STRUCTURAL AND ADSORPTION EFFECTS OF SOME THIOPURINE AND THIOPYRIMIDINE DERIVATIVES ON THEIR CATALYTIC ACTIVITY AT THE MERCURY ELECTRODE

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6-Thiopurine, 6-thiopurine riboside, 6-thioguanine, 6-thioguanosine, 8-thioguanosine, 2-thiouracil, 4-thiouridine, 2-thiocytosine and 2-thiocytidine are investigated by means of DC polarography and coulometry. In acid medium they give a typical catalytic hydrogen discharge wave, as shown by its parameters and the regeneration of the depolarizer during the electrode process. At pH values above 4.2 a second catalytic wave appears. Both catalytic waves possess pronounced surface characteristics, most likely due to adsorption of the molecules with differing orientations on the electrode surface. The catalytic wave overlaps the reduction wave, which is placed in evidence under conditions where the catalytic effect is absent. Under the same conditions, 2-thiouracil was found to be catalytically inactive, but it induces catalytic hydrogen evolution in Co(II)-containing ammonia buffer. The present results indicate that the position of thio group yields also such distinct change in the catalytic activity of the investigated compounds.

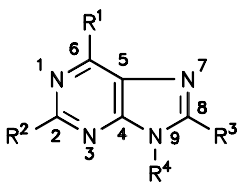
The importance of thiopurines and thiopyrimidines in biochemistry, pharmacology and organic synthesis has been reported[1–3]. Many pharmacologically important sulfur compounds, mainly thiols, give catalytic reduction waves with and without cobalt salts in the analyte solution[4]. A large number of substances that lower the hydrogen overvoltage[5] could be listed. However, the question of catalytically active compounds and of functional groups responsible for catalytic activity is more important. The catalytic effects caused by sulfur-containing compounds were originally ascribed to thiol groups[6]. Among the compounds producing catalytic hydrogen discharge, thiopurines and thiopyrimidines are the most typical. Little work has been done on the catalytic activity of some thiopyrimidine derivatives[7–11]. It is interesting to note that hydrogen evolution at the catalytic hydrogen wave evokes the reduction of hydrogen ion catalyzed by hydrogenase[12]. This enzyme contains a nickel center coordinated by sulfur ligands, which was demonstrated at least for several varieties[13]. In this context, further studies in the field of the catalytic hydrogen wave may reveal structural and mechanistic details useful in understanding the properties of hydrogenase.

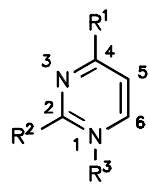
The purpose of this work is to clarify the appearance and origin of the catalytic waves for compounds under investigation. A further aim of this paper was to study the differences in catalytic behaviour of thiopurine and thiopyrimidine derivatives. These differences are discussed on the basis of the electronic structure of compounds *I* – *IX* and their surface activity.

EXPERIMENTAL

Reagents and Solutions

6-Thiopurine monohydrate (*I*), 6-thiopurineriboside (*II*), 2-amino-6-thiopurine (6-thioguanine) (*III*), 2-amino-6-thiopurine riboside (6-thioguanosine) (*IV*), 2-amino-6-hydroxy-8-thiopurine riboside (8-thioguanosine) (*V*), 4-hydroxy-2-thiopyrimidine (2-thiouracil) (*VI*), 2-hydroxy-4-thiopyrimidine riboside (4-thiouridine) (*VII*), 4-amino-2-thiopyrimidine (2-thiocytosine) (*VIII*) and 4-amino-2-thiopyrimidine riboside (2-thiocytidine) (*IX*) were obtained from Sigma (U.S.A.) and were used without further purification. These compounds have the following general structure.





	R^1	R^2	R^3	R^4
I	SH	H	H	H
II	SH	H	H	ribose
III	SH	NH_2	H	H
IV	SH	NH_2	H	ribose
V	OH	NH_2	SH	ribose

	R^1	R^2	R^3
VI	OH	SH	H
VII	SH	OH	ribose
VIII	NH_2	SH	H
IX	NH_2	SH	ribose

The solutions containing different concentrations of thiol compounds were prepared by dissolving a known amount of chemically pure product in a specific volume of Britton–Robinson buffer. This buffer was brought to constant ionic strength of 0.5 mol l^{-1} by the addition of NaCl and adjusted to the desired pH. It also served as a supporting electrolyte. Brdicka solutions, have the composition: 0.2 mM $CoCl_2$, 0.1 M NH_4OH and 0.1 M NH_4Cl were used. A stock 0.1% solution of Triton X-100 was used. Twice-distilled deionized water served as the solvent. The pH was measured with a digital Radiometer pH-meter Model M64, accurate to ±0.05.

Apparatus and Methods

A Princetone Applied Research (PAR) Model 303 DME was used in conjuction with a PAR Model 174A polarographic analyzer and Hewlett–Packard 7045 X–Y recorder for sampled DC polaro-

graphy. For controlled potential electrolysis experiments, the PAR Model 173 potentiostat is used to control the potential of the working electrode at which electrolysis of interest is proceeding. The potentiostat is provided with a Model 178 Electrometer probe to monitor the reference electrode (SCE). The 179 digital coulometer was designed to plug directly into 173 potentiostat. The experimental results are read directly from the 179 digital meter.

The cell used for the polarographic studies is the thermostatted PAR cell equipped with a three electrodes system. This system contained a dropping mercury electrode (DME) as the working electrode, Ag/AgCl/KCl as the reference electrode and a platinum wire as a counter electrode. The cell was maintained at 22 ±0.5 °C. The solution was oxygenated with pure (99.998%) nitrogen before contact with mercury and start of electrochemical experiments.

RESULTS

The electrochemical behaviour of the thiol compounds *I – IX* was followed in universal buffer solutions of pH 3.2 – 11.2 by DC polarography. At potentials close to 0 V, all compounds investigated were found to produce anodic currents. The ability of thiopurines and thiopyrimidines to yield anodic polarographic current arising from the formation of a sparingly soluble mercury compound[14]. In addition to the anodic wave, a typical catalytic hydrogen discharge wave or peak appears at more negative potential of ca −1.4 V. This wave appears in acid medium with an extraordinary current values and strong pH-dependence. The dependence of its height on the depolarizer concentration exhibits a saturation effect at higher concentration. These properties in conjuction with the disappearance of the wave in the presence of surfactants and the formation of gas bubbles at the mercury electrode all indicate that this is a surface catalytic hydrogen wave.

Catalytic Activity of Thiopurine Derivatives

The pH dependence of the catalytic wave of the thiopurine derivatives (*I – V*) was studied and represented in Fig. 1 and Table I. The catalytic waves decreased rapidly with increasing pH and completely disappeared at pH > 6. Significant catalytic waves commenced at pH values of 2.1 or 3.2. This catalytic wave overlaps the reduction wave of thiopurine derivatives; the latter is revealed only under conditions where catalytic effects disappear. According to the reduction mechanism clarified by Janik and Elving[15–17] the N(1)=C(6) and afterwards the N(3)=C(2) double bond undergoes at pH range from acid to neutral, a totally irreversible electrode reaction with a total uptake of 4 electrons and 4 protons. Accordingly, it is suggested that the reduction products of the compounds *I – V* are 1,6-dihydro- or 1,2,3,6-tetrahydrothiopurine derivatives. Because of the catalytic activity of the reduction products, a second catalytic wave appears for compound (*IV*) at pH values above 4.2 (Table I).

The dependence of the catalytic wave on the bulk concentration of the above-mentioned substances (*I – V*) is carried out at pH 3.2 and it is represented in Fig. 2. The

height of the catalytic wave linearly dependent on concentration c to a limit value, whereas a nonlinear function of concentration at high values of c was observed. These results indicate that the character of the catalytic process will depend on the nature of the adsorption of the various investigated compounds.

Since catalytic waves are frequently seriously affected by surfactants[18] the effect of Triton X-100 on the catalytic wave was studied at pH 3.2 (Fig. 3). Addition of small amount of Triton X-100 was found to depress the height of the catalytic wave. In presence of 0.003% Triton X-100 the catalytic waves for compounds *IV* and *V* completely disappeared, whereas for compounds *I – III* the catalytic waves height reduced by 58.3%, 88.8% and 32.1%, respectively. This results indicate that the catalytic wave is a surface catalytic wave.

TABLE I

Influence of pH (Britton–Robinson buffer) on the normalized wave height, i_n, and the half-wave potential, $E_{1/2}$, for sigmoidal-shaped waves, or the peak potential, E_p, for peak-shaped waves in sampled DC polarography for some thiopurine derivatives

Compound	pH	c mmol l^{-1}	i μA	i_n μA mmol^{-1} l	$E_{1/2}$ or E_p V
I	2.1	0.46	16.5	35.87	–1.32[a]
	3.2	0.46	7.5	16.30	–1.40[a]
	4.2	0.46	6.5	14.13	–1.42
	5.2	0.46	6.0	13.04	–1.54
	6.2	0.46	5.5	11.95	–1.63
II	3.2	0.46	26.00	56.50	–1.40[a]
	4.2	0.46	6.25	13.58	–1.42[a]
	5.2	0.46	1.87	4.06	–1.45
III	3.2	0.576	15.00	26.04	–1.43[a]
	4.2	0.576	15.50	26.91	–1.51[a]
IV	3.2	0.0065	11.75	1 807.70	–1.42[a]
	4.2	0.0065	3.50	538.50	–1.37[b]
		0.0065	6.00	923.10	–1.50[c]
	5.2	0.4615	14.07	30.5	–1.37[b]
		0.4615	15.00	65.00	–1.60[c]
	6.2	0.4615	14.00	30.30	–1.65[c]
	7.2	0.4615	0.30	0.50	–1.65[c]
V	3.2	0.967	16.50	17.06	–1.50
	4.2	0.967	4.00	4.13	–1.52
	5.2	0.967	2.25	2.32	–1.54

[a] Peak potential E_p; [b] 1st catalytic wave. [c] 2nd catalytic wave.

A large temperature coefficient was observed for the catalytic wave over the range 0 – 40 °C at pH 3.2. The catalytic wave height increased linearly with temperature up to about 30 °C, with temperature coefficient of 1.52, 2.70, 3.29, 1.38 and 1.76% deg^{-1} for compounds *I* – *V*, respectively. Such large temperature coefficients are often typical for the catalytic waves[18], where competing effects of catalyst desorption and increased reaction rates determine the overall temperature effect.

During the course of electrolysis at the wave potential of compounds *I* – *V*, a high current flow was observed. Furthermore, it was not possible to eliminate the polarographic wave even after many hours of electrolysis. The electrolysis current remained at a high value and the solution pH increased. It was also found that with prolonged electrolysis, the starting compound was the only product detectable, testifying to complete regeneration of the depolarizer and pointing to a major part of the current-controlling process which is the catalytic reduction of hydrogen ions. As a result coulometric measurements of faradaic n-values were unsuccessful.

Catalytic Activity of Thiopyrimidine Derivatives

In aqueous buffered medium (3.2 < pH > 7.2) thiopyrimidine derivatives exhibit two polarographic waves **a** and **b**. In the pH range 8 – 11 there is an additional wave **c** which is obscured by the background discharge, as a result of which, its characterization is difficult (Table II).

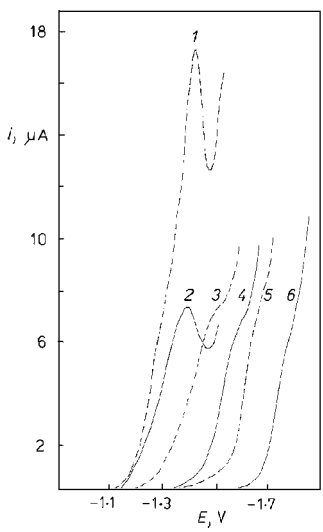


FIG. 1
DC polarography of 4.6 . 10^{-4} mol l^{-1} of *I* at different pH values: *1* 2.1; *2* 3.2; *3* 4.2; *4* 5.2; *5* 6.2; *6* 7.2. Universal buffer, 20 °C, scan rate 5 mV s^{-1} and drop time 2 s

Wave **a** shows up in acid medium at pH < 5.2 with a maximum current considerably in excess of that anticipated for a diffusion wave under these conditions (Table II). The temperature coefficients for the wave **a** are 1.62, 1.81 and 2.13% deg^{-1} for compounds *VII – IX*, respectively. The dependence of wave height on depolarizer concentration exhibited a saturation effect (Fig. 4). Addition of surfactants, such as Triton X-100, led to a decrease of the wave, which disappeared entirely in the presence of an excess of surfactant. Even prolonged electrolysis at the wave **a**, which was accompanied by a high current flow, had no effect; the starting compound was the only product detectable, testifying to complete regeneration of the depolarizer and pointing to the purely catalytic character of wave **a**.

The properties of wave **b** are markedly pH-dependent (Table II). From acid to neutral medium this wave initially appears, whereas in strongly alkaline medium (pH 12) the wave completely disappears. The dependence of the limiting current on depolarizer concentration exhibits a saturation effect. $E_{1/2}$ for wave **b** is dependent on pH, the wave is shifting to more negative potential with increase in pH. Surfactants affect wave **b** to a considerably lesser extent than wave **a**, e.g. addition of Triton X-100 to a concentration of 0.004% leads to elimination and formation of a normal wave with a simultaneous shift of $E_{1/2}$ by about 0.04 V to more negative potentials. As regards wave **b**, its properties show that it is due to two simultaneous proceses, the catalytic liberation of hydrogen and the process of reduction of the pyrimidine ring. In this context, thiopyrimidine derivatives exist in aqueous solutions in the thione form. This form is readily electro-reducible by a mechanism very similar to that observed for other pyrimidines

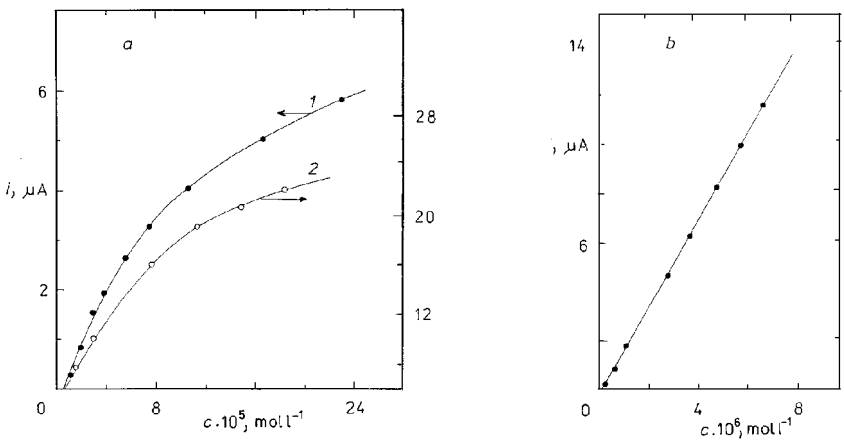


FIG. 2
Dependence of the hight of DC polarographic catalytic wave at pH 3.2 on the concentration of compound *a*: *1 I*, *2 II* and *b*: *IV* in the low concentration range

TABLE II

Influence of pH (Britton–Robinson buffer) on the normalized wave height, i_n, and the half-wave potential, $E_{1/2}$, for sigmoidal-shaped waves, or the peak potential, E_p, for peak-shaped waves in sampled DC polarography for some thiopyrimidine derivatives

Compound	pH	Wave	c mmol l^{-1}	i μA	i_n μA $mmol^{-1}$ l	$E_{1/2}$ or E_p V
VII	3.2	**a**	0.166	7.25	43.6	–1.32[a]
	4.2	**a**	0.166	3.25	19.6	–1.32[a]
		b	0.166	3.50	21.0	-1.44
	5.2	**a**	0.166	0.75	4.5	-1.34
		b	0.166	1.70	10.2	–1.45
	6.2	**b**	0.166	1.50	9.0	–1.46
	7.2	**b**	0.166	1.00	6.0	–1.48
	8.2	**b**	0.166	0.90	5.4	–1.50
	9.2	**c**	0.166	0.50	3.0	–1.55
	10.2	**c**	0.166	0.44	2.6	–1.60
	11.2	**c**	0.166	0.37	2.3	–1.67
VIII	3.2	**a**	0.967	75.00	77.6	–1.45
	4.2	**a**	0.967	70.00	72.4	–1.52
	5.2	**a**	0.967	21.00	21.7	–1.53
	6.2	**a**	0.967	11.25	11.6	–1.57
	7.2	**a**	0.967	8.12	8.4	–1.59
	8.2	**b**	0.967	6.00	6.2	–1.80
	9.2	**b**	0.967	4.50	4.6	–1.87
IX	3.2	**a**	0.0215	5.60	260.5	–1.45
	4.2	**a**	0.0215	4.00	186.0	–1.50
	5.2	**a**	0.0215	3.00	139.5	–1.52
	6.2	**a**	0.0215	2.80	130.2	–1.57
	7.2	**a**	0.0215	0.90	41.8	–1.65
	8.2	**a**	0.0215	0.80	37.2	–1.70
	9.2	**a**	0.0215	0.50	23.2	–1.76
		b	0.0215	0.50	23.2	–1.90
	10.2	**b**	0.0215	1.00	46.5	–1.92
	11.2	**b**	0.0215	2.50	116.3	–1.94

[a] Peak potential E_p.

having similar structure[9,10,19–21]. For 2-thiopyrimidine derivatives *VI*, *VIII* and *IX* the double bond N(3)=C(4) is the electroactive center of molecules. Initial 1 e/1 H^+ attack is directed on the N(3)=C(4) bond; free radical formed dimerize or undergo further 1 e / 1 H^+ reduction to 3,4-dihydro-2-thiopyrimidine derivatives. However, for 4-thiouridine (*VII*) the reduction wave is a 4 e/4 H^+ process involving reduction of the 4-thiouridine ring to 5,6-dihydropyrimidone-2. This indicates that both double bonds C(5)=C(6) and C=S are reduced simultaneously. Accordingly, it is to be noted that the wave **b** is probably due to electroreduction of the investigated compounds accompanied by the catalysis of the hydrogen evolution by the products of their reduction. Moreover, the reduction product of 4-thiouridine (5,6-dihydropyrimidone-2) give catalytic effects at more negative potential (wave **c**). This indicates a superposition of catalytic effects on the reductive process.

Under the same conditions, 2-thiouracil (*VI*) was found to be catalytically inactive in the pH range 3.2 – 11.2, but it induces catalytic hydrogen wave in Co(II)-containing ammonia buffer (pH 9.2). This indicate that the formation of a complex of the thiol

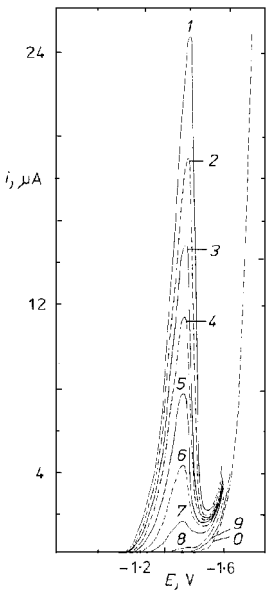


FIG. 3
Effect of Triton X-100 concentration on the catalytic wave height of 9.9 . 10^{-6} mol l^{-1} *IV* at pH 3.2. *0* background, *1* 0.0; *2* 0.0002; *3* 0.0006; *4* 0.001; *5* 0.0014; *6* 0.0018; *7* 0.0022; *8* 0.0026; *9* 0.003%. Other conditions as in Fig. 1

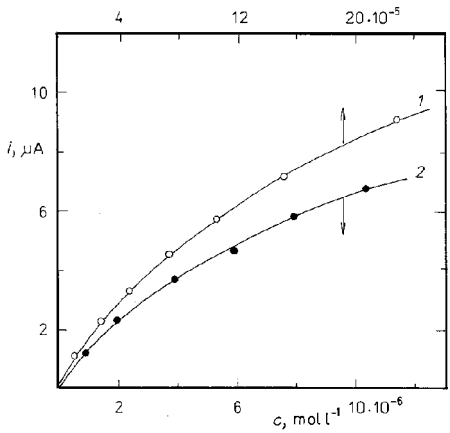


FIG. 4
Dependence of the catalytic wave height on the concentration of *1 VII* and *2 VIII* at pH 3.2. Other conditions as in Fig. 1

containing substance with cobalt ions must be regarded as a further condition necessary for the formation of a catalytic wave.

DISCUSSION

All the investigated compounds exhibit similar electrochemical properties. In acid medium there is a typical catalytic wave, as shown by its parameters and the regeneration of the depolarizer during the electrode process (except 2-thiouracil). Concentration studies of this wave and its disappearance in the presence of surfactants suggest that it is a typical catalytic surface wave.

The general mechanism for catalytic hydrogen evolution by thiols was formulated previously[9]. It was found that the catalytic activity is due to the unshared pair of electrons on the sulfur, oxygen or nitrogen atoms to which proton may attach itself. For thiopurine derivatives *I* – *V* the catalytically active center is S[6]. Since neither an exocyclic oxygen nor a ring nitrogen participate in the liberation of hydrogen. Based on the above observations, it is suggested that the enhanced catalytic current for 6-thioguanosine compared to that for 8-thioguanosine (Table I) results from the different position of thio group (–SH) in the purine moiety. This demonstrates that the introduction of sulfur into the 8-position of purine moiety markedly decreases the basic properties and consequently decreases the catalytic hydrogen wave according to the reaction mechanism[9]. On the other hand, although both 6-thioguanine and 6-thioguanosine possess the structure suitable to produce catalytic waves (–SH at C(6) and $-NH_2$ at C(2), the catalytic activity of the 6-thioguanosine is much higher (Table I), because of its higher adsorbability on the electrode surface. The AC voltammetric measurements[22] confirm the strong adsorption of the thiopurine and thiopyrimidine derivatives at mercury electrode surface. Over the pH range 3.2 – 9.2 and at about 10^{-5} M thiopurine derivatives, a very well-defined pit is observed around the electrocapillary zero of the blank supporting electrolyte. The pit reflects the formation of a compact adsorbed film due to pronounced lateral interactions of adsorbed species. However, in neutral and alkaline buffer solutions (pH > pK_a) a new depression appears at about –0.15 V (anodic pit). This anodic pit is located at a positively charged electrode and its occurrence at pH above pK_a value of the investigated compounds indicates that it corresponds to the adsorption of the anionic species. The AC voltammetric results indicate that the presence of a ribose moiety in the nucleoside facilitates the formation of the perpendicular and stacked layer at the electrode surface. In this context, the surface activity for thiopurine derivatives decreases in the order 6-thioguanosine > 6-thiopurine riboside > 8-thioguanosine > 6-thioguanine > 6-thiopurine. On the other hand, the catalytic activity is most likely related to adsorption with the planes of the molecules perpendicular to the electrode surface, confirmed by the decrease of catalytic activity in the same order as above (Table I), with the exception of 8-thioguanosine which appears to be less active than 6-thioguanine. The foregoing results indicate that, in the case of surface catalytic

waves, the adsorption of a sulfur catalyst on the electrode is the factor limiting the rate of the electrode process. Any change in adsorption leads to an increase of activity or inhibition of the electrochemical step.

For thiopyrimidine derivatives *VI – IX* the catalytic activity decreases in the order: 2-thiocytidine > 2-thiocytosine > 4-thiouridine (Table II), while 2-thiouracil was found to be catalytically inactive. In this case the catalytically active center is S(4). Neither an exocyclic oxygen nor a ring nitrogen participate in the liberation of hydrogen. It is shown by e.g. absence of catalytic activity for uracil and 2-thiouracil, the unchanged catalytic activity of *N*-methyl derivatives of 4-thiouracil[23], and by the absence of catalytic effects of 4-thiomethyluracil[23]. On the other hand, the catalytic activity of 2-thiocytosine or 2-thiocytidine may be explained by the introduction of amino group ($-NH_2$) to C(4). This confirmed by the replacement of the amino group by the hydroxyl group, would be responsible for the catalytical inactivity in the case of 2-thiouracil. Furthermore, the presence of a sulfur substituent at position 2 of pyrimidine system (uracil or cytosine) decreases the energy of the lowest unoccupied molecular orbital (LUMO) and, correspondingly, increases the electron acceptor properties of the molecules[11]. This indicates that the position of SH group yields distinct changes in the catalytic activity of the investigated compounds. Moreover, the catalytic activity of thio group is also affected by neighboring groups.

Additional information can be obtained from the adsorption behaviour of the investigated compounds. The adsorption behaviour of thiopyrimidine derivatives[22] shows that in acid buffer solutions, pH ≤ 3.2, 2-thiouracil and 4-thiouridine are not associated on the electrode at any bulk concentration and a "dilute" adsorption layer is formed, which reflects a flat orientation of the adsorbed species at the electrode surface[24]. However, at higher bulk concentration of 2-thiocytosine or 2-thiocytidine a sharp decrease in AC capacity current is observed, giving a sharply defined pit, as evidence of a "compact" adsorption layer of stacked vertically oriented adsorbed molecules. It is remarkable that small steric differences in the adsorbed molecules cause a distinct change in the catalytic activity of the thiopyrimidine derivatives *VI – IX*. The foregoing discussion has revealed that the electronic structure and the adsorption of the thiols are essential for the formation of the catalytic hydrogen wave.

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