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Oscillopolarography of Mercaptopurine and Its Synthesis Intermediates Using a Commercial Oscilloscope and an Adapter Circuit

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Abstract □ Details are given of an adapter circuit to be used with a storage oscilloscope fitted with a camera back for the recording of oscillopolarograms with the dropping mercury electrode. The satisfactory operation of the unit was shown by studies with zinc, cadmium, cupric, and plumbous ions in various supporting electrolytes. The unit was also used for an oscillopolarographic study of 6-purinethiol (mercaptopurine) and its synthesis intermediates: purine-6(1H)-one (hypoxanthine), 4,5-diamino-6-hydroxypyrimidine (4,5-diaminohypoxanthine), 4,5-diamino-6-hydroxy-2-thiopyrimidine (4,5-diamino-2-thiouracil), and 4-amino-6-hydroxy-2-thiopyrimidine (4-amino-2-thiouracil).

Keyphrases □ Oscillopolarography—mercaptopurine and synthesis intermediates, using commercial oscilloscope and adapter circuit □ Mercaptopurine and synthesis intermediates—oscillopolarographic study

The first experiments in oscillopolarography (1, 2) used alternating current to investigate the electrode processes in polarography and followed the changes in electrode potential on the oscilloscope. Since the commercial introduction¹ of the polaroscope, papers have shown that the method is applicable to qualitative and quantitative analyses of inorganic and organic compounds, to the determination of decomposition kinetics, and to following fast electrode processes. Many substances that are inactive in dc polarography give characteristic ac oscillopolarograms. The technique supplements classical polarography but has its own established theory and instrumentation (3-6).

This study was an investigation of 6-purinethiol

(mercaptopurine) (I), an antitumor agent, and four synthesis intermediates which might be present in mercaptopurine: purine-6(1H)-one (hypoxanthine) (II), 4,5-diamino-6-hydroxypyrimidine (4,5-diaminohypoxanthine) (III), 4,5-diamino-6-hydroxy-2-thiopyrimidine (4,5-diamino-2-thiouracil) (IV), and 4-amino-6-hydroxy-2-thiopyrimidine (4-amino-2-thiouracil) (V) (7).

EXPERIMENTAL

Equipment—The following were used: adapter circuit to give the desired function, $dE/dt = f(E)$ as illustrated in Fig. 1 (any oscillator capable of a 10-v peak-to-peak output can be used in the circuit); type 546 B storage oscilloscope with type 2B67 time base and 3A3 dual-trace differential amplifier, fitted with an oscilloscope camera² having a film pack holder³; dropping mercury electrode⁴, 3-sec drop time; and refrigerated, heated water bath and circulator⁵.

Technique—The oscillopolarogram was recorded by photographing the oscilloscope face just before detachment of the drop. To test the proper functioning of the equipment, oscillopolarograms were obtained for zinc, plumbous, cadmium, and cupric ions in various supporting electrolytes including 1 N solutions of hydrochloric acid, sulfuric acid, lithium chloride, sodium hydroxide, lithium hydroxide, and potassium nitrate. Best results were obtained in 1 N lithium chloride. Polarograms for the four ions are shown in Fig. 2. The *Q* values for the ions are shown in Table I; calibration curves for quantitative determination are shown in Fig. 3.

A study was then carried out on Compounds I-V, using as the supporting electrolyte solutions of 1 N H₂SO₄, NaOH, LiCl, KNO₃, and LiOH and also potassium chloride solutions buffered

² Tektronix, Inc., Portland, Ore.

³ Polaroid.

⁴ Catalog No. 3-2942, F. H. Sargent Co., Springfield, N.J.

⁵ Forma Jr., Forma Scientific, Inc., Marietta, Ohio.

¹ Zavody Prumyslove Automatizace, Prague, Czechoslovakia, 1950.

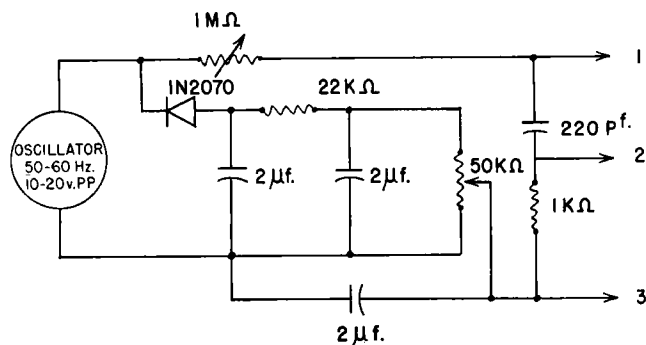


Figure 1—Oscillopolarographic adapter circuit. To record $dE/dt = f(E)$, connect 1 to mercury pool reference and oscilloscope horizontal, 2 to oscilloscope vertical, and 3 to mercury cathode and oscilloscope ground.

at pH 1.0, 2.5, 4.5, 6.2, 7.3, 8.4, 9.0, 11.0, and 13.5. The best separations for Q values were found in 1 N H_2SO_4 (Table II). Oscillopolarograms of the five substances are shown in Figs. 4 and 5.

DISCUSSION

The qualitative and quantitative results for the inorganic ions are in agreement with the literature (8, 9). This indicates that the unit is performing as intended.

In the classical polarography of mercaptopurine, pyrimidine, and 2-thiopyrimidine, only a catalytic wave is observed (10). The forte of oscillopolarography is its capability of detecting substances that are neither oxidizable nor reducible but that form capacitive incisions due to adsorption on the surface of the mercury. Figures 4 and 5 show that all five compounds are oscillopolarographically active and show different behavior.

Mercaptopurine shows a sharp, irreversible, capacitive indentation at $Q = 0.45$. Hypoxanthine and Compound III have extremely negative values of 0.95 and 0.94, respectively. Compound IV has $Q = 0.46$ and Compound V has $Q = 0.47$, lobe-like rather than sharp. The results suggest that both the sulfhydryl group and the pyrimidine ring are polarographically active. All compounds are characterized by irreversible electrode processes.

Even though there are sufficient differences in Q values to allow quantitative determination of hypoxanthine in a mixture with mercaptopurine, the relative depth of the incisions indicates

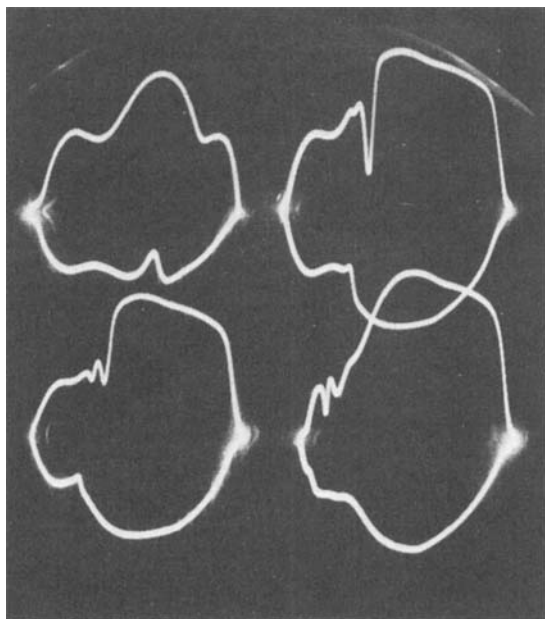


Figure 2—Oscillopolarograms in 1 N $LiCl$, 4.5×10^{-4} M solutions. Key: top left, zinc; bottom left, plumbous; top right, cadmium; and bottom right, cupric.

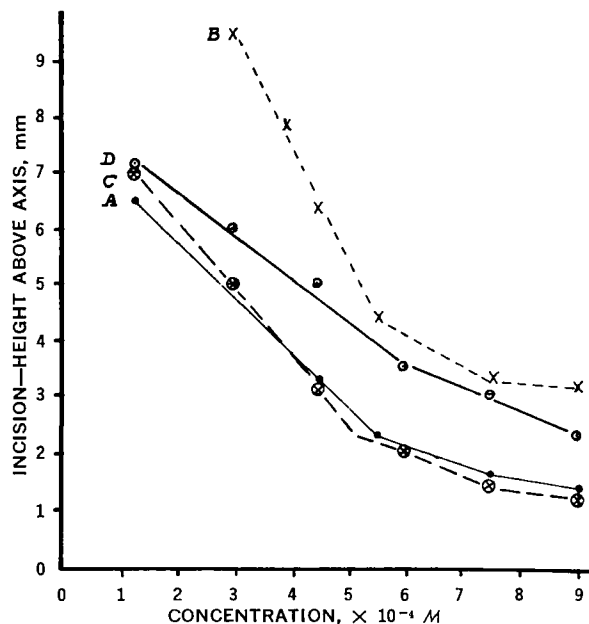


Figure 3—Relation between concentration and depth of incision. Key: A, zinc, $Q = 0.60$, anodic; B, plumbous, $Q = 0.35$, cathodic; C, cadmium, $Q = 0.40$, cathodic; and D, cupric, $Q = 0.15$, cathodic.

that it is unlikely that the determination can be made when the hypoxanthine-mercaptopurine ratio is smaller than 1:10.

CONCLUSIONS

1. The adapter circuit suggested was shown to be functional. The sensitivity of the apparatus is such that it is expected to be useful for solutions at a concentration of $1-5 \times 10^{-4}$ M .
2. Mercaptopurine, which yields only a catalytic wave in classical polarography, gives a characteristic oscillopolarogram, different from that of four possible contaminants.

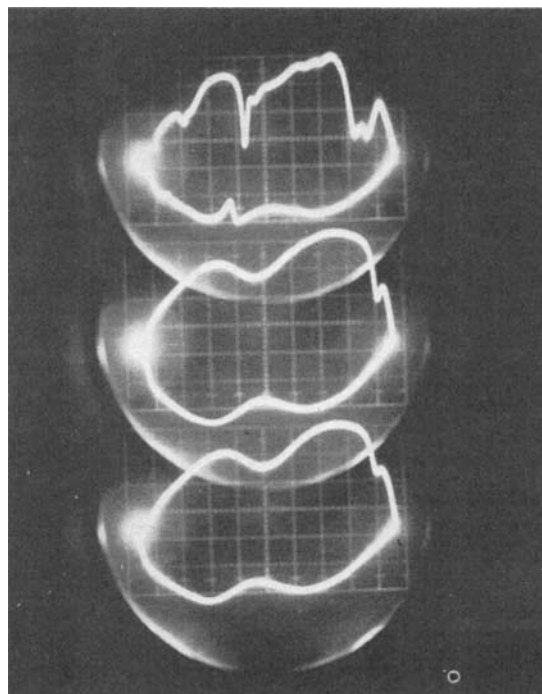


Figure 4—Oscillopolarograms of (from top to bottom) Compound I, Compound II, and Compound III; $dE/dt = f(E)$ (3×10^{-4} M in 1 N H_2SO_4 , dropping mercury electrode).

Table I—Q Values in 1 N Lithium Chloride

Ions	Q Cathodic	Q Anodic
Zinc	0.85	0.60
Plumbous	0.30	0.20 ^a
Cadmium	0.35	0.30 ^a
	0.40	
Cupric	0.15	—
	0.25	

^a Lobe-like, a flat indentation instead of a sharp one.

Table II—Q Values in 1 N H₂SO₄

Compound	Cathodic	Anodic
I	0.20	0.35
	2.45	
	0.85	
	0.90	
II	0.95	—
III	0.94	—
IV	0.46	—
V	0.20	0.20
	0.47	

3. Because of the differences in Q values shown by mercaptopurine and the possible contaminants, it might be possible to develop a quantitative measure of mercaptopurine in the presence of these contaminants. No advantage is foreseen over the more conventional spectrophotometric determination.

4. Because of the relative depth of the incisions, the determination by oscillography of the contaminants at trace levels in mercaptopurine does not seem possible.

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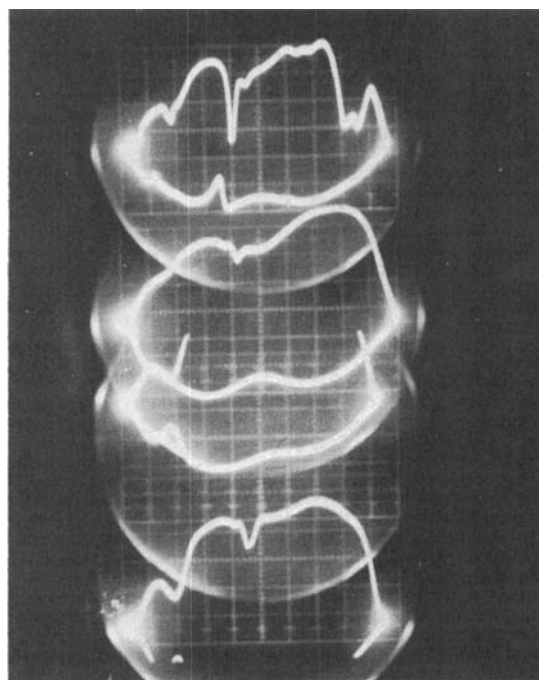


Figure 5—Oscillograms of (from top to bottom) Compound I, Compound IV, Compound V (anodic branch), and Compound V (cathodic branch); $dE/dt = f(E)$ (3×10^{-4} M in 1 N H₂SO₄, dropping mercury electrode).

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