Table III—Concentration[a] of Δ⁹-Tetrahydrocannabinol

	Gibberellic Acid				Indoleacetic Acid			
Control	25	25F	250	250F	25	25F	250	250F
1.04	0.90	0.60	0.89	0.65	0.78	0.96	0.90	0.91

[a] Average percent dry weight of two samples.

leaves and in the content of III. There was no effect on the stem weight, which suggests that the significant increase in height was due to a change in the growing habit from a branching plant to one main stem.

The concentrations of II used were not high enough to induce any effect on the plants. Similar results were observed on *Hyoscyamus niger* L., where concentrations of 10 and 50 p.p.m. of I induced significant effects on rosette plants while concentrations up to 500 p.p.m. of II applied in the same manner failed to induce any stem elongation.

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Kinetics of Hydrolysis of Hypoglycemic 1-Acyl 3,5-Dimethylpyrazoles

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Abstract □ Other investigators suggested that various 1-acyl 3,5-dimethylpyrazoles might owe their hypoglycemic activity to a nonenzymatic hydrolysis *in vivo* to the potent compound 3,5-dimethylpyrazole. As a test of this hypothesis, relative rates of hydrolysis at pH 2.0 and 6.7 (37.6°) were determined for a representative series of compounds covering a wide range of hypoglycemic potencies. No correlation between hydrolysis rate and activity was observed. 3,5-Dimethylpyrazole-1-carboxamide and 3,5-dimethyl-pyrazole-1-*N,N*-dimethylcarboxamide possess equivalent biological activity; the former was rapidly hydrolyzed (half-life about 1 hr. at pH 2.0 and 6.7), whereas the latter was totally stable. Differences in biological activity reflect intrinsic potencies of the various compounds or differences in their absorption and/or metabolism.

Keyphrases □ 3,5-Dimethylpyrazoles, 1-acyl series—kinetics of hydrolysis, hydrolysis rate-hypoglycemic activity correlation □ Hypoglycemic activity-hydrolysis rate correlation—1-acyl 3,5-dimethylpyrazoles, kinetics of hydrolysis □ Hydrolysis rates—1-acyl 3,5-dimethylpyrazole series, correlated with hypoglycemic activity, kinetics

Wright *et al.* (1) reported the hypoglycemic activities of a large number of pyrazoles in the intact, fasted, glucose-primed rat. The most active compounds were 3,5-dimethylpyrazole (I) (2) and various 1-acyl derivatives. Preliminary experiments showed that 3,5-dimethylpyrazole-1-carboxamide (II) was readily hy-

drolyzed to I at pH's encountered *in vivo* in the GI tract and the blood. As a consequence, it was suggested that the various 1-acyl derivatives of I might owe their activities either to nonenzymatic hydrolysis *in vivo* or to metabolic transformation to I (1). To test the hypothesis of nonenzymatic conversion, relative rates of

Table I—Reaction Conditions and Kinetic Constants for the Hydrolysis of 1-Acyl 3,5-Dimethylpyrazoles at 37.6°

Com-pound	pH 2.0 Concentration, $M \times 10^5$	λ, nm.	k', min.$^{-1}$	$t_{1/2}$	pH 6.7 Concentration, $M \times 10^5$	λ, nm.	k', min.$^{-1}$	$t_{1/2}$	Hypo-glycemic Activity[a]
II	7.83	235	1.09×10^{-2}	63 min.	7.23	235	1.12×10^{-2}	62 min.	25
III	6.21	239	1.18×10^{-4}	4.1 days	6.89	238	7.57×10^{-5}	6.4 days	4–8
IV	10.64	236	—[b]	—	9.92	234	—[b]	—	20–30
V	7.39	237	1.54×10^{-2}	45 min.	8.55	237	1.79×10^{-3}	6.5 hr.	16
VI	6.10	255	6.78×10^{-3}	102 min.	6.10	254	1.22×10^{-3}	9.5 hr.	100

[a] Reference 1, tolbutamide = 1. [b] No reaction.

hydrolysis were determined (pH 2.0 and 6.7, 37.6°) for a series of representative compounds (II–VI) covering a wide range of hypoglycemic potencies (Table I).

EXPERIMENTAL

Samples of II–VI were dissolved in aqueous ethanol and diluted with 0.01 N HCl or 0.08 M phosphate buffer, preequilibrated at 37.6°, to yield the final concentrations and pH values shown in Table I. The final ethanol concentration was 0.4% (v/v). Each solution was placed in a UV spectrophotometer equipped with a constant-temperature cell compartment maintained at 37.6°. For the rapidly hydrolyzed compounds (II, V, and VI), absorbances (A_t) were determined at appropriate times at the characteristic absorption maxima (Table I) until no further absorbance decreases occurred (A_∞). Pseudo-first-order reaction rate constants, k', were obtained from the slopes of plots of log $(A_t - A_\infty)$ versus time. For the slowly hydrolyzed III, k' was estimated from the initial slope of log A_t versus time.

RESULTS AND DISCUSSION

The method utilized for the determination of the kinetics of hydrolysis of Compounds II–VI is based on the differences in their UV spectra from the spectrum of the product, I (λ_{max}: 218 nm. at pH 2.0, 214 nm. at pH 6.7). In each case, first-order kinetics were observed and permitted calculation of the pseudo-first-order reaction rate constants, k', and corresponding half-lives, $t_{1/2}$, shown in Table I.

Examination of the data in Table I shows no correlation between rates of nonenzymatic hydrolysis and hypoglycemic activity. Compounds II and IV possess the same hypoglycemic activity; nevertheless, II was rapidly hydrolyzed in both acid and neutral solutions

while IV was totally stable under the conditions studied. Thus, differences in biological activities of these compounds appear to reflect differences in their intrinsic potencies, in their absorption, or in their metabolism. The last is considered the most likely. Metabolism to I via N-dealkylation and/or hydrolysis and the oxidation of the 3-methyl group to a 3-carboxylic acid are possible. 5-Methylpyrazole-3-carboxylic acid, a metabolite of I in the rat (3, 4), is also a potent hypoglycemic agent (5). Efficient absorption of these compounds from the GI tract would be expected based on the complete absorption after oral administration found for I in the rat (4) and for the highly polar 5-methylpyrazole-3-carboxylic acid in the rat, dog, and man (6).

Scott (7) reported the kinetics of ethanolysis of a number of 1-acyl 3,5-dimethylpyrazoles, including II; Staab (8) and Hüttel and Kratzer (9) determined the kinetics of neutral hydrolysis and of aminolysis of 1-acetyl-3,5-dimethylpyrazole. However, the nature of the catalysis involved in the hydrolysis reaction has not been elucidated. Data in Table I for Compounds III, V, and VI indicate that hydronium-ion catalysis is involved. In addition, the magnitude of the pseudo-first-order reaction rate constants at pH 6.7 relative to those at pH 2.0 implicates hydroxide-ion and/or direct water catalysis. Results for Compounds II–IV indicate a correlation with the degree of steric hindrance by substituents on the carboxamide nitrogen. A similar relationship was observed by Scott (7) in the ethanolysis reaction and is consistent with the mechanism involving attack on the acyl group proposed by Meloche and Laidler for the hydrolysis of aromatic amides (10).

Compound II was unique in that its hydrolysis rates at the pH's studied were essentially identical. The kinetics and mechanism of the facile hydrolysis of II will be the subject of a separate report.

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I: R = —H
II: R = —C(=O)—NH$_2$
III: R = —C(=O)—NHC$_4$H$_9$
IV: R = —C(=O)—N(CH$_3$)$_2$
V: R = —C(=O)—NH—NH$_2$
VI: R = —C(=O)—phenyl