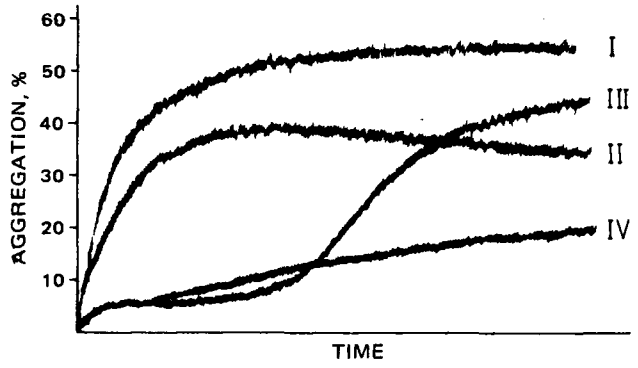

Figure 2—*Aggregation induced by adenosine 5′-diphosphate in filtered dog platelets (I) and in filtered dog platelets previously treated with tetracycline hydrochloride (II) and aggregation induced by collagen in filtered dog platelets (III) and in filtered dog platelets previously treated with tetracycline hydrochloride (IV).*

conclusion that solutions of 10^{-4} *M* adenosine 5′-diphosphate gave identical results to solutions of 2×10^{-5} *M* adenosine 5′-diphosphate in human plasma. The 0.2-mg/ml collagen solutions used in human plasma gave similar results in dog plasma, although the latency period for aggregation with platelet-rich dog plasma was approximately twice that obtained with platelet-rich human plasma. No significant aggregation in platelet-rich dog plasma was obtained with epinephrine, so this aggregating agent was not used for *in vivo* tests in dogs.

Platelet aggregation *in vivo* was studied at 0, 30, 60, and 90 min and 2, 24, 48, and 72 hr after intramuscular administration of tetracycline hydrochloride. As shown in Fig. 1, tetracycline hydrochloride inhibited 40% of collagen-induced platelet aggregation in blood extracted 30 min after the intramuscular administration of 5 mg of antibiotic/kg and 30% of adenosine 5′-diphosphate-induced platelet aggregation in blood extracted 30 and 45 min after a similar dose of the antibiotic. Platelet factor 3 values in dog plasma were similar to those found in human plasma. In dogs treated with tetracycline hydrochloride, factor 3 release was decreased significantly ($p < 0.0001$), with a mean value of 32.5 sec, compared to liberation in untreated dogs, where the mean value was 26 sec.

Electrophoretic mobility was identical for platelets from treated and untreated dogs, indicating unaltered platelet membrane potentials. The results were $x/N = 12.65 \pm 0.05$ and $x_1/N = 13.15 \pm 0.05$, where x is the time required for dog platelets to travel 64 μm (32 μm to the left and 32 μm to the right by varying the polarity), x_1 is the time required for tetracycline-treated dog platelets to travel the same distance, and N is the number of determinations (in this case, 20).

To determine whether the alteration in platelet aggregation produced by tetracycline was due to the platelets themselves or to the surrounding plasma, the platelets were filtered. The activity of normal filtered platelets with tetracycline-treated dog plasma and of tetracycline-treated platelets with untreated dog plasma in the presence of adenosine 5′-diphosphate and collagen was studied; the aggregation of untreated filtered platelets was greater than that of tetracycline-treated platelets (30% inhibition in the presence of adenosine 5′-diphosphate and 85% in the presence of collagen) (Fig. 2).

In conclusion, high concentrations of antibiotics usually completely inhibit the *in vitro* aggregation of platelets induced by adenosine 5′-diphosphate. At concentrations equivalent to plasma levels after administration of a therapeutic dose, only cephaloridine, rifamycin sodium, tetracycline hydrochloride, and amphotericin B partially inhibit adenosine 5′-diphosphate-induced aggregation and only tetracycline hydrochloride maintains this activity in the presence of other aggregating agents. In addition, tetracycline hydrochloride exerts an inhibitory effect *in vivo* on platelet aggregation in dog blood, affecting the platelets themselves and not the surrounding plasma, as shown by the results obtained with platelets filtered in Sepharose columns. It appears to alter the liberation of platelet factor 3.

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ACKNOWLEDGMENTS

Supported by a grant from the Comisión Asesora de Investigación Cientifica y Técnica, Spain.

Binding of Toxic Metabolites of Isoniazid by Aconiazide

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Received October 17, 1979, from **Connaught Laboratories Limited, Willowdale, Ontario, Canada*, and the ‡*Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada.* Accepted for publication May 12, 1980.

Abstract □ Isoniazid, the hydrazide of isonicotinic acid, is widely used in the treatment and prophylaxis of tuberculosis. The toxicity and carcinogenicity of isoniazid have been attributed to the action of its metabolites, hydrazine and acetylhydrazine. Aconiazide, the isonicotinylhydrazone of 2-formylphenoxyacetic acid, has been used in the treatment and prophylaxis of tuberculosis. Aconiazide is hydrolyzed in the body to isoniazid and 2-formylphenoxyacetic acid. 2-Formylphenoxyacetic acid has been shown to bind hydrazine and acetylhydrazine. This binding could explain the lower toxicity of aconiazide and also could provide a reason for postulating its lack of carcinogenicity.

Keyphrases □ Isoniazid—binding of toxic metabolites by aconiazide □ Aconiazide—binding of toxic metabolites of isoniazid, potential as antitubercular agent □ Antitubercular agents, potential—aconiazide, binding of toxic metabolites of isoniazid

Isoniazid (isonicotinic acid hydrazide) was introduced in 1952 as a drug for the treatment of tuberculosis and it soon became recognized as the most effective drug for the treatment and prevention of tuberculosis. It later became apparent that prolonged treatment with isoniazid may affect the peripheral and central nervous systems, and

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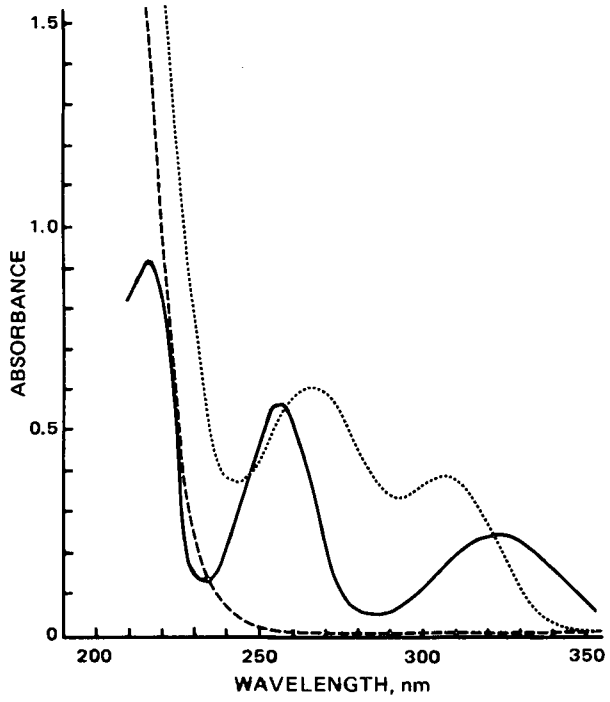

Figure 1—*UV spectra of 2-formylphenoxyacetic acid (0.05 mM) (—), hydrazine (25 mM) (– –), and a mixture of 2-formylphenoxyacetic acid (0.05 mM) and hydrazine (25 mM) (• • •). The spectra were taken within 2 min after the solutions were prepared in 0.1% $NaHCO_3$.*

numerous reports indicated that isoniazid could induce hepatitis (1, 2). Furthermore, it has been well established that isoniazid is carcinogenic in animals (3).

Isoniazid (I) is metabolized to isonicotinyl acetylhydrazine (II), which is metabolized further to isonicotinic acid (III), acetylhydrazine (IV), and hydrazine (V). Free IV and V undergo enzymatic conversion to hepatotoxic (4) and carcinogenic products (3). Therefore, there is obviously a need for antituberculous drugs with the same efficacy as I but with no carcinogenicity and reduced toxicity.

In a search for such drugs, many derivatives of I have been prepared. One compound, aconiazide (the isonicotinylhydrazone of 2-formylphenoxyacetic acid, VI) (5–12),

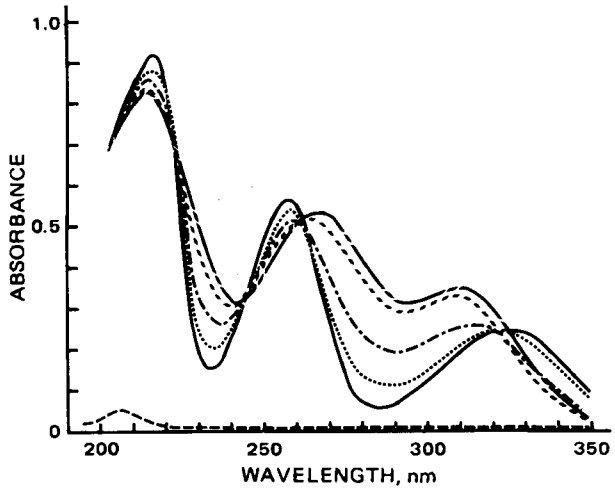

Figure 2—*UV spectra of 2-formylphenoxyacetic acid (0.05 mM) (—), hydrazine (0.05 mM) (– –), and a mixture of the two compounds taken after 30 min (• • •), 4 hr (• – •), 24 hr (– – –), and 3 days (— – —) after its preparation. All solutions were prepared in 0.1% $NaHCO_3$.*

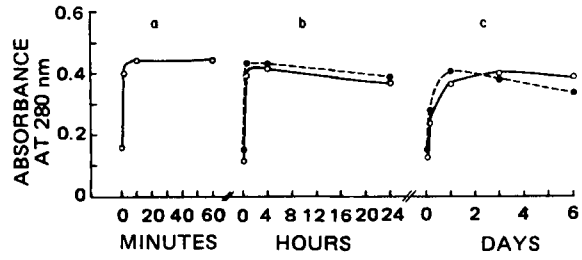

Figure 3—*Reaction of 2-formylphenoxyacetic acid (0.05 mM) with different concentrations of hydrazine in 0.1% $NaHCO_3$ at 23 (—) and 37° (– –). Key: a, 5 mM; b, 0.5 mM; and c, 0.05 mM.*

formed from I and 2-formylphenoxyacetic acid (VII), showed antituberculous activity in animals (6) and humans (8) equivalent to that of I and had a much lower toxicity (5, 7, 8). Aconiazide[1] was used in humans in Canada as a substitute for I (8). The manufacturing of this antituberculous drug was discontinued for economic reasons, although it is still listed in the Compendium of Pharmaceuticals and Specialties (9). However, economic considerations alone should not prevent the use of an antituberculous drug that may have no carcinogenic activity and reduced side effects as compared with I. Therefore, studies were undertaken to determine if the carcinogenic metabolites (IV and V) of I could be bound and hence inactivated by VII, which is a component of VI.

EXPERIMENTAL

Reagents—Isoniazid[2] (I) (mol. wt. 137.14), aconiazide[3] (VI) (mol. wt.

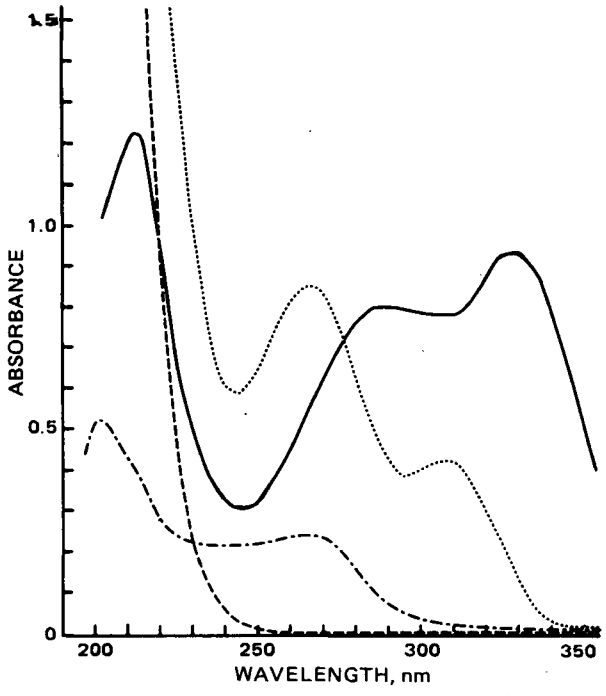

Figure 4—*UV spectra of aconiazide (0.05 mM) (—), hydrazine (25 mM) (– –), and a mixture of aconiazide (0.05 mM) and hydrazine (25 mM) (• • •). The spectrum of isoniazid (0.05 mM) (• – •), which is a component of aconiazide, also is shown. The spectra were taken within 2 min after the solutions were prepared in 0.1% $NaHCO_3$.*

[1] Also known as Compound 377 (6, 7) and later as phenoxalid or aconiazide (9, 10) by the World Health Organization.
[2] Farbenfabriken Bayer A.G., Leverkusen, West Germany.
[3] Connaught Laboratories Ltd., Toronto, Ontario, Canada.

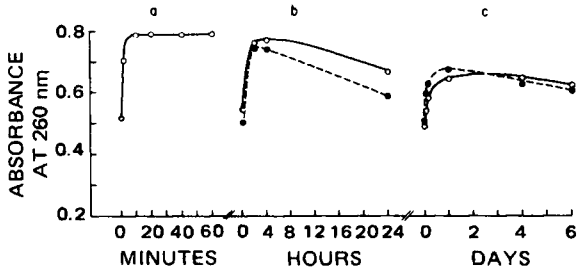

Figure 5—*Reaction of aconiazide (0.05 mM) with different concentrations of hydrazine in 0.1% $NaHCO_3$ at 23 (—) and 37° (- -). Key: a, 5 mM; b, 0.5 mM; and c, 0.05 mM.*

299.28), 2-formylphenoxyacetic acid[4] (VII) (mol. wt. 180.16), acetylhydrazine[5] (IV) (mol. wt. 74.08), and hydrazine[6] (V) (95%) were obtained commercially.

Buffer Solutions—A 0.1% $NaHCO_3$ solution was used to dissolve the reagents and record their UV spectra (Figs. 1–8). The pH of a freshly prepared sodium bicarbonate solution was ~8.4; a 1.25% phosphate buffer of pH 7.2 also was used. It was prepared by dissolving 27.4 g of monobasic potassium phosphate and 72.6 g of dibasic sodium phosphate in 8 liters of water.

UV Spectrophotometry—Due to the sensitivity and characteristics of their UV absorption spectra, the progression of the binding of hydrazine (V) or acetylhydrazine (IV) by 2-formylphenoxyacetic acid (VII) or its isonicotinylhydrazone (VI) can be followed easily. A UV spectrophotometer[7] was used to obtain the spectra.

The progression of the reaction between IV or V and VII in solution was studied by recording the absorbance at 280 nm at various intervals. The progression of the reaction between IV or V and VI in solution was studied at various intervals by recording the absorbance at 260 nm.

RESULTS

Reaction of 2-Formylphenoxyacetic Acid (VII) with Hydrazine (V)—Figure 1 shows the UV spectra of VII and of V at 0.05 and 25 m*M*, respectively. The UV spectrum of a solution containing both substances also is shown. From this spectrum, it appears that a compound, most likely a hydrazone of VII, formed within 2 min after mixing VII and V at final concentrations of 0.05 and 25 m*M*, respectively.

Figure 2 shows the UV spectrum of a solution containing VII and V, both at concentrations of 0.05 m*M*, taken at various time intervals. At this concentration of V (0.05 m*M*), the same new compound was formed but at a much slower rate than when a higher concentration of V (25 m*M*) was used (Fig. 1).

The speed at which VII (0.05 m*M*) reacts with V at different concentrations can be seen more clearly if the absorbance at 280 nm is plotted *versus* time. In fact, Fig. 3 shows that at 23°, the reaction was complete in ~20 min, 2 hr, and 3 days for V at concentrations of 5, 0.5, and 0.05 m*M*, respectively. At 37°, the reaction was approximately two to three times faster than at room temperature and was complete in ~1 hr and 1 day for V at concentrations of 0.5 and 0.05 m*M*, respectively.

Reaction of Aconiazide (VI) with Hydrazine (V)—Figure 4 shows the UV absorption spectra of a solution of VI at 0.05 m*M*[8] (15 μg/ml) and of VI in an excess of V (25 m*M*). Figure 4 also shows that a new compound formed from VI and V within 2 min after these two substances were mixed. Since this new compound has its UV maxima at exactly the same wavelengths as the compound formed from VII and V (Fig. 1), it indicated that the 2-formylphenoxyacetic acid component of VI had reacted with V. Furthermore, Fig. 5 shows that the reaction between VI and V slowed down in exactly the same way as was observed for VII and V when lower concentrations were used.

Reaction of 2-Formylphenoxyacetic Acid (VII) with Acetylhydrazine (IV)—Figure 6 shows the UV absorption spectra of solutions of VII and of IV at 0.05 and 25 m*M*, respectively. Figure 6 also shows the UV spectrum of a solution containing both substances. From this spectrum, it appears that a compound, most likely an acetylhydrazone of VII,

[4] Aldrich Chemical Co., Milwaukee, Wis.
[5] Acethydrazide, Aldrich Chemical Co., Milwaukee, Wis.
[6] Distillation Products Industries, Eastman Organic Chemicals Department, Division of Eastman Kodak Co., Rochester, N.Y.
[7] Pye Unicam SP1800 with linear recorder model AR25.
[8] This concentration can be expected in the serum of patients treated with VI.

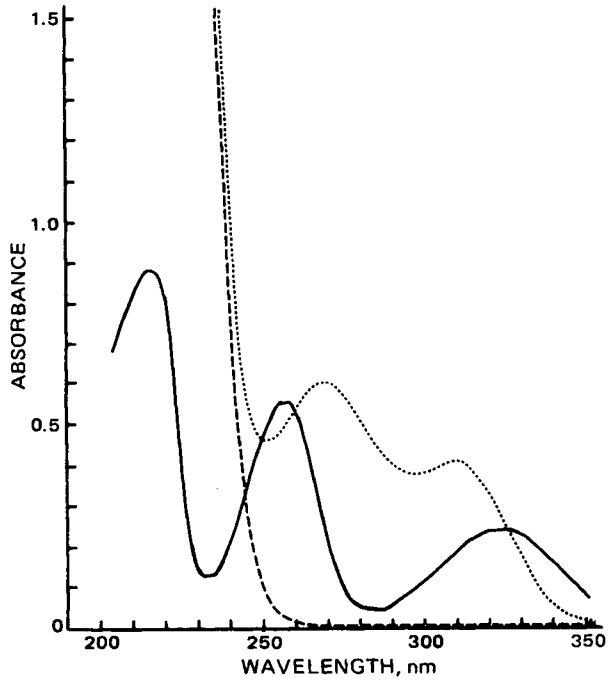

Figure 6—*UV spectra of 2-formylphenoxyacetic acid (0.05 mM) (—), acetylhydrazine (25 mM) (- -), and a mixture of 2-formylphenoxyacetic acid (0.05 mM) and acetylhydrazine (25 mM) (· · ·). The spectrum of the mixture was taken 4 hr after preparation. All solutions were prepared in 0.1% $NaHCO_3$.*

formed within 4 hr after mixing of VII and IV at final concentrations of 0.05 and 25 m*M*, respectively.

Figure 7 shows the speed at which VII reacted with IV at three concentrations (25, 0.5, and 0.1 m*M*). By comparing this reaction with the reaction of VII with V (Figs. 1–3), it is obvious from Figs. 6 and 7 that IV reacted with VII at a much slower rate than V.

Reaction of Aconiazide (VI) with Acetylhydrazine (IV)—Figure 8 shows the UV absorption spectra of VI and of IV at 0.05 and 25 m*M*, respectively. Figure 8 also shows the UV spectrum of a solution containing both substances. From this spectrum, it appears that a new compound formed within 4 hr after mixing VI and IV at final concentrations of 0.05 and 25 m*M*, respectively. Since this new compound has its UV maxima at exactly the same wavelengths as the compound formed from VII and IV (Fig. 6), it can be surmised that the 2-formylphenoxyacetic acid component of VI had reacted with IV.

Reaction of 2-Formylphenoxyacetic Acid (VII) with Hydrazine (V) and Acetylhydrazine (IV) at pH 7.2—The reactions between VII and V or IV were carried out in freshly prepared 0.1% $NaHCO_3$ at a pH of ~8.4. Therefore, it was of interest to find out if the same reactions also would occur at the physiological pH of 7.2 in a phosphate-buffered solution. At this pH, the reactions were not different from those obtained in 0.1% $NaHCO_3$, except that they proceeded at a significantly faster rate.

DISCUSSION

Aconiazide (VI) is formed from I and VII, and VII is a derivative of

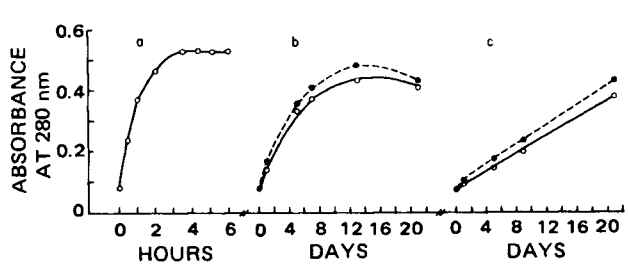

Figure 7—*Reaction of 2-formylphenoxyacetic acid (0.05 mM) with different concentrations of acetylhydrazine in 0.1% $NaHCO_3$ at 23 (—) and 37° (- -). Key: a, 25 mM; b, 0.5 mM; and c, 0.1 mM.*

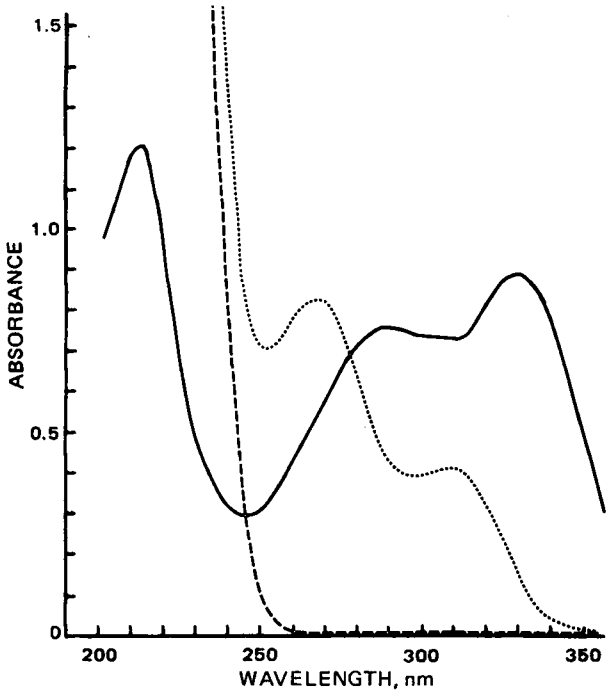

Figure 8—*UV spectra of aconiazide (0.05 mM) (—), acetylhydrazine (25 mM) (– –), and a mixture of aconiazide (0.05 mM) and acetylhydrazine (25 mM) (· · ·). The spectra of the solutions were taken 4 hr after preparation in 0.1% $NaHCO_3$.*

salicylaldehyde. It is well known that aldehydes react with hydrazines. Therefore, the reaction between VII and V or IV, as evidenced by the spectra in Figs. 1–3 and Figs. 4 and 5, respectively, could result in the formation of the hydrazone or acetylhydrazone of VII. Regardless of whether VII or VI was used in the presence of V or IV, the same new compounds were formed, although their levels of absorbance in the 265- and 305-nm regions were different (Figs. 1 and 4 and Figs. 6 and 8). This difference in the level of absorbance is due to the fact that I, which also has an absorption maximum around 265 nm (Fig. 4), is liberated from VI.

The reaction product between VII and V, namely, the sodium salt of the hydrazone of VII, could easily be obtained in crystalline form by mixing a fairly strong solution of the sodium salt of VII with an excess of V and evacuating the mixture to dryness. The preparation so obtained showed a UV absorption spectrum identical to the spectra shown in Figs. 1 and 2.

The fact that V and IV react with VI to form new compounds indicates that VII has a greater affinity for V and IV than for isoniazid (I). One interesting feature of VI is that for each liberated amount of I, the equivalent amount of VII is liberated simultaneously, making it available to bind an equivalent amount of the toxic metabolites released from I, namely, V and IV. This binding could account for the lower toxicity of VI compared to I (7, 8).

It also was shown conclusively that lung tumors can be induced in mice fed isoniazid (I) over 6–12 months and that the tumors are caused by the metabolites of I, namely, acetylhydrazine (IV) and hydrazine (V) (1, 13–15). The demonstration by UV spectrophotometry that a dissociation product of aconiazide (VI) (namely, 2-formylphenoxyacetic acid, VII) binds the carcinogenic metabolites of I strongly suggests that VI may not be carcinogenic. Hence, a comparative study between VI and I in animals is warranted to ascertain whether VI is carcinogenic.

Should these studies show that aconiazide (VI) is noncarcinogenic, and since clinical studies (8), although limited, have shown that VI is as effective as and less toxic than isoniazid (I) in patients with tuberculosis, there should be no reason why VI could not be used as a substitute for I.

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