A_0}{V_B(k_2 - V_A k_1/V_B)} (\exp^{-V_A k_1 t} - \exp^{-k_2 t}) \quad (5)$$

$$C = A_0\left[1 - \frac{1}{k_2 - V_A k_1/V_B}\left(k_2 \exp^{-k_1 t} - k_1 \exp^{-k_2 t} - \frac{V_A k_1}{V_B}\exp^{-k_1 t} + k_1 \exp^{-V_A k_1 t/V_B}\right)\right] \quad (6)$$

Eqn 4 results from a straightforward integration of eqn 1 and is correct as given. Eqns 5 and 6, however, cannot be obtained by integrating the corresponding differential eqns 2 and 3. The volume terms in the integrated equations are inconsistent with the differential equations. This can be shown by inserting eqn 4 into eqn 2 to give:

$$dB/dt = \frac{V_A}{V_B} k_1 A_0 \exp^{-k_1 t} - k_2 B \quad (7)$$

$$\text{or } dB + k_2 B dt = \frac{V_A}{V_B} k_1 A_0 \exp^{-k_1 t} dt \quad (8)$$

which is a linear differential equation of the first order. It can, therefore be