Inhibition of Epinephrine Oxidation in Weak Alkaline Solutions

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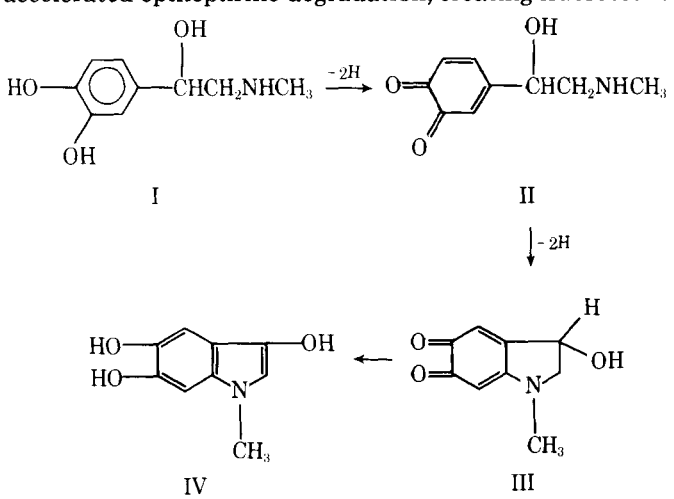
Abstract □ The effect of sodium metabisulfite and cysteine on the oxidation of epinephrine in weak alkaline solutions was studied. The fluorescent intensity of the reacting mixture also was measured. Sodium metabisulfite in 2×10^{-3} *M* NaOH at concentrations lower than 3×10^{-5} *M* had no effect on epinephrine oxidation; however, in concentrations from 5×10^{-5} to 3×10^{-4} *M*, it greatly accelerated this process. Its inhibitory action appeared only in concentrations greater than 5×10^{-4} *M*. The mechanism of these reactions also was explained. Cysteine in 2×10^{-3} *M* NaOH inhibited the oxidation of epinephrine even in concentrations of 7×10^{-5} *M*.

Keyphrases □ Epinephrine—oxidation in alkaline solutions, effect of sodium metabisulfite and cysteine □ Oxidation—epinephrine in alkaline solutions, effect of sodium metabisulfite and cysteine □ Sodium metabisulfite—effect on oxidation of epinephrine in alkaline solutions □ Cysteine—effect on oxidation of epinephrine in alkaline solutions □ Adrenergics—epinephrine, oxidation in alkaline solutions, effect of sodium metabisulfite and cysteine

The stabilization of epinephrine (I) is a problem because present technological methods do not permit long storage of preparations including it (1, 2). The instability of epinephrine in solution is related to its spontaneous oxidation, caused by the oxygen from the air, which in alkaline solutions proceeds according to Scheme I (3–6). In addition to decreasing the pharmacological activity of the preparation, epinephrine oxidation yields epinephrine quinone (II), adrenochrome (III), and adrenolutine (IV), which are toxic (5).

The mechanism of the reaction between epinephrine and bisulfite leading to the formation of sulfonate was described previously (7, 8). Loss of epinephrine activity was determined by a modified triacetyl derivative procedure. The results of the investigations into accelerated loss in optical activity induced by bisulfite are in agreement with the triacetylation procedure (7, 8).

In this study, the oxidative changes of epinephrine under the influence of sodium metabisulfite were followed by the fluorescence method. Under certain conditions, bisulfite accelerated epinephrine degradation, creating fluorescent products. The objective of this work was to determine the activity of sodium metabisulfite and cysteine on epinephrine oxidation in weak alkaline solutions.



Scheme I

EXPERIMENTAL

Materials—All chemicals were either reagent or analytical grade. Epinephrine[1] (BP 1973 and USP XVIII), (−)-1-(3,4-dihydroxyphenyl)-2-methylaminoethanol, was dissolved in an equal amount of hydrochloric acid.

Cysteine chloride monohydrate[2], quinine sulfate (Ph. Jug. II), and sodium metabisulfite[2] were used as received.

Instrumentation—The intensity of the fluorescent reacting mixture was measured by the photoelectric fluorometer based on the principle of ray penetration (9).

Filter paper[3], 8 cm in diameter, was used as a fluorescence standard. It was soaked in 10 mg % quinine sulfate and 0.1 *M* H_2SO_4, air dried, and fixed to the bottom of a petri dish for easier handling while measuring. The photocurrent strength was regulated by a resistor so that the fluorescence standard showed a decline of 500 parts on the scale.

All measurements were performed in a 50-ml glass beaker (5.5 cm high and 3.5 cm in diameter). To maintain a constant thickness of the layer, the total volume of the reacting mixture was always a constant 10 ml. The oxidative changes of epinephrine were followed in free air and in nitrogen atmospheres at 22°.

RESULTS AND DISCUSSION

When following the spontaneous oxidation of epinephrine in relation to the concentration of sodium hydroxide, all measurements were performed with identical concentrations of epinephrine of 1.09×10^{-5} *M*. Figure 1 shows the changes in the intensity of fluorescence in relation to time in solutions of sodium hydroxide from 2×10^{-3} to 2×10^{-1} *M*. The acceleration of the oxidation of epinephrine and the intensity of the fluorescence of the reacting mixture increased correspondingly to the increase in the concentration of sodium hydroxide.

Under the same conditions in relation to the concentration of epi-

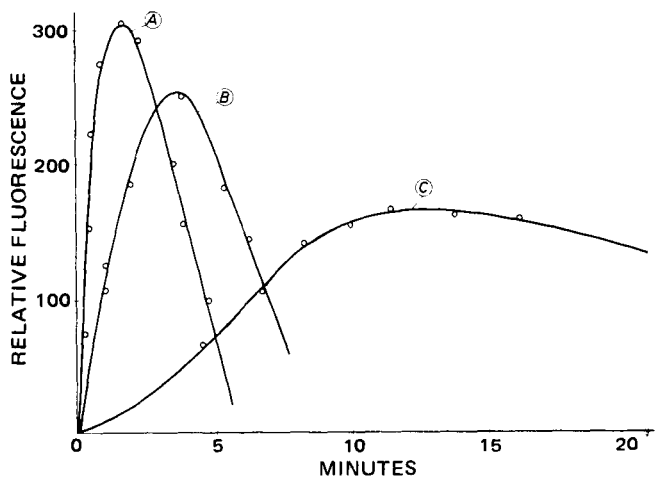


Figure 1—*Changes in fluorescent intensity of the* 1.09×10^{-5} M *epinephrine solution in relation to time and sodium hydroxide concentration. Key: A,* 2×10^{-1} M *NaOH, pH 13.2; B,* 2×10^{-2} M *NaOH, pH 12.2; and C,* 2×10^{-3} M *NaOH, pH 10.7.*

[1] Adrenalin.
[2] Merck.
[3] Whatman No. 1.

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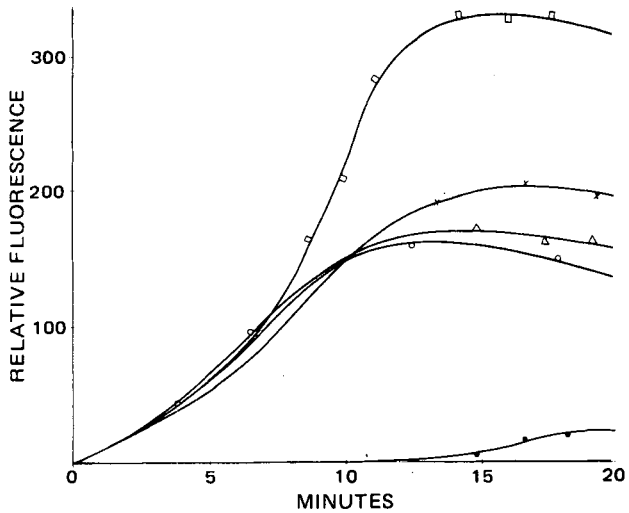


Figure 2—*Changes in fluorescent intensity in relation to time of the 1.09 × 10⁻⁵ M epinephrine solution in 2 × 10⁻³ M NaOH in the presence of different concentrations of sodium metabisulfate. Key:* ○, *control (2 × 10⁻³ M NaOH), pH 10.7;* △, *3 × 10⁻⁵ M $Na_2S_2O_5$, pH 10.5;* □, *5 × 10⁻⁵–3 × 10⁻⁴ M $Na_2S_2O_5$, pH 10.3–10.7;* ×, *4.5 × 10⁻⁴ M $Na_2S_2O_5$, pH 9.5; and* ●, *8 × 10⁻⁴ M $Na_2S_2O_5$, pH 8.4.*

nephrine and sodium hydroxide, the effects of different concentrations of sodium metabisulfite on the oxidation of epinephrine were followed (Figs. 2–4). Sodium metabisulfite in concentrations less than 3×10^{-5} *M* had no effect on the oxidation of epinephrine in 2×10^{-3} *M* NaOH. Increasing the concentration of sodium metabisulfite to 5.0×10^{-5} or 3×10^{-4} *M* doubled the effects of oxidation. However, these effects were decreased by an increase in the concentration of sodium metabisulfite of more than 5×10^{-4} *M*. At 8×10^{-4} *M*, the inhibition of the oxidation of epinephrine almost reached its maximum. Epinephrine oxidation was inhibited almost completely in higher concentrations of sodium metabisulfite.

If the sodium hydroxide concentration was increased 10 times (2×10^{-2} *M*), sodium metabisulfite had no stimulus on epinephrine oxidation, and the effects of inhibition were perceivable only when the sodium metabisulfite concentration in the reacting mixture was increased above 2.5×10^{-3} *M*. When the sodium hydroxide concentration was increased to 2×10^{-1} *M*, the effects of sodium metabisulfite were even less visible. To slow oxidation under such conditions, sodium metabisulfite concentrations greater than 1×10^{-2} *M* were needed.

From analysis of the results, it can be assumed that sodium metabisulfite, under the conditions in which it accelerates epinephrine oxidation, reacts according to Scheme II. Sodium metabisulfite dissolved in an alkaline solution in the presence of oxygen produces SO_4^{2-} ions. Increasing the bisulfite concentration increases the concentration of SO_3^{2-} ions, by which the oxidation of epinephrine is stopped.

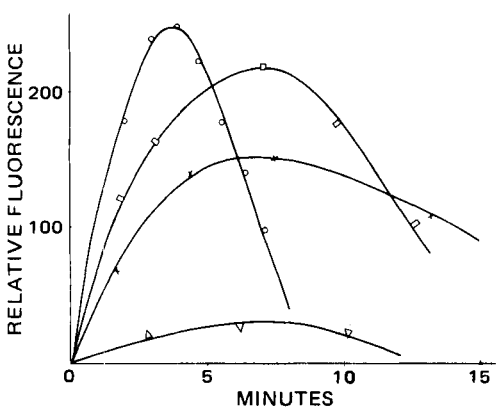


Figure 3—*Changes in fluorescent intensity in relation to time of the 1.09 × 10⁻⁵ M epinephrine solution in 2 × 10⁻² M NaOH in the presence of different concentrations of sodium metabisulfite. Key:* ○, *control (2 × 10⁻² M NaOH), pH 12.2;* □, *2.5 × 10⁻³ M $Na_2S_2O_5$, pH 12.1;* ×, *5 × 10⁻³ M $Na_2S_2O_5$, pH 12.0; and* △, *7 × 10⁻³ M $Na_2S_2O_5$, pH 11.9.*

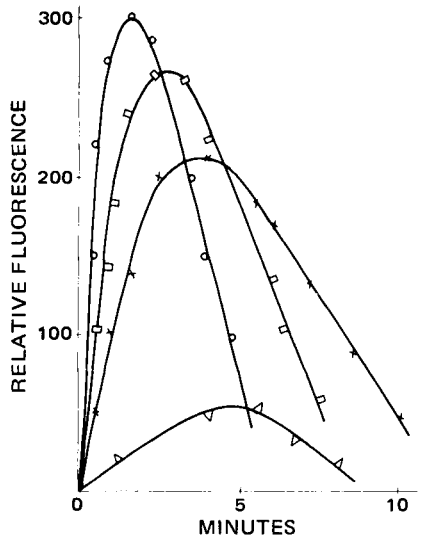


Figure 4—*Changes in fluorescent intensity in relation to time of the 1.09 × 10⁻⁵ M epinephrine solution in 2 × 10⁻¹ M NaOH in the presence of different concentrations of sodium metabisulfite. Key:* ○, *control (2 × 10⁻¹ M NaOH), pH 13.2;* □, *1 × 10⁻² M $Na_2S_2O_5$, pH 13.0;* ×, *1.5 × 10⁻² M $Na_2S_2O_5$, pH 12.8; and* △, *4 × 10⁻² M $Na_2S_2O_5$, pH 12.5.*

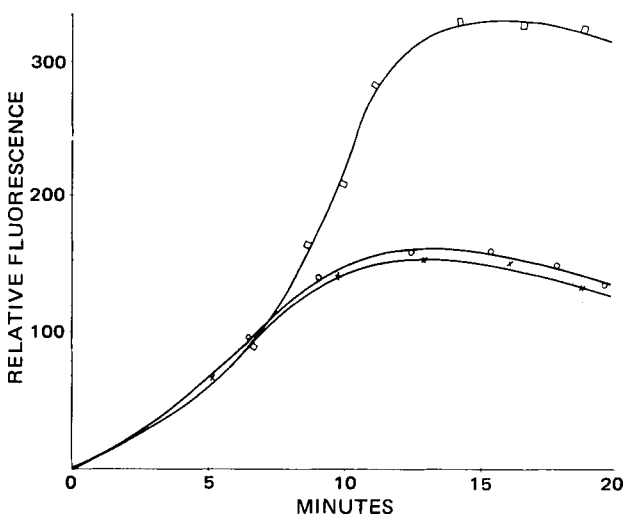


Figure 5—*Changes in fluorescent intensity in relation to time of the 1.09 × 10⁻⁵ M epinephrine solution in 2 × 10⁻³ M NaOH in the presence of the same concentration of sodium metabisulfite, in the presence of air, and in the nitrogen atmosphere. Key:* ○, *control (2 × 10⁻³ M NaOH, air), pH 10.7;* □, *1 × 10⁻⁴ M $Na_2S_2O_5$ (air), pH 9.8; and* ×, *1 × 10⁻⁴ M $Na_2S_2O_5$ (nitrogen), pH 9.8.*

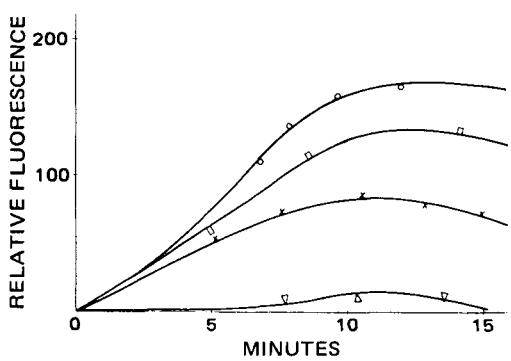
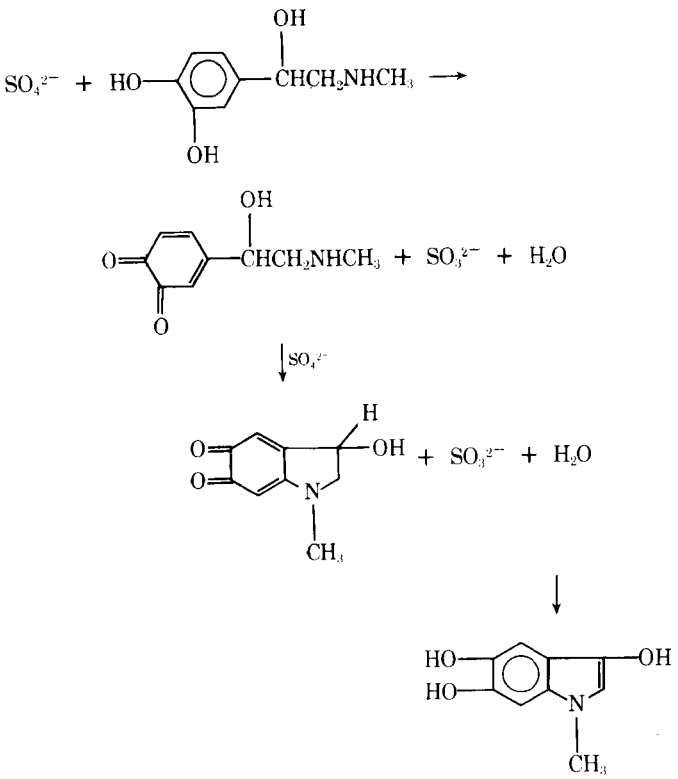


Figure 6—*Changes in fluorescent intensity in relation to time of the 1.09 × 10⁻⁵ M epinephrine solution in 2 × 10⁻³ M NaOH in the presence of different concentrations of cysteine. Key:* ○, *control (2 × 10⁻³ M NaOH), pH 10.7;* □, *2.5 × 10⁻⁵ M cysteine, pH 10.4–10.6;* ×, *5 × 10⁻⁵ M cysteine, pH 10.4–10.6; and* △, *7 × 10⁻⁵ M cysteine, pH 10.4–10.6.*



Scheme II

Previous success (10) in stabilizing different water solutions of epinephrine up to 5 years by preparing them in a nitrogen atmosphere confirms this hypothesis.

The results of the present experiments obtained in the nitrogen atmosphere show that oxygen in the reacting mixture is the most decisive factor for the stimulation of epinephrine oxidation in the presence of sodium metabisulfite in the given concentrations. Namely, the stimulating action of sodium metabisulfite on epinephrine oxidation is absent when dissolved oxygen from the air is eliminated from the solution of the reactant and when the reacting process goes on in the nitrogen atmosphere. In a highly alkaline medium, this process does not occur because of the strong influence of the hydroxide ions on epinephrine oxidation (Fig. 5).

Under the same experimental conditions, numerous experiments were performed using cysteine in different concentrations instead of sodium metabisulfite. Cysteine concentrations greater than 7×10^{-5} *M* efficiently inhibited epinephrine oxidation (Fig. 6). Since cysteine is an integral part of the human organism and since it maintains a kind of balance with the epinephrine concentration (11), it would be worthwhile to study its application in the stabilization of aqueous epinephrine solutions.

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Cardiovascular Effects of *Azadirachta indica* Extract

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Abstract □ A crude extract of the leaves of *Azadirachta indica* was studied for its effects on the cardiovascular system of anesthetized guinea pigs and rabbits. These effects include profound hypotension and a minimal negative chronotropic effect, which increased at higher doses. In one rabbit, 200 mg of extract/kg decreased the heart rate from 280 to 150 beats/min. The extract also exhibited a weak antiarrhythmic activity in rabbits against ouabain-induced dysrhythmia.

Keyphrases □ *Azadirachta indica*—extract of leaves, cardiovascular effects evaluated in guinea pigs and rabbits □ Cardiovascular effects—*Azadirachta indica* extract of leaves, evaluated in guinea pigs and rabbits

There is scanty literature information concerning the pharmacological properties of *Azadirachta indica*, an evergreen flowering plant belonging to the family Meliaceae. In Nigeria, this plant, called "dongoyaro," is used primarily to treat malaria, fever, abdominal disorders, and hemorrhoids and as an anthelmintic (1). Recent studies *in vivo* (2) failed to confirm the antimalarial effects attributed to this plant.

A preliminary pharmacological study (3) revealed that the extract acted as a spasmogen on isolated guinea pig ileum. It also increased the respiratory rate in the dog without a change in depth.

The present experiments were undertaken to elucidate further the pharmacological effects of an extract of the leaves of *A. indica* on the heart and circulatory system.

EXPERIMENTAL

Preparation of Crude Aqueous Extract—Air-dried powdered leaves[1] (1.8 kg) of *A. indica* were extracted with 3 liters of distilled water in a soxhlet apparatus for 1 week. The extract volume was then reduced to about 200 ml on low heat with constant stirring. The final 200 ml of

[1] The leaves were collected from plants on the University of Ife campus, Ile-Ife, Nigeria, during January. The plant material was identified as *A. indica* A. Juss. (Meliaceae) by Dr. E. A. Sofowora, Department of Pharmacognosy Incorporating Drug Research Unit, University of Ife, Ile-Ife, Nigeria, and authenticated by comparison with specimens in the Institute of Forest Research Herbarium, Ibadan, Nigeria. Voucher (preserved) specimens (No. UNIFE 426) are on deposit in the Museum and Herbarium of Nigerian Medicinal Plants of the University of Ife.

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