Electroreduction of Some Potential Antimicrobial Thiosemicarbazide Derivatives of Quinazolin-4(3*H*)-one

Mohamed I. Ismail
Department of Chemistry, Faculty of Science, University of Ain Shams, Cairo, Egypt

Polarographic data for a series of 3-phenyl-2-substituted thiocarbamoylhydrazonomethylquinazolin-4(3*H*)-ones (with antimicrobial activity), in 50% ethanolic aqueous buffers covering a wide range of pH are reported and discussed. A mechanism interpreting the electrode process, in both acidic and alkaline media, is proposed and confirmed *via* the identification of CPE products, Hammett's σ–$E_{\frac{1}{2}}$ relation and pK_a determination.

Various thiosemicarbazones have been reported as effective antineoplastic agents which block DNA synthesis in mammalian cells, presumably *via* chelation to the iron required by the enzyme ribonucleoside diphosphate reductase.[1–3] Thiosemicarbazones can be readily converted into 1,3,4-thiadiazoles[4] which are known to be CNS depressants, bactericides, fungicides and herbicides.[5–7] The pharmacological action of sulfur-containing compounds against Bilharzis snails is also well known.[8] In addition, antiinflammatory, anticonvulsant and antimicrobial activities have been ascribed to several quinazolin-4(3*H*)-ones.[9–11] As part of our studies on the electrochemical behaviour of various heterocyclic compounds of specific biological activity,[12–14] the present study was undertaken to follow the polarographic reduction of a novel series of compounds containing a thiosemicarbazide moiety attached at the 2-position of quinazolin-4(3*H*)-one (**1a–g**) as potential antimicrobial agents. The study included:

(i) Elucidation of the electrode mechanism of compounds **1a–g** in 50% aqueous alcohol buffers covering a wide range of pH values.

(ii) Finding a simple electrochemical route for the synthesis of related compounds through a study of their controlled potential electrolysis (CPE).

Experimental

Organic Synthesis.—The starting compound, namely, 2-formyl-3-phenylquinazolin-4(3*H*)-one (**2**) was prepared as described elsewhere.[15] The designed compounds; 3-phenyl-2-substituted thiocarbamoylhydrazonomethylquinazolin-4(3*H*)-ones (**1a–g**) were synthesized according to the following procedure.[4,16] To a solution of 2-formyl-3-phenylquinazolin-4(3*H*)-one (0.002 mol) in ethanol–chloroform (2:1) mixture (30 cm^3) was added the equivalent amount of the substituted thiosemicarbazide (0.002 mol). The mixture was heated under reflux for 3 h, partially concentrated and cooled. The separated product was filtered off and recrystallized from the appropriate solvent (Table 1). The purity and structure were checked by measurements of m.p., NMR, IR and mass spectra, and elemental analysis.

Antimicrobial Testing.—The prepared compounds were evaluated for *in vitro* antimicrobial activity using the agar diffusion method.[17] A 0.1% solution of each compound in propylene glycol was tested against *Staphylococcus aureus* (NCTS 4163), *Escherichia coli* (NCTC 5933) and *Candida albicans* (3501). The resulting inhibition zones indicated that none of the tested compounds showed superior activity to streptomycin in the performed test.

Voltammetric Investigation.—The DC-polarograms were recorded using a Sargent Welch polarograph Model XVI. A cell of our own design with a separated saturated calomel electrode (SCE) was used.[12] The characteristics of the capillary in 0.1 mol dm^{-3} KCl at open-circuit were m = 1.68 mg/s, t = 4.76 s/drop at h = 45 cm. The temperature was maintained at 25 ± 0.1 °C using an ultrathermostat. The depolarizer concentration studied was 5×10^{-4} mol dm^{-3} in 50% (v/v) ethanolic-Thiel buffer mixtures. Prior to each run oxygen-free nitrogen gas was bubbled through the polarographic cell.

Polarographic Analyzer model 264A (PARC) and the electrode assembly model 303A with hanging mercury drop electrode (area = 2.6×10^{-2} cm^2) as working electrode, Ag/AgCl as reference electrode and Pt wire as counter electrode were used for cyclic voltammetry. The X-Y recorder model RE 0089 (Houston instrument) was used for recording the voltammograms.

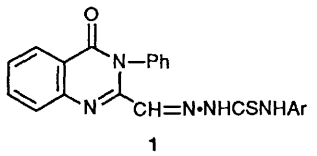
The controlled potential electrolysis (CPE) was verified using a potentiostat TACUSSEL of type 4/60. The electrolytic cell used was that described by Peltier *et al.*[18] The number of electrons involved in the electrode processes of the reduction waves was computed from i–t curves following the method outlined by Lingane.[19] The number of electrons was calculated and found to be 4 at pH < 6.4 and 2 at pH > 9.1. After complete electrolysis (10^{-3} mol dm^{-3}), the electrolytic products were isolated and identified by IR spectroscopy and TLC, as well as elemental analysis.

Potentiometric Determination of the Apparent Dissociation Constants (pK_a).—40 cm^3 of a 10^{-3} mol dm^{-3} solution of the compound (solvent composition was 1:1 EtOH–H_2O) were titrated against standardized 10^{-2} mol dm^{-3} aqueous NaOH. The apparent pH values were read on a TS4H Prolabo pH-meter type titrimeter accurate to ±0.02 units. The apparent pK_a values (Table 2) were calculated from the Henderson–Hasselbach equation.[20]

Results and Discussion

Voltammetric Measurements.—The DC-polarography of compounds **1a–f** was investigated in 50% ethanolic-Thiel buffer solutions of different pH. The polarograms of 5×10^{-4} mol dm^{-3} solutions of **1a** were taken as a typical representative example for the studied series (Fig. 1). At pH < 6.4, this compound exhibits a well-defined wave. As pH is increased up to 9.1, the limiting current (i_l) starts to decrease and the wave seems to be unstable. Consequently, an irregular-shaped polarogram is produced. At pH > 9.1, a well developed wave is obtained whose limiting current is practically half that observed in the acidic medium.

Table 1 List of 3-phenyl-2-substituted thiocarbamoylhydrazonomethylquinazolin-4(3*H*)-ones **1a–g**



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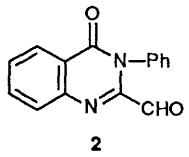

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Compound (Ar)	M.p./°C	Yield (%)	Molecular formula	Solvent of crystallization	Analysis, Calc./Found (%) C	H	N	S
1a (H)	221	65	$C_{16}H_{12}N_5OS$	Ethanol	59.62 59.65	3.73 3.6	21.74 21.6	9.94 9.75
1b (C_6H_5)	226	70	$C_{22}H_{17}N_5OS$	Ethanol	66.17 65.9	4.26 4.4	17.54 17.6	8.02 7.9
1c (*p*-$C_6H_4CH_3$)	234	75	$C_{23}H_{19}N_5OS$	$CHCl_3$–light petroleum (40–60 °C)	66.83 66.85	4.60 4.55	16.95 16.9	7.75 7.7
1d (*p*-$C_6H_4OCH_3$)	232	65	$C_{23}H_{14}N_5O_2S$	$CHCl_3$–light petroleum (40–60 °C)	64.34 64.3	4.43 4.4	16.32 16.2	7.46 7.4
1e (*p*-C_6H_4Cl)	229	90	$C_{22}H_{16}ClN_5OS$	Dioxane–H_2O	60.90 60.95	3.69 3.65	16.15 16.1	7.38 7.4
1f (*p*-C_6H_4Br)	236	80	$C_{22}H_{16}BrN_5OS$	Dioxane–H_2O	55.24 55.2	3.35 3.3	14.65 14.6	6.70 6.65
1g (*p*-$C_6H_4NO_2$)	233	75	$C_{22}H_{16}N_6O_3S$	Ethanol	59.46 60.0	3.60 3.65	18.92 18.85	7.21 7.15

Table 2 Polarographic results and pK_a values of some 3-phenyl-2-substituted thiocarbamoylhydrazonomethylquinazolin-4(3*H*)-ones **1a–g**

Compound	pH	$-E_{\frac{1}{2}}$	$D/10^{-6}$ $cm^2\ s^{-1}$	S^a	αn	$\Delta E_{\frac{1}{2}}/\Delta pH$	Z_H^+	Ke°/ $Cm\ s^{-1}$	$\Delta G^\ddagger$/ $kJ\ mol^{-1}$	pK_a
1a	2.3	0.73	2.375	14.34	0.85	0.061	1.03	2.2×10^{-9}	85.27	8.52
	5.5	0.91		15.93	0.94			3.5×10^{-10}	101.75	
	10.2	1.18		16.58	0.99			4.1×10^{-12}	127.63	
1b	2.3	0.70	2.636	12.57	0.74	0.058	0.98	7.5×10^{-8}	81.43	8.41
	5.5	0.84		14.82	0.87			1.6×10^{-9}	99.78	
	10.2	1.05		16.17	0.95			6.2×10^{-11}	117.08	
1c	2.3	0.78	2.812	13.01	0.77	0.060	1.02	6.6×10^{-8}	88.63	8.72
	5.5	0.97		14.93	0.88			9.1×10^{-9}	108.45	
	10.2	1.21		16.68	0.98			1.5×10^{-12}	131.52	
1d	2.3	0.79	2.934	13.71	0.81	0.063	1.07	5.7×10^{-8}	90.13	8.90
	5.5	0.03		15.22	0.90			1.3×10^{-10}	110.96	
	10.2	1.28		16.11	0.95			3.9×10^{-12}	135.11	
1e	2.3	0.64	2.448	14.53	0.86	0.056	0.95	5.4×10^{-7}	77.53	7.91
	5.5	0.71		15.84	0.93			7.3×10^{-8}	92.27	
	10.2	0.92		16.29	0.96			1.7×10^{-10}	115.17	
1f	2.3	0.62	2.984	14.11	0.83	0.064	1.08	3.8×10^{-7}	74.90	7.82
	5.5	0.69		14.76	0.87			5.2×10^{-8}	91.37	
	10.2	0.90		15.74	0.93			9.8×10^{-9}	109.87	
1g	2.3	0.54	2.679	12.96	0.76	0.054	0.92	8.6×10^{-6}	63.52	5.94
	5.5	0.66		13.77	0.81			3.3×10^{-7}	84.17	
	10.2	0.83		14.68	0.87			4.9×10^{-9}	108.51	

[a] S = The slopes of the correlation of log $i/(i_l - i)$ with E; the slopes are calculated by the least squares method.

Compound **1g** showed an additional 4-electron irreversible diffusion controlled wave at more positive potential than the main wave. The behaviour of the latter wave is comparable to that of the well known aromatic *p*-nitro group.[21] It is not unreasonable to attribute this extra wave to the reduction of the nitro group to hydroxylamine.[22] At pH >10.2, a further ill-defined 2-electron wave appears which may be attributed to the successive reduction of the hydroxylamine to the amine.[21]

The cyclic voltammograms of the compounds under investigation in acidic and alkaline media at different sweep rates (20–200 mV s^{-1}) show two peaks in the cathodic scan (Fig. 2) but do not show any anodic peaks in the reverse scan, which indicates the irreversible nature of the reduction waves.

Nature of Wave.—The dependence of both limiting current (i_l) and half-wave potential ($E_{\frac{1}{2}}$) on pH is illustrated in Fig. 3. The limiting current of the wave decreases with increase of pH in the form of a well-defined dissociation curve.[23] The effect of mercury height variation on i_l indicates that this wave is mainly controlled by diffusion in the pH range where i_l is practically constant. At pH values where i_l decreases with height, the slopes of the linear plots of log i_l *vs.* log h give values <0.5, a direct indication that this wave is of a kinetic nature.

The shift of $E_{\frac{1}{2}}$ to more negative values on increasing the pH indicates that the proton transfer most probably precedes the electrode process. The number of protons (Z_{H^+}) involved in the rate determining step was calculated from a $E_{\frac{1}{2}}$–pH plot.[24] The diffusion coefficient (D) of the depolarizer was determined

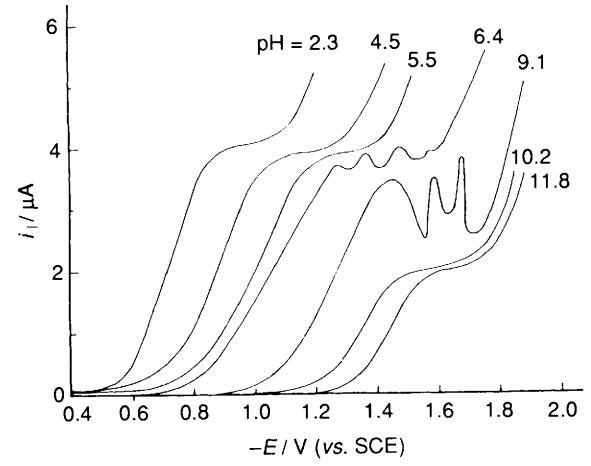

Fig. 1 Polarograms of 5 × 10^{-4} mol dm^{-3} solutions of **1a** in 50% ethanolic-Thiel buffers solutions of different pH

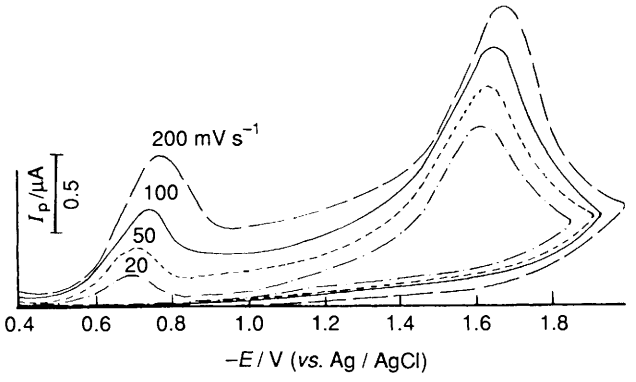

Fig. 2 Cyclic voltammograms of **1a** (1 × 10^{-4} mol dm^{-3}; pH = 4.5) at different sweep rates

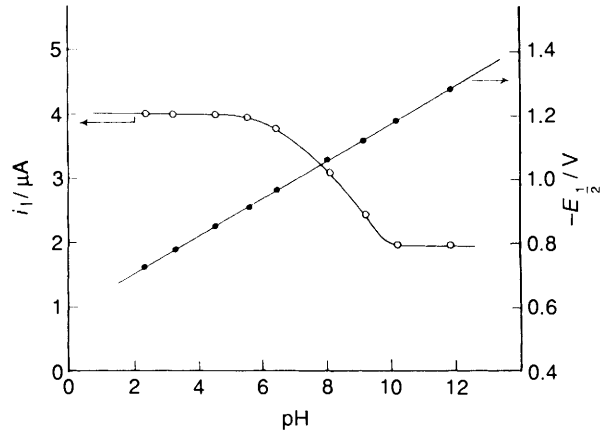

Fig. 3 The correlation of $E_{\frac{1}{2}}$ and i_l with pH

experimentally by applying the Stokes–Einstein equation.[25] The electrode reduction process was found to be irreversible in nature as indicated by logarithmic analysis of the wave, the shift of $E_{\frac{1}{2}}$ to more negative values with the increase in the concentration of the depolarizer,[26] the shift of the peak potential (E_p) to more negative values with the increase in the sweep rate, the lower values of the electron rate constant (Ke°) and the higher values of the free energy of activation ($\Delta G^\ddagger$) calculated by applying the different approaches proposed for the analysis of irreversible waves.[27–29]

Reduction Mechanism.—The polarographic study of the model compound 2-formyl-3-phenylquinazolin-4(3*H*)-one **2** measured under the same experimental conditions as that of **1a** showed that it is not electroactive, indicating that the exocyclic group attached to the heterocyclic ring in position 2 of compound **1a** is the only electroactive centre. Owing to the tautomeric effect, the active centre may exist in the azo (–N=N–) or in the hydrazono (–CH=N–NH–) form. However, the reduction of the nitro group of compound **1g** prior to the main reduction wave is a direct indication that the reduced species is the hydrazono linkage, not the azo form.[30,31]

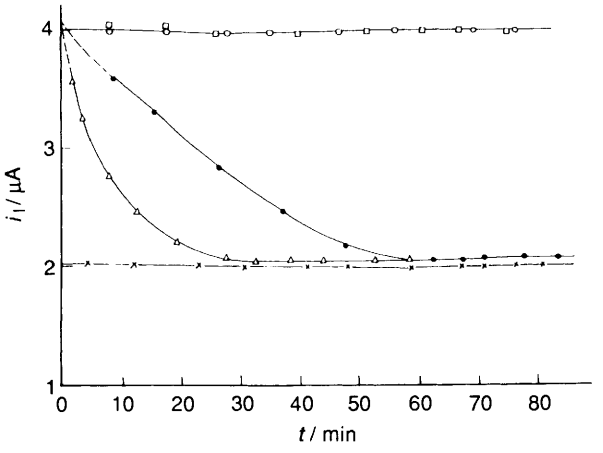

Fig. 4 Effect of time on the limiting current of 5 × 10^{-4} mol dm^{-3} solution of **1a** at pH = 2.3, □; 4.5, ○; 6.4, ●; 9.1, △; 10.2 and 11.8, ×

The decrease in the limiting current which occurred on raising the pH of the medium may be attributed to the hydrolysis of the compounds under study, or of their reduced species. For this reason, the stability of the studied compounds at different pH's was tested spectrophotometrically. The intensity and the position of the adsorption bands at a fixed pH value were not changed by time over more than 3 h. This result excludes the possibility that the decrease of the current at pH >6.4 is due to the hydrolysis of the compounds under study but may be due to the hydrolysis of a species produced at the electrode surface. This fact was further supported by a study of the effect of time on the plateau of the reduction wave at different pH's. The relationship between i_l and time is illustrated graphically in Fig. 4. At pH <6.4, the limiting current remains constant for a period exceeding 80 min. However, at 9.1 > pH > 6.4, the limiting current decreases noticeably in the form of a decay curve, achieving a constant limiting value very close to that characteristic for pH >9.1. Such experimental results reveal that the compound produced at the electrode surface is hydrolysable only in alkaline media; *i.e.*; it is most probably a base-catalysed hydrolysis.

Both the obtained polarographic data and the coulometric analysis indicate that the course of the electroreduction of the compounds **1a–g** is dependent on the pH of the buffer solutions used. A scheme representing the reduction of the compounds under investigation at the electrode surface may thus be proposed as follows:

(*a*) pH <6.4. The polarogram revealed a single 4-electron step which represents the reduction of the protonated form of **1a** through the reductive cleavage of the hydrazono linkage into thiourea (**IV**) and 2-aminomethyl-3-phenylquinazolin-4(3*H*)-one (**V**). This mechanism was confirmed by CPE of a 10^{-3} mol dm^{-3} solution of **1a** at pH 2.3. The TLC of the resulting solution indicated the presence of two components. The water insoluble compound 2-aminomethyl-3-phenylquinazolin-4(3*H*)-one (**V**) was extracted from the electrolytic mixture with diethyl ether and identified by IR spectroscopy and elemental analysis.[16] The appearance of only a 4-electron wave may be due to the fact that the energies of the individual steps (E_1, E_2) are very close.

Table 3 Statistical treatment of $E_{\frac{1}{2}}$–σ data for compounds **b** **g**[a]

pH	ρ	r	s
2.3	0.090	0.754	0.097
5.5	0.110	0.881	0.129
10.2	0.096	0.826	0.087

[a] r = Correlation coefficient, s = Standard deviation.

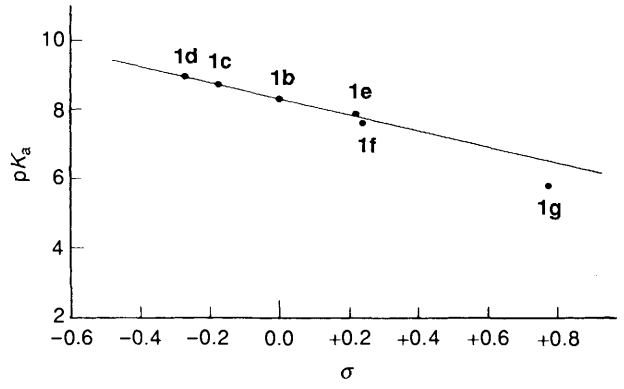

Fig. 5 pK_a–σ relation for compounds **1b**–**g**; ρ = 2.86; r = 0.98; s = ±0.23

Accordingly, the intermediate electroactive imino product **III** is regarded as so reactive that it is immediately reduced to the corresponding amino derivative (**V**).

(*b*) pH >9.1. The height of the wave is almost half that obtained at lower pH, *i.e.*, it represents a 2-electron step, producing initially 2-iminoformyl-3-phenylquinazolin-4(3*H*)-one (**III**) which undergoes further hydrolysis at higher pH to form 2-formyl-3-phenylquinazolin-4(3*H*)-one (**VI**). It is worth mentioning that the hydrolysis of the carbon–nitrogen double bond involves initial addition of water and elimination of a nitrogen moiety.[32] This conclusion is supported by the formation of ammonia as being detected during CPE at the voltage of the limiting current plateau taking place at pH 10.2. The exponential decrease of the limiting current with time which occurs in the pH range 6.4–9.1 seems to be due to the partial hydrolysis of compound **III** which becomes much more pronounced at pH >9.1. That is to say that, in alkaline media, the rate of hydrolysis of the electroactive imino compound **III** is greater than that of its reduction at the electrode surface. Additional evidence for the suggested mechanism is that the IR spectrum of the product resulting from CPE at pH 10.2 is identical to that of the authentic specimen of 2-formyl-3-phenylquinoazolin-4(3*H*)-one (**2**). Therefore, the proposed mechanism may be summarized as follows:

$$\underset{\mathbf{I}}{\text{Y–CH=N–NHCSNHAr}} \overset{H^+}{\rightleftharpoons} \underset{\mathbf{II}}{[\text{Y–CH=N·NHCSNHAr}]H^+}$$

At pH <6.4

$$\mathbf{II} + 2e + H^+ \xrightarrow{E_1} \underset{\mathbf{III}}{\text{Y–CH=NH}} + \underset{\mathbf{IV}}{NH_2CSNHAr}*$$

$$\mathbf{III} + 2e + 2H^+ \xrightarrow{E_2} \underset{\mathbf{V}}{\text{Y–CH}_2\text{–NH}_2}*$$

$$E_1 = E_2 = E_{\frac{1}{2}}$$

At pH >9.1

$$\mathbf{III} \xrightarrow[\text{base catalysis}]{H_2O} \text{Y–CHO}* + NH_3*$$

Y = 3-phenylquinazolin-4(3*H*)-one moiety

* Identified product

In order to obtain further insight into this mechanism, the most reliable $E_{\frac{1}{2}}$ values of compounds **1b**–**g** at a selected pH have been correlated with Hammett's constant (σ) as tabulated by Ritchie and Sagar.[33] Statistical treatment of the data was made using Jaffe calculations[34] and the results are given in Table 3. The obtained $E_{\frac{1}{2}}$–σ plots exhibited reasonable linearity with small positive values of the reaction constant ρ *viz.*, 0.09–0.11. These values are close to those previously reported for hydrazono derivatives.[35] Such small values of ρ suggest that the conjugation of the substituents with the reducible centre is not important enough to produce a pronounced effect. The pK_a values obtained are in agreement with those previously reported for hydrazones.[14,36] Inspecting these pK_a values (Table 2), one may conclude that electron attracting groups enhance the ionization of the compounds under study in which the negative charge is delocalized over the whole molecule. The linearity of the pK_a–σ plot and the calculated reactivity parameters shown in Fig. 5 imply that the resonance interaction between the substituents and the ionizable site is significant and that the phenyl group bearing substituents is nearly coplanar with the hetero ring. It is clear also from Fig. 5 that the point characterizing the *p*-NO_2 substituent, **1g**, deviates from the line. However, if a special σ^- value is introduced instead of σ the point fits the correlation. This is a further indication that the reaction centre is strongly conjugated with the aryl group and the negative charge builds up on the reaction centre in the transition state.[37]

Acknowledgements

The author thanks Mrs. Hala M. Refat, Department of Microbiology, National Research Center (NRC), Cairo, Egypt, for the antimicrobial testing.

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Paper 1/05241A
Received 15*th October* 1991
Accepted 6*th January* 1992