ACTA CHEMICA SCANDINAVICA 27 (1973) 868–874

Acid-Base Properties of Atropine, Scopolamine and Some Glycolic Acid Esters

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The dissociation constants of atropine and ten related anticholinergic compounds have been obtained from emf titrations in 0.1 M NaCl medium at 25°C using glass electrodes. All the compounds studied were tertiary amines and the acids corresponding to them had a $pK_a = 8-10$. One compound has been studied at different concentrations of Na^+, Ca^{2+}, and Cl^-. No association with these ions could be detected. The data were treated by graphical as well as computer methods such as the least squares program LETAGROP ETITR, allowing for small acid impurities.

This investigation is part of a research program with the intention to investigate possible relationships between structure and activity of anticholinergic drugs.

The degree of ionization and the lipophilic character of a drug influence the passage of the drug through cell membranes. There are experimental results, which indicate that glycolates affect membrane processes in the central nervous system.[1] In connection with this problem it has been questioned whether these drugs can form complexes with calcium.[2]

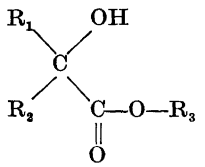
This investigation has been undertaken in order to determine dissociation constants of atropine, scopolamine, and some glycolic acid esters, and the possible formation of calcium complexes with such compounds.

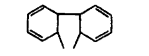
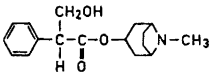
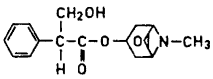
EXPERIMENTAL

Chemicals and analysis. The compounds studied in this paper are listed in Table 1. *Atropine* and *scopolamine hydrobromide* were commercial products from Vitrum AB, Stockholm. All the other drugs were synthetized and analysed for C, H, N at the Research Institute of National Defence, Sweden (Table 1, Nos. 1–7 analysed by Flormark and

* The measurements have been made by A.M. at FOA and the calculations have been performed by A.M. and O.W.

Table 1. Name, code number, formulae and melting points of the compounds studied.



Code number	Name	R_1	R_2	R_3	M.p. (°C)	Notes
1	1-Methyl-4-piperidyl benzilate				166	
2	3-Tropyl benzilate	-"-	-"-		237 – 241	Melting point of the hydrochloride
3	3-Quinuclidinyl benzilate	-"-	-"-		166 – 168	
4	3-Quinuclidinyl cyclopentyl phenylglycolate	-"-		-"-	98 – 102	
5	3-Quinuclidinyl phenyl 2-thienylglycolate	-"-		-"-	237 – 240	
6	3-Quinuclidinyl 9-hydroxy 9-fluorenecarboxylate			-"-	213 – 216	
7	3-Tropyl 9-hydroxy 9-fluorenecarboxylate	-"-			225	Melting point of the hydrochloride
8	3-Quinuclidinyl di-2,2'-thienylglycolate				154 – 155	
9	3-Quinuclidinyl di-3,3'-thienylglycolate			-"-	139	
10	Atropine				115 – 116	
11	Scopolamine				55	Melting point of the hydrobromide

Larsson [3] and Nos. 8 – 9 by Nyberg *et al.*[4]). Melting points of the substances are tabulated in Table 1. *Sodium hydroxide.* A 50 % stock solution was prepared from the EKA Bohus AB, analytical grade product. From this stock solution portions were taken and diluted with de-aerated water. NaOH solutions were standardized by titration with potassium hydrogen phthalate, using phenolphthalein as an indicator.[5] *Hydrochloric acid.* Merck AG (Darmstadt) Titrisol stock solutions were used. The concentration of HCl was determined from the first acid points in each titration using the Gran method of extrapolations to the equivalence point.[6] *Sodium chloride* and *calcium chloride.* Merck's analytical grade products were used.

Apparatus. Potentiometer: Radiometer, Copenhagen, *PHM 26c* and Autoburet *AMU 1b.* The emf values could be read to a precision of ±0.2 mV. *Electrodes:* Glass electrode *202C* from Radiometer. Ag,AgCl electrode prepared according to Brown.[7] *Thermostat:* A water mantle at 25.00±0.02°C. The equilibrium solution was stirred with a magnet and commercial nitrogen gas was bubbled through.

Emf measurements and titration procedure. The emf of the following cell was measured:

$$-\text{Ag,AgCl/S/glass electrode}+$$

The solution S contained A mol/l of a weak acid and the analytical (total excess) concentration of H^+ over A and $H_2O = H$ mol/l. The concentrations in the starting solution, before the addition of buret solution, are called A_0 and H_0 mol/l, respectively. In order to keep the activity coefficients approximately constant, NaCl was added so that $[Cl^-] = 0.1$ M (except for one acid, *cf.* Table (2b)).

The emf of the cell can be written:

$$E = E_0 + 59.155 \log h \quad (1)$$

where $h = [H^+]$. Usually we started with 30 ml of an acid solution containing HCl + A and then 2 – 3 ml of NaOH solution was added in small portions from a buret to change H. A solution of 0.2 M NaCl was added from a second buret. The acids studied were very slightly soluble in water (≈ 1 mM of A). At the end of the titrations a precipitate was often formed causing a drift in the emf values.

Each titration was carried out within 30 min in order to avoid decomposition by hydrolysis. The emf usually reached a stable value ($\pm$ 0.2 mV) within 1 min. The alkaline hydrolysis of some of the glycolates has recently been studied.[8] By separate experiments, it was checked that the decomposition during the titrations did not exceed 2 %. For 3-quinuclidinyl di-3,3′-thienylglycolate forward and back titrations agreed within the limits of experimental error (*cf.* Fig. 1, a and b). Thus the reversibility of the equilibria was confirmed.

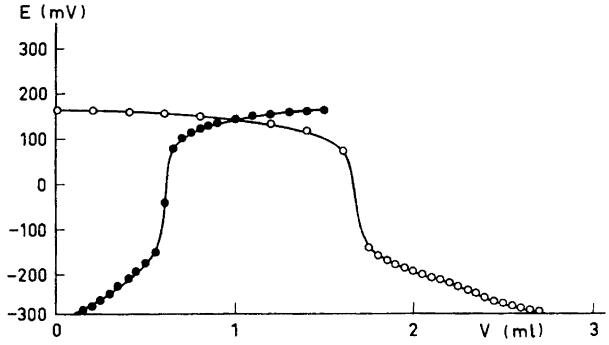


Fig. 1a. Titration curves for 3-quinuclidinyl di-3,3′-thienylglycolate. The filled symbols represent a back titration. E = the measured emf. V = the volume added from a buret to V_0 ml of a solution S_0. The buret contained 0.0457 M NaOH for the forward titration and 0.1004 M HCl for the back titration. The solution S_0 for the forward titration contained the total concentration 0.993 mM weak acid ($= A_0$) and the analytical concentration of $H^+ = 3.46$ mM ($= H_0$). $E_0 = 320.4$ mV.

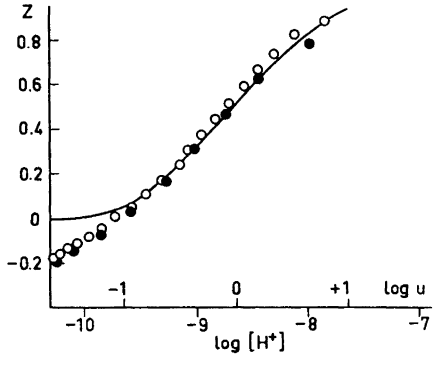


Fig. 1b. The data in Fig. 1a transformed to $Z(\log h)$ with $pK_w = 13.8$ (*cf.* eqn. (3c)). Z = the average number of H^+ bound per A. The line corresponds to $pK_{a1} = 8.6$.

Hydrogen electrodes could not be used in these experiments due to the rather rapid hydrolysis of the esters studied. The glass electrodes used were checked against hydrogen electrodes.

By using a coulometer one can avoid possible impurities of the sodium hydroxide solution. The accuracy of a coulometric titration is usually very high.[9] However, in the present case, it was not possible to get reproducible results by the coulometric technique, possibly due to decomposition of the compounds studied during the electrolysis.

TREATMENT OF DATA

The reaction studied can be written

$$HA^+ + H_2O \rightleftharpoons H_3O^+ + A \quad (2)$$

The law of mass action gives for this equilibrium $[HA^+] = \beta_1 h[A]$, where $h = [H^+]$. The total concentration of the weak acid ($= A$) and the concentration of H^+ bound to the weak acid ($= ZA$) can be written as

$$A = [A] + \beta_1 h[A] \quad (3a)$$

$$ZA = \beta_1 h[A] \quad (3b)$$

The experimental values of Z were calculated from

$$ZA = H - h + K_w h^{-1} \quad (3c)$$

Note that Z = the average number of H^+ bound per A. For the ionic product of water in 0.1 M NaCl at 25°C we have used the value of $\log K_w = -13.80$.[10]

The primary data are values of volume and emf (V,E) at different total concentrations H and A (*cf.* Fig. 1a). Experimental points in the range $-2.5 \geq \log h \geq -3.5$ were used in a Gran [6] plot to correct H_0 and for calculation of E_0. The primary data (V,E) were then transformed to $Z(\log h)$ using eqns. (1) and (3c) (*cf.* Fig. 1b).

Graphical determination of the dissociation constants. If it is assumed that HA^+ and A are the only A-species present in the solution, then from eqns. 3 (a–b)

$$Z = \beta_1 h/(1 + \beta_1 h) \quad (4a)$$

By introducing the substitution $u = \beta_1 h$ one obtains

$$Z = u/(1 + u) \quad (4b)$$

$Z(\log u)$ is a normalized function,[11] which could be very well fitted to the experimental curves $Z(\log h)$ for $\log h > -10$. The last points of each titrations revealed a small additional buffer capacity (*cf.* Fig. 1b). This can be due to (i) imperfections of the glass electrodes; (ii) acid-base impurities, such as carbonic acid or silicic acid, with pK_a values of ~ 10 (*cf.* Ref. 12); (iii) decomposition of the compound studied; or (iv) a second dissociation step of the acids. Since the equilibria were found to be reversible a decomposition can be ruled out.

For 3-quinuclidinyl di-3,3′-thienylglycolate the graphical method gave the best fit with

$$pK_a = \log \beta_1 = 8.6 \pm 0.1 \quad (4c)$$

Since all the compounds studied have very similar titration curves, the graphical treatment was carried out only for a few acids. Instead all equilibrium constants were obtained using the generalized least squares program LETAGROP.[13,14]

Computer refinement of the dissociation constants. In the computer treatment the error squares sum $U = \sum(H_{calc} - H_{exp})^2$ was minimized.[13,14] For each titration we have assumed small errors δH and δE_0 in H and E_0, respectively. These errors correspond to small shifts in the curve $Z(\log h)$.

In order to correct for the extra buffer capacity at high pH values (see above), we have adjusted one parameter together with pK_{a1} and the systematic errors δH and δE_0. The same values of pK_{a1} were obtained either a second dissociation constant pK_{a2}, or the ionic product of water, pK_w, was adjusted, or if an acid impurity with a concentration of 0.5 – 1.0 mM was assumed. Such a large amount of impurities is rather unlikely. Values of pK_w ranging from 12.6 to 13.6 were necessary to explain the extra buffer capacities in all the titrations.

Table 2a. Results of the least squares calculations (LETAGROP). $pK_{a1} = \log \beta_1$. The standard deviations of $H = \sigma(H)$, and the total concentrations of the weak acids in the starting solutions, A_0 are in mM.

Substance	Number of points	log ($\beta_1 \pm 3\,\sigma$)	$\sigma(H)$	A_0
1	19	8.37 ± 0.17	0.021	0.517
2	25	9.39 ± 0.08	0.013	0.488
3	34	8.72 ± 0.05	0.020	1.200
4	30	8.81 ± 0.07	0.031	1.031
5	24	8.48 ± 0.11	0.012	0.511
6	34	8.62 ± 0.08	0.010	0.469
7	36	9.73 ± 0.08	0.020	0.527
8	27	8.64 ± 0.04	0.008	1.111
9	36	8.63 ± 0.08	0.022	0.993
10	32	9.76 ± 0.07	0.023	0.906
11	39	7.81 ± 0.02	0.009	1.253

Table 2b. Calculations of $pK_{a1} = \log \beta_1$ for 3-quinuclidinyl benzilate for various total concentrations and media. The standard deviations of $H = \sigma(H)$, and the total concentrations of the weak acid in the starting solutions, A_0 are in mM. The extra buffer capacity may be explained with $pK_{a2} = 10.0 \pm 0.1$ (and $pK_w = 13.8$) or $pK_w = 13.3 \pm 0.1$ (assuming one dissociation step). No correction for the varying ionic medium has been introduced.

Medium	Number of points	A_0	log ($\beta_1 \pm 3\,\sigma$)	$\sigma(H)$
0.1 M NaCl	34	1.200	8.72 ± 0.05	0.020
»	29	0.770	8.79 ± 0.07	0.014
»	43	0.536	8.88 ± 0.10	0.024
»	27	0.230	8.67 ± 0.22	0.015
0.3 M NaCl	30	1.132	8.82 ± 0.06	0.021
0.5 M NaCl	30	1.076	8.88 ± 0.08	0.028
0.25 M $CaCl_2$	32	0.978	8.90 ± 0.10	0.031

The analytical errors in H are very small, ~ 0.02 mM, and so is the standard deviation of H, *viz.* $\sigma(H) \sim 0.02$ mM (*cf.* Table 2, a and b).

RESULTS AND DISCUSSION

The final equilibrium constants given in Table 2a are valid in 0.1 M NaCl solution at 25°C. Most of the quinuclidinyl glycolates have $pK_a = 8.6 \pm 0.1$, while the pK_a of the tropyl derivates are 9.4 – 9.8. For atropine $pK_a = 9.8 \pm 0.1$, and for scopolamine $pK_a = 7.81 \pm 0.05$. These values are consistent with previously determined values, *viz.* 9.85 (18°C) [15,16] and 10.20 (16.5°C) [17] for atropine, 7.62 (21 ± 2°C) [18] and 7.55 (23°C) [19] for scopolamine. Abood [1] has found $pK_a = 7.8$ for 1-methyl-4-piperidyl benzilate (our value is 8.4 ± 0.2). The rather different media and temperatures used in the investigations make a direct comparison difficult.

No association of 3-quinuclidinyl benzilate with the medium ions Na^+, Cl^+, or Ca^{2+} could be detected (*cf.* Table 2b). The change of pK_a is so small that complex formation to Cl^- and Ca^{2+} can be neglected. Since charges cancel in reaction (2), the variation of $\log \beta_1$ with ionic strength can be expected to be due mainly to the salting out of the base form. The salting out coefficient can be estimated to 0.12, which is not unreasonable.[20]

In fact, the "best" fit to the data was obtained by assuming a second dissociation step. This may be due to the proton in the hydroxyl group (see Table 1). The values of pK_{a2} varied from 9.6 ± 0.5 for the 2-substituted thienylglycolates (5, 8 in Table 2a) to ~ 11 for atropine and scopolamine (10, 11 in Table 2a). However, pK_{a2} needs further documentation before it can be established with certainty. It deserves to be mentioned that a value of $pK_{a2} = 11$ seems very low for the primary alcohols atropine and scopolamine. Spectrophotometric studies might give the elucidations on this point.

Acknowledgements. Thanks are due to Dr. Erik Ekedahl for valuable comments and suggestions. We are indebted to Arne Flormark and Gun Wallerberg for supplying the substances. Dr. Don Koenig has kindly revised the English text.

This investigation has been financially supported by the *Tricentennial Fund of the Bank of Sweden* and by the *Swedish Natural Science Research Council.*

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Received September 20, 1972.