extraction from the plasma samples was 84 ± 9%. The minimum quantifiable amount of I was ~25 pg/injection.

**Human Data**—To demonstrate the application of the assay modification, urine and plasma I concentrations were measured in a human subject receiving IV[10] during a drug interaction study.

The peak plasma I concentration was achieved at ~1.5 hr after an oral dose of IV. Despite the somewhat scattered plasma data, the elimination half-life could be estimated using truncated plasma data, as reported previously (2, 3). When the truncated 8-hr plasma data were evaluated, an apparent half-life of 3.9 hr was noted; other investigators noted a half-life of 2.8 hr (3) and 4.0 hr (2) using similar data. However, when a 24-hr plasma sample was taken into account (Fig. 2), the elimination half-life was 5.4 hr. It becomes apparent that the use of 8-hr plasma data results in an underestimation of the terminal elimination half-life. Since no blood samples were taken between 8 and 24 hr after drug administration, an accurate estimation of the true elimination half-life was hampered using plasma data.

It was possible, however, to assess accurately the I elimination half-life using the urinary excretion rate data. When the urinary excretion rate of I was determined, the peak excretion rate was at 1.5 hr, in agreement with the time required for peak plasma concentrations to occur in this subject (Fig. 3), and the elimination half-life was 6 hr. In the postabsorptive phase of the plasma concentration and urinary excretion rate data, a biexponential decline was observed (Figs. 2 and 3), suggesting that I may follow multicompartment kinetics in humans. Following intravenous administration, Bateman *et al.* (3) reported biexponential kinetics for I, but these investigators may have missed the true terminal elimination phase noted in this subject (Fig. 3). This finding was confirmed by the fact that measurement of the half-life for I using truncated urinary excretion data (0–8 hr only, Fig. 3) yielded a half-life of 3 hr, in agreement with the reported value (3).

When the urinary excretion rate for I was determined in another experiment, the time for the maximal excretion rate was attained more rapidly. Slopes observed in the postabsorptive phase in both experiments (Fig. 3) were virtually identical, yielding a half-life of 6 hr. Thirteen percent of the dose was recovered as the intact drug after 96-hr cumulative urinary excretion. Acid hydrolysis of urine samples yielded a threefold increase in intact drug recovered.

Although urinary excretion rate data strongly suggest a longer half-life for I, the precise determination of the I elimination half-life in human plasma requires more blood samples. Additional studies are planned to confirm this observation.

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## ACKNOWLEDGMENTS


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# Kinetic Studies of Hydralazine Reaction with Acetaldehyde


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**Abstract** □ *In vitro* kinetic studies of the reaction of hydralazine with acetaldehyde at physiological concentrations and pH were conducted. This reaction, which leads to the formation of 3-methyl-*S*-triazolo[3,4-*a*]phthalazine, may occur in the plasma and may represent an alternative pathway for hydralazine metabolism. The reaction of hydralazine with acetaldehyde followed second-order kinetics with an activation energy of 16.9 kcal/mole. At 37°, the half-life of the reaction for a solution containing 2.3 μg of acetaldehyde/ml and 1 μg of hydralazine/ml was 4.5 hr. The rate increased with increasing acetaldehyde concentrations.



**Keyphrases** □ Hydralazine—reaction with acetaldehyde, kinetics, half-life, therapeutic implications □ Antihypertensive agents—hydralazine, reaction with acetaldehyde, kinetics, half-life, therapeutic implications □ Kinetics—hydralazine reaction with acetaldehyde □ Acetaldehyde—reaction with hydralazine, kinetics, half-life, therapeutic implications


Since the early 1950's, hydralazine has been used to treat hypertension by direct peripheral vasodilation. More recently, the drug has been used in the treatment of severe congestive heart failure (1, 2). Its use, however, has been limited due to inadequate pharmacokinetic data on the drug and its metabolites.

## BACKGROUND

The complete metabolism of hydralazine remains undefined. Studies (3, 4) showed that hydralazine undergoes polymorphic *N*-acetylation to form 3-methyl-*S*-triazolo[3,4-*a*]phthalazine as the major metabolic pathway, with phenotypically slow acetylators having higher steady-state plasma concentrations than fast acetylators. However, it was pointed out that the thermal half-life of hydralazine among slow acetylators is only slightly longer than or is not significantly different from that of fast acetylators (5). Thus, it seems doubtful that the enzymatic *N*-acetylation can represent the major metabolic pathway of hydralazine in humans.

Hydralazine metabolism is also more complicated because hydralazine is chemically reactive. It undergoes condensation with biogenic aldehydes and ketones to form hydrazones. Studies (6) in this laboratory showed that the reaction of hydralazine with formaldehyde and acetaldehyde forms *S*-triazolo[3,4-*a*]phthalazine and 3-methyl-*S*-triazolo[3,4-*a*]-phthalazine, respectively. Both products are known hydralazine metabolites (3, 4).

The formation of 3-methyl-*S*-triazolo[3,4-*a*]phthalazine from acetaldehyde and hydralazine was mentioned previously (7). These findings suggest that the formation of the hydralazine metabolites in humans may be at least partially due to the reaction of hydralazine with acetaldehyde. The acetaldehyde concentration in biological fluids is subject to significant increase by alcohol consumption and certain disease states (8). Thus, metabolite formation *via* this chemical reaction is significant with respect to proper drug use, especially in alcoholism, diabetes, and fasting states and on prolonged hydralazine administration.

To examine the effect of this chemical reaction on hydralazine metabolism and pharmacokinetics, the *in vitro* kinetics of 3-methyl-*S*-triazolo[3,4-*a*]phthalazine formation from hydralazine and acetaldehyde were studied. The importance of this reaction is discussed.

## EXPERIMENTAL

**Materials**—Hydralazine (I), hydrochloride salt[1], and acetaldehyde[2] (II) were used as obtained. 3-Methyl-*S*-triazolo[3,4-*a*]phthalazine (III)

[1] Courtesy of Dr. M. Wilhem, Ciba-Geigy, Summit, N.J.
[2] Aldrich Chemical Co., Milwaukee, Wis.




*0022-3549/79/1000-1256$01.00/0*

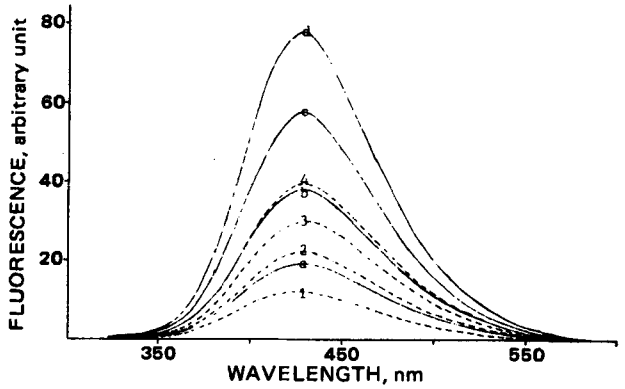
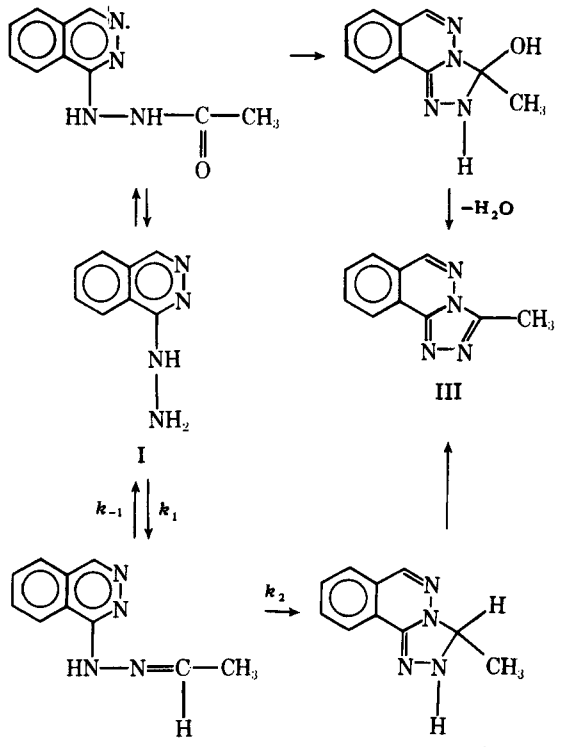



**Figure 1**—*Fluorescence spectrum of 3-methyl-S-triazolo[3,4-a]-phthalazine (III) in pH 7.4 phosphate buffer. The solid lines represent standard solutions of III: a, 1.36 × 10⁻⁶ M; b, 2.72 × 10⁻⁶ M; c, 4.07 × 10⁻⁶ M; and d, 5.43 × 10⁻⁶ M. The broken lines represent formation of III from solutions containing 2.036 × 10⁻⁵ M hydralazine (I) and 5.227 × 10⁻⁵ M acetaldehyde (II) at 25° at the end of 45 (1), 75 (2), 120 (3), and 180 (4) min.*

was prepared and purified according to a literature procedure (9). All other chemicals were analytical or chromatographic grade.

**Methods**—The kinetics of the reaction of I with II were studied by measuring the formation of III using a fluorometric technique. The experiments were carried out in pH 7.4 phosphate buffer with physiological concentrations of I (1–4 μg/ml) and II (2.3 μg/ml) at 25, 30, 35, 37, and 40°. Stock solutions of I and II were prepared daily using chromatographic grade methanol. Sufficient stock solution of I was diluted to 100 ml with the buffer, and the solution was equilibrated at a desired temperature using a temperature-controlled water bath. After the addition of the desired amount of II, samples were taken at selected time intervals and analyzed immediately for III.

The fluorescence measurements were made with a spectrophotofluorometer[3] equipped with a 150-w xenon lamp and a 1P21 photomultiplier tube. The cell compartment of the spectrophotofluorometer also was equilibrated at the desired temperature by a circulatory temperature-controlled water bath. In pH 7.4 buffer, I and II are nonfluorescent, but III exhibits strong fluorescence at 430 nm when excited at 240 nm. The formation of III in solutions containing I and II was monitored by fluorescence measurement, and the concentration of III was determined using a fluorescence calibration curve of known III concentrations in the 5 × $10^{-7}$–1 × $10^{-5}$ *M* range.




*Scheme I—Mechanisms of the formation of III by acetylation and hydrazone pathways*

[3] Aminco-Bowman, American Instruments Co., Silver Spring, Md.

**Table I—Kinetic Results for the Reaction of Hydralazine (I) with Acetaldehyde (II) at Physiological pH and Concentrations**

| Temperature | *k*, liters/mole/min | $t_{1/2}$, hr[a] |
|---|---|---|
| 25° | 18.8 | 12.01 |
| 30° | 26.3 | 8.59 |
| 35° | 39.2 | 5.77 |
| 37° | 50.0 | 4.50 |
| 40° | 62.6 | 3.61 |

[a] Calculated for solutions containing 5.09 × $10^{-6}$ *M* I and 52.27 × $10^{-6}$ *M* II as initial concentrations.

The formation of III from I and II followed second-order kinetics. The overall rate constants of the reaction were determined using:

$$\left(\frac{1}{C_I - C_{II}}\right)\left(\log\frac{C_{II}(C_I - X)}{C_I(C_{II} - X)}\right) = \left(\frac{k}{2.303}\right)(t) \qquad \text{(Eq. 1)}$$

where $k$ is the overall rate constant; $C_I$ and $C_{II}$ are the initial concentrations of I and II, respectively; and $X$ is the concentration of III at time $t$. The relationship between the rate constant and temperature was examined using the Arrhenius equation, from which the activation energy of the reaction also was calculated.

## RESULTS AND DISCUSSION

The fluorescence spectra of 3-methyl-*S*-triazolo[3,4-*a*]phthalazine (III) in pH 7.4 phosphate buffer are shown in Fig. 1. Spectra a, b, c, and d were obtained from solutions prepared with standard Compound III, whereas spectra 1, 2, 3, and 4 represent the formation of III in solutions containing hydralazine (I) and acetaldehyde (II). The formation of III in these solutions was confirmed also by a high-pressure liquid chromatographic study at conditions reported previously (6).

Figure 2 shows the plots of the percentage of I remaining as a function of time for solutions containing 5.227 × $10^{-5}$ *M* (2.3 μg/ml) of II and varying concentrations of I measured at 25°. The reaction half-life was dependent on the concentration of I, indicating that the reaction followed second- or higher order kinetics. When these data were treated with Eq. 1, it was found that the reaction was second order. The plot of Eq. 1 for the data of Fig. 2 is shown in Fig. 3. A straight line was obtained for solutions containing different concentrations of I. Similar results also were obtained for the reaction carried out at other temperatures.

A possible mechanism of the condensation of I with II and comparison of this reaction with the acetylation process in the formation of III are shown in Scheme I. Hydralazine condensation with II forms an intermediate hydrazone, which is subsequently cyclized and oxidized to form

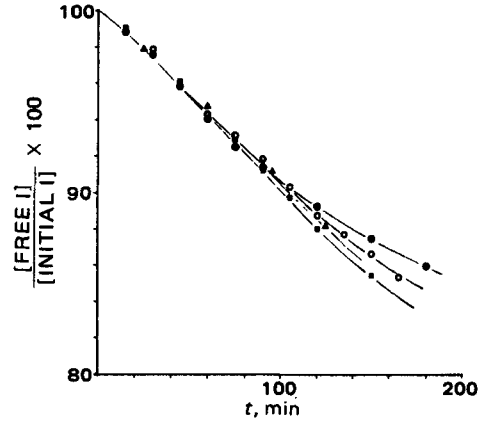



**Figure 2**—*Plot of the ratio of free and initial concentrations of hydralazine (I) as a function of time for solutions containing 52.27 × $10^{-6}$ M acetaldehyde (II) and 5.09 × $10^{-6}$ M I (■), 10.18 × $10^{-6}$ M I (▲), 15.27 × $10^{-6}$ M I (○), and 20.36 × $10^{-6}$ M I (●). The reaction temperature was 25°.*

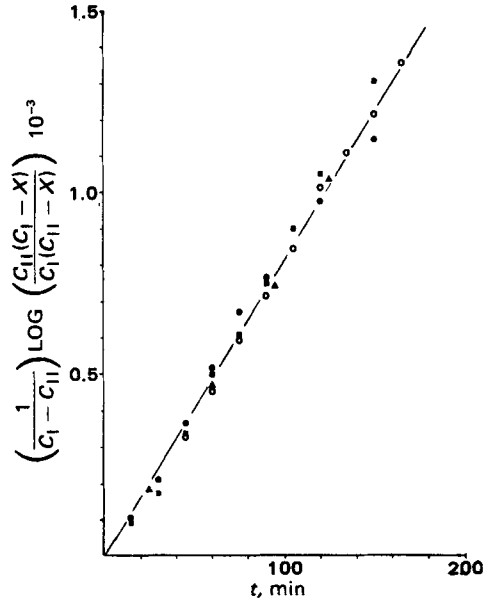



**Figure 3**—*Plot of Eq. 1 for the data shown in Fig. 2.*

III *via* the driving force of the formation of a completely aromatic system. Such oxidation reactions also were reported in analogous heterocyclic systems (7, 10). The acetylation process is enzyme dependent and occurs primarily in the intestine and liver (11), whereas the condensation reaction may occur primarily in the plasma. From the mechanism shown in Scheme I, the kinetics of the reaction of I with II may be approximated by the steady-state approach (Schemes II and III).

$$\text{I} + \text{II} \underset{k_1}{\overset{k_1}{\rightleftarrows}} \text{intermediate}$$

*Scheme II*

$$\text{intermediate} \xrightarrow{k_2} \text{III}$$

*Scheme III*

This approach will also give second-order kinetics with $k_1k_2/(k_1 + k_2)$ equal to the overall rate constant of Eq. 1.

The overall rate constants determined at various temperatures are shown in Table I. When the log values of the rate constants were plotted *versus* $1/T$, where $T$ is the absolute temperature, a straight line was obtained, indicating that the reaction obeys the Arrhenius principle. From the slope of the plot, the activation energy of the reaction is found to be 16.9 kcal/mole.

The half-lives listed in Table I were calculated for solutions containing $5.09 \times 10^{-6}$ *M* (1 μg/ml) I and $5.227 \times 10^{-5}$ *M* (2.3 μg/ml) II, typical physiological concentrations (12). At 37°, the reaction has a half-life of 4.5 hr. The physiological half-life of I in humans was reported to be in the 4–8-hr range (13). Although the half-life measured for the chemical reaction of I is not to be mistaken for the biological half-life, it does indicate that the reaction of I with II may significantly affect drug metabolism and pharmacokinetics. This result is especially true in conditions such as chronic or acute alcohol ingestion, fasting states, and diabetes, which result in elevated levels of II. Since the reaction is second order, an increase of the blood level of II will result in an increase of the formation rate of III. On the other hand, with low levels of I, as in the case of noncompliant patients who take less than the prescribed dose and patients with poor absorption, the reaction also becomes more significant. The effect of II levels on the elimination of I and on the formation of III is being studied in this laboratory.

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