

TABLE III.—COMPONENT FATTY ACIDS IN THE SOLID ACIDS FRACTION OF *I. digitata* LINN. TUBER OIL.^a

Acid	% in Solid Acids	% in Mixed Acids
Palmitic	40.52	8.15
Stearic	55.95	11.25
Oleic	3.53	0.71

^a Results of three analyses.TABLE IV.—COMPOSITION OF THE SOLID, LIQUID, AND MIXED ACIDS FRACTIONS OF *I. digitata* LINN. TUBER OIL

Acid	% in Solid Acids Fraction	% in Liquid Acids Fraction	% in Mixed Acids Fraction
Palmitic	40.52	...	8.15
Stearic	55.95	...	11.25
Oleic	3.53	74.34	60.10
Linoleic	...	24.25	19.38
Linolenic	...	1.51	1.11

with copper acetate and potassium ferrocyanide. The acids appeared as brown-red copper complexes.

Quantitative Estimation of Solid Acids Fraction.

The solid acids fraction was converted into methyl esters by the Fischer and Spier method, and 15.74 Gm. of this methyl ester was distilled under reduced

pressure (4 mm.). The results are shown in Table II.

Saponification and iodine values of each fraction were determined, and the liberated acids from each fraction were identified by paper chromatography. The amount of each acid in different fractions was calculated from these values. The percentage of various acids in the solid acids and in the mixed acids are listed in Table III.

The total percentage of various fatty acids in the solid, liquid, and mixed acids fractions of *I. digitata* Linn. tuber oil are listed in Table IV.

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Mechanism of Degradation of Penicillin G in Acidic Solution

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By analysis of data available in the literature of the degradation of penicillin G in acidic aqueous solution, it is shown that two reactions are involved. One is the acid catalyzed hydrolysis of the undissociated penicillin molecule to form penilloic acid. The second is rearrangement of the penicillin ion following attack by proton or the kinetically equivalent uncatalyzed reaction of undissociated penicillin to form penicillenic acid which rearranges to penillic acid. The significance of this mechanism is discussed.

THE INSTABILITY of penicillin G (I) in acidic aqueous solution has been an important consideration in the design of dosage forms of the antibiotic. It is well known that penicillin decomposes in acidic aqueous solution to form both penillic acid (V) and penilloic acid (III) with a maximum yield of the former at pH 2-3 (1). It is also known that penicillenic acid (IV) is formed in acid solution and subsequently decomposes relatively rapidly (2). It may be detected by its characteristic ultraviolet absorption spectrum with maximum at 320 μ . Recently, it has been proposed that penicillenic acid is responsible for penicillin allergy (3, 4). In the present work it is shown by mathematical analysis of data available in the literature that penicillenic acid, and subsequently penillic acid, arise from reaction of penicillin ion and penilloic acid from undissociated penicillin.

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TREATMENT OF DATA

The data used in this work were from a report by Krejci (5). He determined rate constants for both over-all rate of loss of penicillin (k_P) and rate of formation of penicillenic acid (k_E) by means of polarography. Table I presents these data. The over-all rate constants were resolved into rate constants for proton catalyzed degradation of undissociated penicillin and penicillin ion by the method previously described for pheneticillin (6). The pKa of penicillin G was taken as 2.7 (7), and the plot of k_P/H^+ against fraction penicillin dissociated (f_{A^-}) is shown in Fig. 1. From the intercept and slope, the rate constants k_{HA} and k_{A^-} were evaluated as 2.6 and 10.8, respectively. The over-all equation for acid degradation of penicillin G may then be represented by

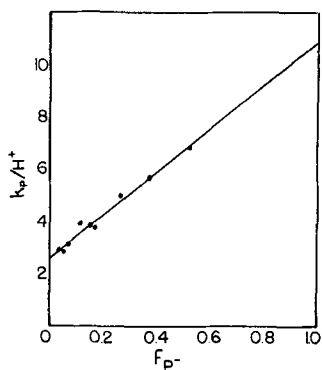
$$k_P = 2.6 [H^+] [f_{HA}] + 10.8 [H^+] [f_{A^-}]$$

or at a single pH, since f_{HA} and f_{A^-} are functions only of pH,

$$k_P = k_1 + k_2$$

TABLE I.—RATE CONSTANTS (MIN.⁻¹) FOR DEGRADATION OF PENICILLIN AND FORMATION OF PENICILLINIC ACID IN ACID AQUEOUS SOLUTION AT 25°

pH	k_P	k_E
1.32	0.142	0.0219
1.47	0.097	0.0178
1.60	0.0792	0.0151
1.82	0.060	0.0151
2.03	0.0351	0.0145
2.28	0.0262	0.0110
2.49	0.0185	(0.0083)
2.76	0.0119	(0.0052)

Fig. 1.—Plot of k_P/H^+ against f_{P-} for penicillin G at 25°, from which k_{HP} and k_{P-} were determined.

where $k_1 = 2.6[H^+][f_{HA}]$ and $k_2 = 10.8[H^+][f_A^-]$.

From Krejci's data, by subtracting k_E from k_P , one obtains the rate constant for reactions other than formation of penicillenic acid, and these have been designated k_0 . In Table II there are compared, at each pH, k_0 with k_1 and k_2 . There is a substantial correspondence between the two sets of values, particularly in their dependence on pH.

DISCUSSION

The correspondence of rate constants indicates that the formation of penicillenic acid is a result of the reaction of penicillin ion with proton or the kinetically equivalent spontaneous rearrangement of undissociated penicillin acid. Which of these reactions is actually occurring cannot be determined from the available data because of the following:

$$k_A \cdot [H^+] [A^-] = k_A \cdot K_a [HA]$$

where K_a represents the acid dissociation constant of penicillin.

Furthermore, that the rate of formation of penicillenic acid does not increase rapidly with decreasing pH (1) suggests that penicillenic acid must be formed from penicillenic acid. Therefore, penilloic acid must be the product of the acid catalyzed reaction of undissociated penicillin. Figure 2 summarizes these reactions. An intermediate in the formation of penilloic acid probably is penicilloic acid (II), which is known to decarboxylate rapidly in acid media.

The conclusions presented here are exactly contrary to those of Krejci (5), who depicted penicillenic acid forming from the acid catalyzed reaction of undissociated penicillin. If this were so, however, the yield of penicillenic acid would not show a maximum as it does at pH 2-3 (1) but would increase con-

TABLE II.—COMPARISON OF CALCULATED AND OBSERVED RATE CONSTANTS^a

pH	k_1	k_0	k_2	k_E
1.32	0.122	0.120	0.0194	0.0219
1.47	0.085	0.0792	0.0194	0.0178
1.60	0.062	0.0641	0.0194	0.0151
1.82	0.035	0.0449	0.0184	0.0151
2.03	0.020	0.0206	0.0172	0.0145
2.28	0.010	0.0152	0.0151	0.0110
2.49	0.0053	(0.0102) ^b	0.0130	(0.0083) ^b
2.76	0.0022	(0.0067) ^b	0.0090	(0.0052) ^b

^a Min.⁻¹. ^b Data in parentheses have a higher degree of uncertainty.

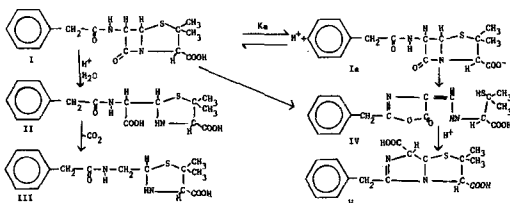
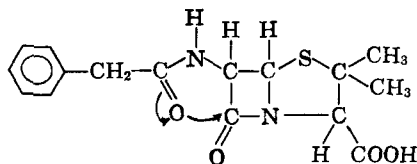


Fig. 2.—Degradation of penicillin G in acidic aqueous solution.

tinuously as pH was decreased. Further support for the present conclusions lies in the similarity of the mechanisms proposed for formation of penicillenic and penicillenic acids by Woodward (8). As the first step, he showed an attack by the amide carbonyl oxygen as follows:



The intermediate formed could then readily isomerize to penicillenic acid. The relative acid stability of certain newer penicillins has been attributed to the effect of polar groups in limiting the extent of the above (1) rather than greatly affecting the rate of formation of penilloic acid. Some of the newer acid stable penicillins appear to produce much less penicillenic acid (1) and thus may prove to be less allergenic than penicillin G. The latter aspect of penicillin degradation is currently under investigation.

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