Stability of Tetrahydrocannabinols I

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Abstract □ Δ^9-Tetrahydrocannabinol, as monitored by flame-ionization GLC at various temperatures, degrades by a biphasic semilogarithmic curve with time in acidic aqueous solutions (<1 mg/liter) below pH 4 to GLC-observable products with separate retention times and the degradations are specific hydrogen-ion catalyzed. The products are considered as Δ^8-tetrahydrocannabinol, P_1, P_2, and P_3 and can be observed and isolated by TLC. These products do not appear above pH 4 in the neutral region, and these degradations are primarily first order, are not biphasic, and are pH independent. The half-life of Δ^9-tetrahydrocannabinol is about 15 min at 37° and pH 1, typical stomach conditions. The product P_1 may give rise to cannabinol by the GLC and TLC procedures since the IR, UV, TLC, NMR, and GLC of thin-layer chromatographed P_1 and cannabinol are coincident, but chloroform extracts do not show the higher absorbances expected if the product that forms in solution to give P_1 is cannabinol. The products P_2 and P_3, isolated by TLC, are consistent with the expected properties of Δ^9-hydroxycannabidiol and 9-hydroxycannabinol, respectively, by IR, UV, NMR, and mass spectroscopy. The final amounts of Δ^8-tetrahydrocannabinol, P_1, P_2, and P_3 are in a constant ratio independent of pH below pH 4. Δ^9-Tetrahydrocannabinol, as monitored by flame-ionization GLC, degrades solely by a first-order process to an equilibrium with P_2 and P_3 at acidic pH values and the process is specific hydrogen-ion catalyzed. The equilibrium appears to be independent of pH below pH 4 and is the same when TLC-isolated P_2 or P_3 is used as the starting material. It follows that the acid-catalyzed isolated double-bond migration favors Δ^8-tetrahydrocannabinol over the Δ^9 compound, and it is most probable that the equilibrating P_2 and P_3 are results of water addition to the isolated double bond and ether solvolysis. The product that gives rise to the P_1 retention time that ultimately gives cannabinol is structurally indeterminate at present.

Keyphrases □ Tetrahydrocannabinols—stability, effects of pH, isolation and identification of degradation products, mechanisms, flame-ionization GLC □ Stability—tetrahydrocannabinols, effect of pH, isolation and identification of degradation products, flame-ionization GLC □ GLC—monitoring of tetrahydrocannabinol stability

The pKa', aqueous solubility and anomalous glass-binding properties of Δ^9-tetrahydrocannabinol were published recently (1). In addition, a preliminary study on the aqueous acidic degradation of Δ^9-tetrahydrocannabinol was presented using the developed GLC analytical methods (2).

The quantified kinetics and stability of the tetrahydrocannabinols in aqueous medium as a function of pH need to be known for the development of elegant dosage forms and for insight into the possible chemical transformations and their rates that can occur in biological fluids. This paper presents the quantitative kinetic studies used to determine the rate–pH profiles of Δ^9- and Δ^8-tetrahydrocannabinols. Evidence is also presented for the structures and kinetics of degradation of the major GLC detectable products of tetrahydrocannabinol degradation.

EXPERIMENTAL

Materials—Both Δ^9-tetrahydrocannabinol[1] and Δ^8-tetrahydrocannabinol[2] were used directly or after TLC purification. Various degradation products were obtained by preparative TLC as will be described. Other chemicals were of analytical reagent grade. The concentrated hydrochloric acid was twice preextracted with chloroform. Double-distilled water stored in glass containers was used.

Kinetic Procedures—Appropriate amounts of 2 *N* NaCl were added to prepared solutions of hydrochloric acid, buffer, or sodium hydroxide of known concentrations to adjust the ionic strength to 0.05 except when the inherent ionic strength exceeded 0.05 as with the concentrated hydrochloric acid solutions. An aliquot (0.5 ml) of an ethanolic solution of 1 mg of Δ^9-tetrahydrocannabinol/ml was introduced into 1 liter of solution after it was purged with nitrogen and the erlenmeyer flask was equilibrated to the temperature of the reaction. At various time intervals and after immediate and vigorous shaking, 20 or 25 ml of solution was delivered quickly to a 125-ml separator by a glass dispenser[3]. The reactions in strongly acid solutions were quenched with phosphate buffer solution, which adjusted the pH to 6. The reactions at pH values above 9 were quenched with 2 *N* acetic acid, which adjusted the pH to 5. The aliquots were extracted by vigorous shaking for 2–3 min with 20 ml of chloroform; they were then separated and extracted again with 10 ml of chloroform. The chloroform extracts, combined in a 50-ml conical glass centrifuge tube, were evaporated to dryness below 45° under a stream of nitrogen. Chloroform (0.10 ml) containing 3.0 μg of tetraphenylethylene/ml as an internal standard was added, and an additional 5 ml of chloroform was used to rinse down the inner surfaces of the tube. The chloroform solution again was evaporated to dryness and the residue was reconstituted in 100 μl of chloroform. After vigorous shaking on a vortex shaker, a 0.5- or 1.0-μl aliquot was injected into the chromatograph for analysis by the flame-ionization GLC procedure described previously (2).

The conditions for the gas chromatograph[4] with flame-ionization detection were: column temperature, 240°; detector and injection temperature, 260°; and 3% OV-225 on Gas Chrom Q, 100–120 mesh, for column material. The nitrogen flow rate was 50 ml/min and the hydrogen flow rate was 26 ml/min.

The methods of determining the pH values of the solutions were reported previously (3).

Preparation and Separation of Acidic Degradation Products—Fifty milliliters of an ethanolic solution of 1 mg of Δ^9-tetrahydrocannabinol/ml was added to a gallon glass bottle containing a 20% alcohol–water solution of 0.1 *N* HCl equilibrated at 60°. After 5 days, the mixture was extracted twice with 4 liters of chloroform; the combined extracts were evaporated to about 1 ml, streaked on preparative TLC sheets[5], and developed for 15 cm with cyclohexane–acetone (10:1). The R_f values that corresponded to Δ^8-tetrahydrocannabinol, Product 1 (P_1), Product 2 (P_2), and Product 3 (P_3) were located with a short wavelength UV light. The pertinent strips were eluted with chloroform to remove Δ^8-tetrahydrocannabinol and P_1 and with 20% methanol in chloroform to remove P_2 and P_3, followed by vacuum filtration to obtain the drug samples of the various compounds. The separation between P_2 and P_3 was effected by three repeated TLC developments separated by drying, using cyclohexane–acetone (10:1) with elution from the chromatogram strips with 20% ethanol in chloroform.

[1] S.S.C. lot 61591 furnished by the National Institute of Mental Health, Department of Health, Education, and Welfare, U.S. Public Health Service, Bethesda, MD 20014

[2] S.S.C. lot 61656.

[3] CaLab, California Laboratory Supply, Emoryville, CA 94608

[4] Varian model 2100.

[5] Chrom AR 1000, Mallinckrodt Chemical Co., St. Louis, MO 63160

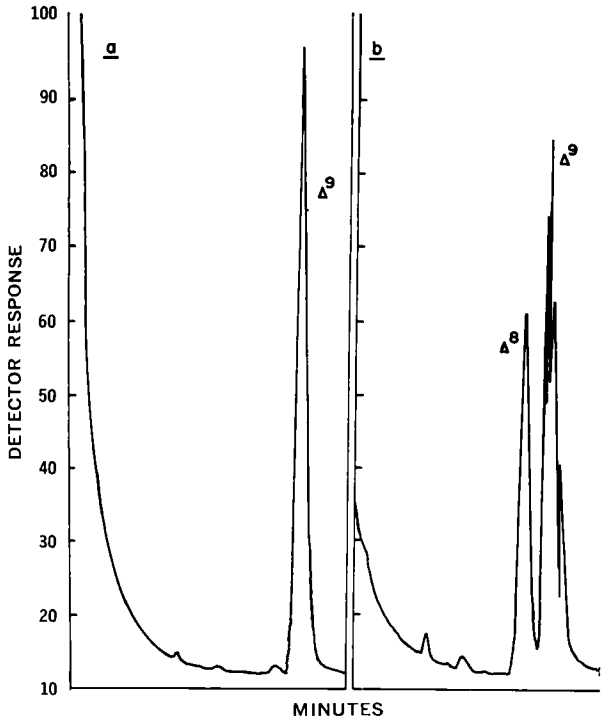


Figure 1—*Gas chromatograms with flame-ionization detection of chloroform-eluted* (a) *lower and* (b) *upper halves of the separated spot with the same* R_f *value as* Δ^9*-tetrahydrocannabinol obtained by TLC of impure* Δ^9*-tetrahydrocannabinol after development with either pure cyclohexane (four repetitive developments to 15 cm separated by drying) or with cyclohexane–acetone (10:1). The impurity of the same retention time as* Δ^8*-tetrahydrocannabinol was observed in the eluted upper half* (b) *of the spot. The segment of* (b) *containing the* Δ^9 *peak was expanded four times in relation to the greater portion of the chromatogram.*

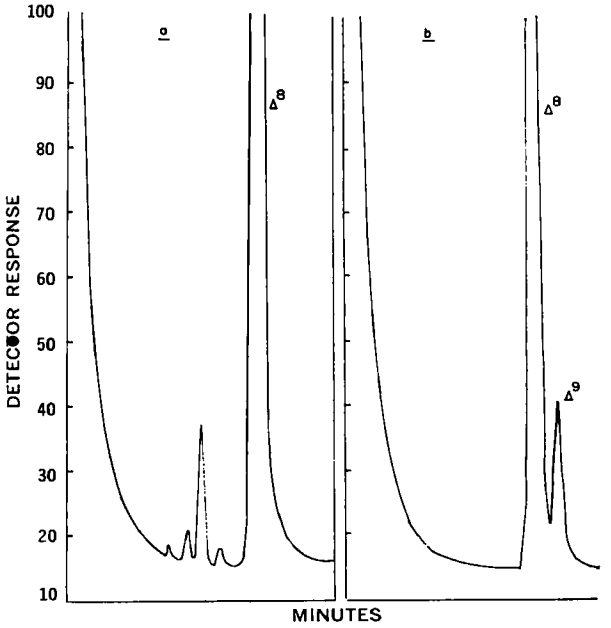


Figure 2—*Gas chromatograms with flame-ionization detection of chloroform-eluted* (a) *upper and* (b) *lower halves of the separated spot with the same* R_f *values as* Δ^8*-tetrahydrocannabinol obtained by TLC of the obtained impure* Δ^8*-tetrahydrocannabinol after development to 15 cm with cyclohexane–acetone (10:1). The impurity of the same retention time as* Δ^9*-tetrahydrocannabinol was observed in the eluted lower half* (b) *of the spot. Further chloroform elution of the lower half of the TLC-developed spot obtained from the chloroform-eluted half spot that gave* (a) *and of the upper half of the TLC spot obtained from the chloroform-eluted half spot that gave* (b) *resulted in chromatographically pure* Δ^8*-tetrahydrocannabinol.*

Separation between large amounts of Δ^8-tetrahydrocannabinol and P_1 was effected by three repeated TLC developments, using cyclohexane–acetone (50:1) with chloroform elution from the chromatogram strips.

Purification of Δ^9- and Δ^8-Tetrahydrocannabinols by TLC—The described TLC method was also used for the purification of Δ^9- and Δ^8-tetrahydrocannabinols. The Δ^9-tetrahydrocannabinol strip obtained from impure material[1] was cut longitudinally in half and the lower half of the strip contained the purified Δ^9-tetrahydrocannabinol extractable by chloroform. The impure Δ^8-tetrahydrocannabinol[2] was also thin layered, and the strip containing it was halved similarly. The strip halves were extracted by chloroform, which was evaporated subsequently at 40° to a small volume (about 100 μl) under a stream of nitrogen. The top half of the strip contained impurities of lower GLC retention times, and the lower half contained an impurity of higher GLC retention time, which most probably was Δ^9-tetrahydrocannabinol. When the condensed chloroform solutions were streaked on a TLC sheet for a second development and the pertinent strips were subsequently halved, purified Δ^8-tetrahydrocannabinol could be extracted from the lower half of the strip obtained from the chloroform extract of the upper half of the strip obtained from the first TLC development and from the upper half of the strip obtained from the chloroform extract of the lower half of the strip obtained from the first TLC development.

Radioactive Monitoring of Tetrahydrocannabinol Degradation—A 0.06 *N* HCl solution (150 ml) was equilibrated at 60.8° in a glass-stoppered 200-ml erlenmeyer flask. Solutions of 2.57×10^6 dpm/ml of ^{14}C-Δ^9-tetrahydrocannabinol (0.40 ml) and 1 mg of purified Δ^9-tetrahydrocannabinol/ml in methanol (0.15 ml) were added. Aliquots (24 ml) were removed at various times and extracted with chloroform, and 1 μl of the final 100 μl of chloroform solution containing the compound and its degradation products from each was assayed by GLC as in the kinetic procedures. The remaining chloroform solution (99 μl out of 100 μl) plus small amounts of chloroform rinses of the container was spotted on a TLC plate[6], which was developed for 15 cm with cyclohexane–acetone (10:1). The radioactivity of the developed plates was quantified by a radiochromatogram scanner[7]. Controls for the system were run with Δ^8- and Δ^9-tetrahydrocannabinols and the degradation products P_1, P_2, and P_3 obtained from the previously described preparative TLC.

Monitoring of UV Spectra of Acid-Degrading Δ^9-Tetrahydrocannabinol—One milliliter of 1 mg of purified Δ^9-tetrahydrocannabinol/ml of ethanol was added to 1 liter of 0.12 *N* HCl maintained at 60.8°. Aliquots (60 ml) were taken after vigorous shaking at 1, 3, 6, 10, 20, 30, 50, 80, and 930 min and twice extracted with 20 ml of chloroform. The combined chloroform extracts were evaporated under nitrogen at 40°, the residue was reconstituted in 2.5 ml of ethanol, and the UV spectrum[8] was monitored. An aliquot (2.0 ml) of this solution was evaporated, 0.1 ml of a chloroform solution of the internal standard tetraphenylethylene was added, and this final solution was analyzed by GLC.

RESULTS AND DISCUSSION

Purification of Δ^9- and Δ^8-Tetrahydrocannabinols by TLC—The Δ^9-tetrahydrocannabinol obtained[1] showed an additional easily distinguishable major peak with the same retention time as Δ^8-tetrahydrocannabinol on flame-ionization GLC. It had 10% of the peak height of the Δ^9 compound. Four other impurities were identifiable by peak separation at 0.5–2% of the peak height of Δ^9-tetrahydrocannabinol (one of the higher peaks had the retention time of cannabinol), and eight others were detectable at the 0.1% level.

TLC (as described under *Experimental*), even with four succes-

[6] Eastman chromagram sheet 6061, Eastman Co., Rochester, N.Y.
[7] Packard model 7201.
[8] Cary model 15, Applied Physics Co., Monrovia, Calif.

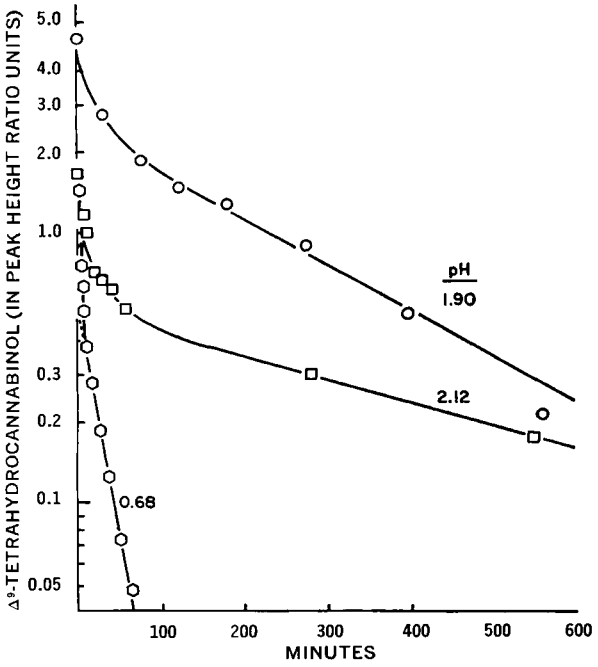


Figure 3—*Semilogarithmic plots of the ratio of the height of the GLC peak for Δ⁹-tetrahydrocannabinol to the peak height of the internal standard tetraphenylethylene against time for the degradation of aqueous solutions (about 1 mg/liter) of purified Δ⁹-tetrahydrocannabinol at 60.8° and at the specified pH values.*

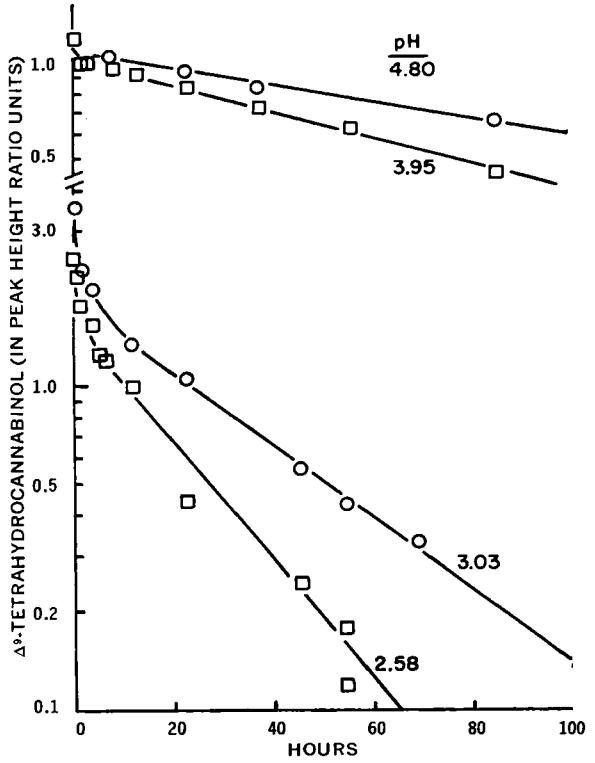


Figure 4—*Semilogarithmic plots of the ratio of the height of the GLC peak for Δ⁹-tetrahydrocannabinol to the peak height of the internal standard tetraphenylethylene against time for the degradation of aqueous solutions (about 1 mg/liter) of purified Δ⁹-tetrahydrocannabinol at 60.8° and at the specified pH values.*

sive developments to 15 cm separated by drying which completely separated the Δ^8- and Δ^9-tetrahydrocannabinol spots, was unable to produce a Δ^9 spot uncontaminated by Δ^8. When this spot was eluted with chloroform and assayed by GLC, a peak with the retention time of Δ^8-tetrahydrocannabinol was still observed. When eluted with chloroform, the lower half of the spot gave a minimal amount of impurity (Fig. 1*a*) whereas the upper half retained it on flame-ionization GLC (Fig. 1*b*).

The Δ^8-tetrahydrocannabinol obtained[2] was even more impure on flame-ionization GLC. Elution of the halves of the TLC spot with the same R_f value as Δ^8-tetrahydrocannabinol showed, by GLC analysis, that the impurities in each half differed. The upper half had the impurities of a smaller retention time (Fig. 2*a*) and the lower half had the impurities of a higher retention time of Δ^9-tetrahydrocannabinol (Fig. 2*b*). A second TLC development of each chloroform-eluted half, with subsequent chloroform elution of the pertinent halves, gave pure Δ^8-tetrahydrocannabinol by GLC.

Kinetics of Δ⁹-Tetrahydrocannabinol Disappearance in Aqueous Solution—The kinetics for nitrogen-purged solutions of purified Δ^9-tetrahydrocannabinol at concentrations below its solubility, *i.e.*, <1 mg/liter, were followed at various pH values from the chloroform extracts of the degrading solution by monitoring the decrease of the ratio of the peak height at the Δ^9 retention time (Fig. 1*a*) obtained with the gas chromatograph using flame-ionization detection (1, 2) to the peak height of the internal standard tetraphenylethylene as a function of time at several constant temperatures. The calibration curves of the peak height ratio against concentration of Δ^9- and Δ^8-tetrahydrocannabinols were linear. Typical semilogarithmic plots of the obtained data at various pH values are given in Figs. 3–5 for studies at 60.8°. These plots are not completely linear, especially in the more highly acidic region below pH 3; they indicate two sequential reactions where the concentration of Δ^9-tetrahydrocannabinol conforms to the time (t) dependency of:

$$C = C_1e^{-k_1t} + C_2e^{-k_2t} \qquad \text{(Eq. 1)}$$

The k_2 values were estimated from the terminal linear slopes of such plots in accordance with:

$$\log C = \log C_2 - k_2t/2.303 \qquad \text{(Eq. 2)}$$

where $\log C_2$ is the extrapolated intercept.

The k_1 values were estimated from the linear slopes of the feathered plots in accordance with:

$$\log (C - C_2e^{-k_2t}) = \log C_1 - k_1t/2.303 \qquad \text{(Eq. 3)}$$

where $\log C_1$ is the intercept of the semilogarithmic plots of the difference between the observed concentration and the antilogarithms of values obtained by the extrapolation of the terminal linear segment of the plot of $\log C$ against time. Above pH 4.0, a sim-

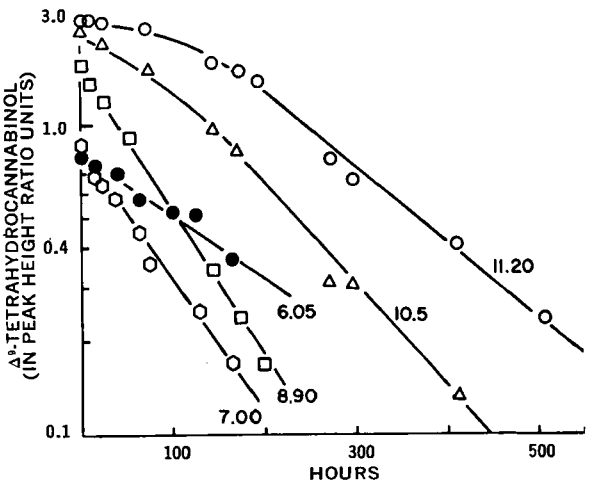


Figure 5—*Semilogarithmic plots of the ratio of the height of the GLC peak for Δ⁹-tetrahydrocannabinol to the peak height of the internal standard tetraphenylethylene against time for the degradation of aqueous solutions (about 1 mg/liter) of purified Δ⁹-tetrahydrocannabinol at 60.8° and at the specified pH values.*

Table I—Rate Constants (in Minutes^{-1}) for the Degradation of Δ^9-Tetrahydrocannabinol in Aqueous Solutions at 0.05 Ionic Strength when Possible in Accordance with $C = C_1e^{-k_1t} + C_2e^{-k_2t}$ below pH 4 and in Accordance with $C = C_0e^{-kt}$ above pH 4

60.8°						70.0°					
pH	10^2k_1	10^4k_2	10^4k	C_1[a]	C_2[a]	pH	10^2k_1	10^4k_2	10^4k	C_1[a]	C_2[a]
0.68	59	398	—	0.7	0.3	1.68	25	243	—	0.7	0.3
1.40	10	85	—	0.7	0.3	2.50	4.3	30.4	—	0.7	0.3
1.90[b]	6.6	36.3	—	0.7	0.3	4.0	—	3.37	—	0.3	0.7
2.12	5.6	20.3	—	—	—	6.95	—	—	2.88	0.0	1.0
2.58	1.1	7.74	—	0.6	0.4	7.97	—	—	3.15	0.0	1.0
3.03	—	4.67	—	0.5	0.5	—	—	—	—	—	—
3.95[c]	—	1.51	—	0.2	0.8	—	—	—	—	—	—
4.80	—	—	1.03	—	—			45.0°			
5.25	—	—	0.66	—	1.0						
6.05	—	—	0.75	—	1.0	0.70	25	99.1	—	0.6	0.4
7.00	—	—	1.70	—	1.0	1.50	3.7	26.7	—	—	—
7.90	—	—	1.90	—	1.0	2.50	3.5	2.73	—	—	—
8.90	—	—	1.82	—	1.0						
9.68	—	—	1.69	—	1.0						
10.5	0.029	—	1.43	—	—						
11.2	0.027	—	1.00	—	—						

[a] Estimated as the fraction of total initial concentration $C_0 = C_1 + C_2$. [b] No significant differences among rate constants evaluated at 0.05 and 0.01 ionic strength. [c] No significant differences among rate constants evaluated at 0.017, 0.051, and 0.10 *M* acetate buffer.

ple first-order decay of Δ^9-tetrahydrocannabinol was observed, which can be characterized by the rate constant k. The rate constants obtained are listed in Table I.

The log k–pH profiles for the apparent first-order rate constants k_1, k_2, and k for the degradation of Δ^9-tetrahydrocannabinol are given in Fig. 6. There are greater errors in the estimates of k_1 from the feathering process.

Except for a few points in the 60.8° plot between pH 4.5 and 6.5 that fall below the reasonably expected pH-independent degradation rate:

$$k = k_0 f_u \qquad \text{(Eq. 4)}$$

The data for k are consistent with the premise that the solvolytic degradation in this pH range is due to the unionized fraction, f_u, of the drug where the pKa′ of Δ^9-tetrahydrocannabinol is 10.6 at 25° (1).

The data for k_1 and k_2 appear to conform to:

$$k_i = k_{H_i^+} a_{H^+} \qquad \text{(Eq. 5)}$$

since:

$$\log k_i = \log k_{H_i^+} - \text{pH} \qquad \text{(Eq. 6)}$$

where:

$$\text{pH} = -\log a_{H^+} \qquad \text{(Eq. 7)}$$

and a_{H^+} is the hydrogen-ion activity.

The values of $k_{H_1^+}$ and $k_{H_2^+}$ were obtained from the antilogarithms of the intercepts of the respective log k *versus* pH plots at the various temperatures; the $k = k_0$ values were estimated from the plateau values between the pH range of 7–9. The microscopic rate constants and their derived Arrhenius parameters are given in Table II. Preliminary studies (1) on acid degradation of Δ^9-tetrahydrocannabinol in 0.01–0.5 $\bar{N}$ HCl gave an apparent $k_{H_2^+}$ value at 55° of 0.12 liter/mole/min, which is consistent with the values listed in Table II.

The few experimental points that fall below the fit to the postulated Eq. 4 at 60.8° between pH 4.5 and 6.5 are difficult to reconcile with existing theory since Δ^9-tetrahydrocannabinol has no pKa′ in this range. This anomaly must either be attributed to some technological artifact or to the buffers exerting an inhibition of degradation rates. However, there were no significant differences among rate constants at pH 3.95 evaluated over a fivefold range of acetate buffer concentrations or a fivefold range of ionic strength at pH 1.90.

A reasonable explanation for the observed biphasic phenomena below pH 4.0 is that the first phase characterized by the apparent first-order rate constant k_1 represents the loss of Δ^9-tetrahydrocannabinol to various products (Scheme I), which includes a *Y* that fortuitously has the same GLC retention time as Δ^9-tetrahydrocannabinol when extracted from the reaction mixture by the specified procedures. This product *Y* subsequently vanishes by a first-order rate at a constant acidity characterized by the rate constant k_2. This would imply that Eqs. 4 and 5 could be combined so that:

$$k_1 = [k_{H_1^+} a_{H^+} + k_0] f_u \qquad \text{(Eq. 8)}$$

where the estimated microscopic rate constants are given in Table II and the dependence of Eq. 8 on k_1 at 60.8° is demonstrated by the dotted line in Fig. 6.

$$\Delta^9\text{-tetrahydrocannabinol} \xrightarrow{k_1} \text{products} + Y \qquad Y \xrightarrow{k_2} Z$$

Scheme I

The fact that only one phase exists above pH 4.0 may be rationalized by postulating that *Y* is only produced by hydrogen-ion attack on the Δ^9-tetrahydrocannabinol whereas the pH-independent degradation above this pH value does not produce *Y*.

Alternatively, the fact that two sequential reactions predominated in the degradation of Δ^9-tetrahydrocannabinol below pH 4 (Figs. 3 and 4) but only the slower phase appeared to persist at the higher pH values to pH 10 can be rationalized by Scheme II.

$$\text{products} \xleftarrow{k_2} \left(\Delta^9\text{-tetrahydrocannabinol} \underset{k_{-1}'}{\overset{k_1'}{\rightleftharpoons}} X\right)$$

Scheme II

Then:

$$k_1 = k_1' + k_{-1}' \qquad \text{(Eq. 9)}$$

and (4):

$$k_2 = \frac{k_2}{1 + k_1'/k_{-1}'} = \frac{k_2'}{1 + [X]_{eq}/[\Delta^9]_{eq}} =$$

$$\frac{k_2'}{1 + C_1/C_2} = \frac{k_2'}{1 + 2.333} = 0.3k_2' \qquad \text{(Eq. 10)}$$

from the rough estimate of the C_1 and C_2 values given in Table I.

Scheme II proposes that the GLC observed Δ^9-tetrahydrocannabinol readily equilibrates with a compound *X* where the sum of the backward and forward rate constants, k_1 (Eq. 9), is given by the rate dependency of Eq. 5 and that both microscopic rate constants k_1' and k_{-1}' would have to be hydrogen-ion dependent so that their ratio is not. The C_1 and C_2 values (Table I) obtained from the intercepts of the phases in the plots of Figs. 3 and 4 permit estimates of:

$$k_1'/k_{-1}' = [X]_{eq}/[\Delta^9]_{eq} = C_1/C_2 = 0.7/0.3 = 2.33 \qquad \text{(Eq. 11)}$$

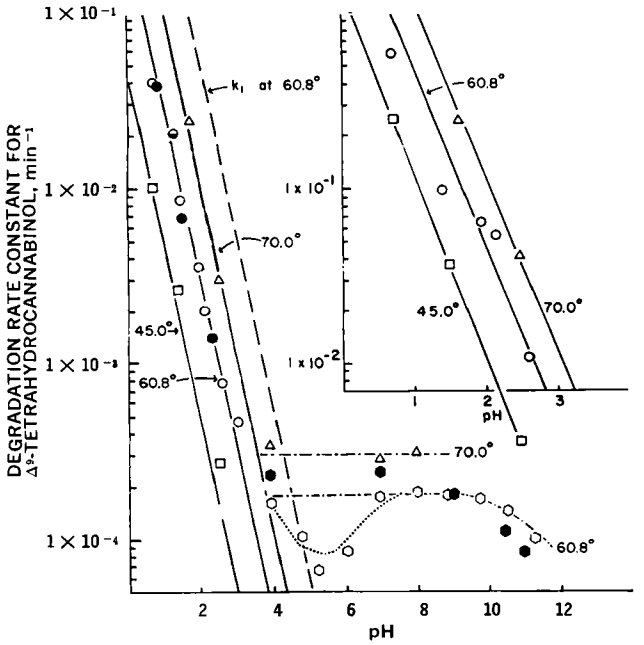


Figure 6—*Log* k–*pH profiles for the degradation of* Δ^9*-tetrahydrocannabinol at several temperatures, where the concentration could be expressed as* $C = C_1e^{-k_1t} + C_2e^{-k_2t}$ *as a function of time,* t*, in minutes. The open symbols were obtained from studies on material purified by TLC, and the solid symbols were obtained from studies on unpurified material. The half-open symbol was obtained from a study on a synthesized mixture of purified* Δ^9*- and* Δ^8*-tetrahydrocannabinols. The larger graph is for the smaller rate constant,* k_2*, and inset is for the larger rate constant,* k_1*, obtained by graphical analysis or appropriate feathering of the plots of the logarithms of the GLC peak height ratios of* Δ^9*-tetrahydrocannabinol to the internal standard tetraphenylethylene against time. For purposes of comparison with the smaller rate constant,* k_2*, the dashed line for* k_1 *at 60.8° is inserted in the larger graph. The dot-dashed lines represent rate constants from reactions at neutral pH values that do not show biphasic semilogarithmic plots.*

so that the concentration of X at the high acidities is more than twice that of Δ^9-tetrahydrocannabinol when equilibrium is established in acidic solutions.

If the apparent rapid equilibration of Δ^9-tetrahydrocannabinol with X characterized by k_1 (Eqs. 5 and 9) is only catalyzed by hydrogen ions but its degradation to products characterized by k_2 (Eq. 10) is not pH dependent above pH 4, then Eqs. 4 and 5 could be combined to:

$$k_2 = [k_{H_2^+}a_{H^+} + k_0]f_u \qquad \text{(Eq. 12)}$$

The rate of loss of Δ^9-tetrahydrocannabinol to products would exceed the postulated rate of its equilibration to X above this pH value and the rapid loss characterized by k_1 would tend to disappear at the higher pH values.

The half-life of Δ^9-tetrahydrocannabinol at pH 1 and 37° can be estimated from the k_1 data of Tables I and II as approximately 15 min and implies that the drug may be significantly degraded in the normal stomach.

GLC and TLC Characterization of Products of Δ^9-Tetrahydrocannabinol in Acid Solution—When Δ^9-tetrahydrocannabinol reacted in aqueous acid solution was analyzed by the stated extraction and GLC procedures, five distinct peaks were observed by flame-ionization detection. Figure 7 shows typical chromatograms of aliquots of solutions of Δ^9-tetrahydrocannabinol at less than 1.0 mg/liter reacted in 0.1 *N* HCl for 4 days at 60.8°. Under the stated experimental conditions, the retention times of the first two peaks, other than the internal standard tetraphenylethylene at 1.3 min, corresponded to Δ^8-tetrahydrocannabinol (2.1 min) and Δ^9-tetrahydrocannabinol (2.34 min). Subsequently, three others were observed and were labeled P_1 (3.5 min), P_2 (4.2 min), and P_3 (5.22 min). The chromatograms of Fig. 7 were obtained from reacting solutions of purified and nonpurified Δ^9-tetrahydrocannabinol in aqueous and in 20% ethanol solutions. There was some difference between the effect of 0 and 20% ethanol on the relative heights of the observed peaks in that the presence of alcohol appeared to favor the products with retention times assigned to Δ^8- and Δ^9-tetrahydrocannabinols. This fact is consistent with the previously stated rationalization (Scheme I) that a product Y may result with the same retention time as Δ^9-tetrahydrocannabinol. Alcohol content did not appear to affect the relative amounts of P_1, P_2, and P_3. The yields of P_2, P_3, and a product with the same retention time as Δ^8-tetrahydrocannabinol increased and the yield of P_1 decreased when impure Δ^9-tetrahydrocannabinol was used as the starting material rather than the purified compound.

Table II—Microscopic Rate Constants (in Liters per Mole per Minute) for the Degradation of Δ^9-Tetrahydrocannabinol in Aqueous Solutions in Accordance with $k_1 = k_{H_1^+}a_{H^+}$ and $k_2 = k_{H_2^+}a_{H^+}$, where $a_{H^+} = 10^{-pH}$ and where k is Independent of pH

Temperature	$k_{H_1^+}$	$k_{H_2^+}$	10^4k
45.0°	1.2	0.045	—
60.8°	5.0	0.265	1.80
70.0°	12.0	1.00	3.05
ΔH_a, kcal/mole	21	23	13
log P, min^{-1}	14.4	14.6	4.9

When the spontaneously formed aqueous emulsion (1) of the original impure Δ^9-tetrahydrocannabinol was reacted in 0.1 *N* HCl at 60° for 5 days and the extracts were analyzed by GLC, the primary product had the same retention time as Δ^8-tetrahydrocannabinol and appeared to have relatively the same peak height ratio to the internal standard as did the Δ^9-tetrahydrocannabinol in the original solution. The peak heights assigned to P_1, P_2, and P_3 were relatively small and formed slowly. The primary Δ^9-tetrahydrocannabinol transformation apparently is to Δ^8, not only in acidified hydrocarbon solutions (5–7) but in the hydrocarbon phase formed

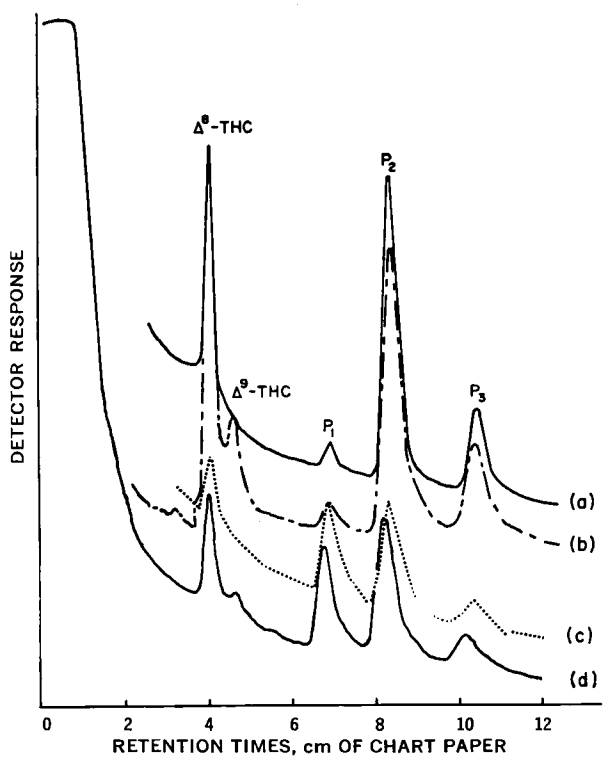


Figure 7—*Gas chromatograms of chloroform-extracted acid-degraded solutions of* Δ^9*-tetrahydrocannabinol (*Δ^9*-THC) in 0.1* N *HCl at 60.8° for 4 days for* (a *and* b) *unpurified compound and* (c *and* d) *TLC-purified compound in 0% ethanol* (a *and* c) *and 20% ethanol* (b *and* d).

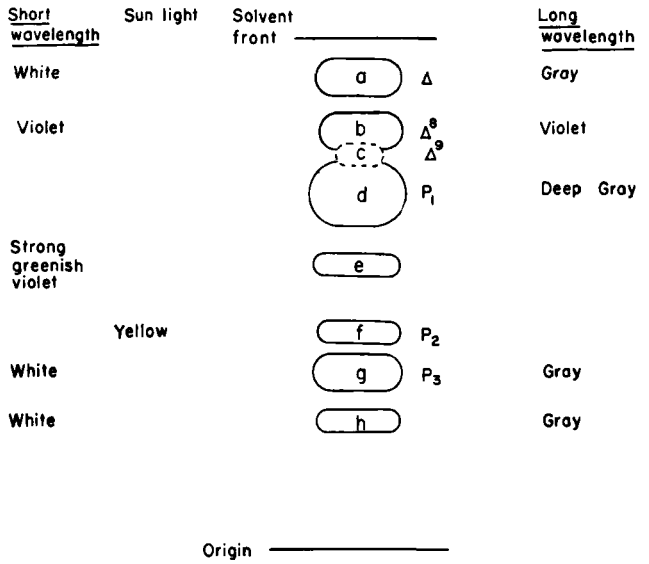
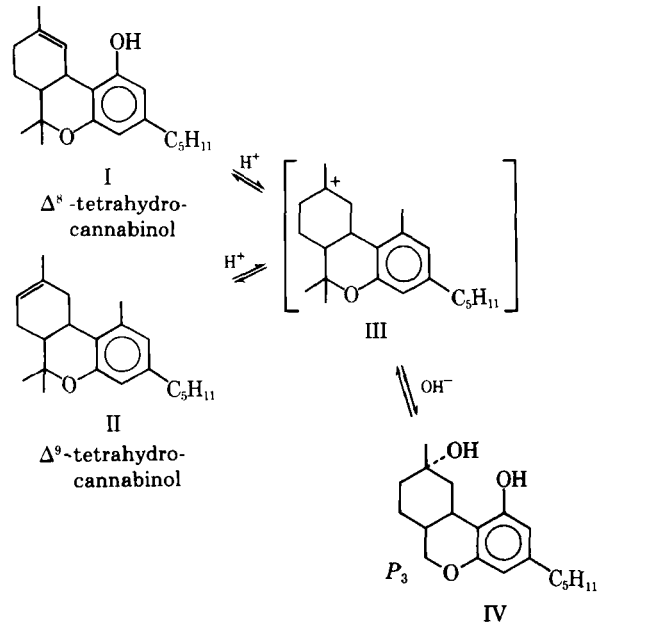


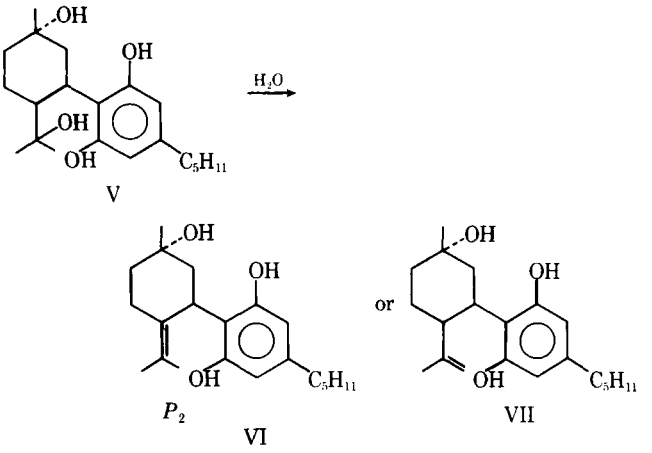
Figure 8—*Thin-layer chromatogram, developed with cyclohexane–acetone (10:1), of chloroform extracts of degraded unpurified Δ⁹-tetrahydrocannabinol (12.5 mg/liter in 20% ethanolic 0.1 N HCl after 5 days). Elution of the pertinent spots and subsequent GLC analyses identified Δ⁸-tetrahydrocannabinol* (b), P_1 (d), P_2 (g), *and* P_3 (h). *The three additional spots observed when the starting material was impure were* (a), *which gave irregular GLC peaks (Fig. 2a), and* (e) *and* (f), *which did not give observable GLC peaks on elution. The dashed spot* (c) *was where Δ⁹-tetrahydrocannabinol would appear if present.*

by its own emulsification (1). The fact that the peak height of Δ^8-tetrahydrocannabinol increased with the increasing alcohol content of true solutions is consistent with the premise that P_1, P_2, and P_3 result from the attack of the components of water on tetrahydrocannabinol in true solution.

TLC [Chrom AR, cyclohexane–acetone (10:1)] effectively separated the chloroform-extracted products of a 20% ethanol solution of Δ^9-tetrahydrocannabinol (12.5 mg/liter) reacted in 0.1 *N* HCl at 61° for 5 days. The spots of R_f values 0.86 *(b)*, 0.80 *(c)*, 0.75 *(d)*, 0.39 *(g)*, and 0.28 *(h)* (Fig. 8), when eluted and gas chromatographed, permitted their identification with the retention times consistent with Δ^8-tetrahydrocannabinol, Δ^9-tetrahydrocannabinol, P_1, P_2, and P_3, respectively. When impure Δ^9-tetrahydrocannabinol was used as the starting material, three additional spots were observed (Fig. 8). One spot *(a)* of R_f 0.97 near the solvent front, when eluted, gave the irregular GLC peaks prior to the Δ^8-tetrahydrocannabinol peak observed when the top half of the spot for Δ^8-tetrahydrocannabinol, obtained by TLC from impure Δ^8-tetrahydrocannabinol, was eluted and gas chromatographed (Fig. 2*a*). A second spot *(e)* of R_f 0.59 showed strong greenish violet fluorescence under short UV wavelength (254 nm); this fluorescence decayed rapidly in air. The third spot *(f)* appeared yellow. Neither spot *(e)* nor spot *(f)* gave an observable GLC peak on elution.



Scheme III



Scheme IV

Characterization of Separated Products—The products were separated and purified by preparative TLC. The separated product, as well as the impurity in the original impure sample of Δ^9-tetrahydrocannabinol that had the same GLC retention time as Δ^8-tetrahydrocannabinol, had the same UV (in methanol) and NMR spectra as pure Δ^8-tetrahydrocannabinol (5, 6).

Tentative Identification of Cannabinol as P_1—The GLC retention times of the TLC isolated P_1 (3.5 min at 245°, 9.5 min at 220°) were coincident with those of cannabinol. Product P_1 and cannabinol had the same R_f values in four different TLC systems: benzene (0.88), chloroform (0.94), cyclohexane (0.04), and cyclohexane–acetone (10:1) (0.79). No spots were observed with either material when isoamyl alcohol was used as the developing solvent. A yellow band was observed at R_f 0.34, and there appeared to be a band at the solvent front in both cases.

The UV spectra[8] of P_1 and cannabinol in methanol had two absorption maxima at 220 and 285 nm, and the ratio of their molar absorptivities was the same ($\epsilon_{220}/\epsilon_{285}$: cannabinol 1.98, P_1 2.18). The IR spectra[8] of P_1 and cannabinol (5) in KBr pellets were coincident. It is well known that both Δ^9- and Δ^8-tetrahydrocannabinols form cannabinol on chloranil dehydrogenation (6).

Tentative Identification of 9-Hydroxycannabidiol as P_2—The UV spectrum[8] of P_2 in methanol had two absorption maxima (275 and 282 nm) very similar to those of Δ^9- and Δ^8-tetrahydrocannabinols, cannabicyclol, cannabigeral, and cannabidiol (5). However, both the IR[9] (Fig. 9*a*) and NMR[10] (Fig. 10*a*) spectra of P_2 exclude its being any of these compounds (5). NMR shows a singlet at δ 6.1 for the two hydrogens on the phenol ring, indicating that these two hydrogens are in the same environment. This can happen only when the ether ring is opened as in cannabinidiol (5). NMR also suggests that the vinylic hydrogen of the double bond at position 9 is absent in P_2 and that the CH_3 peak adjacent to the double bond originally located at δ 1.68 for Δ^9-tetrahydrocannabinol has disappeared. The product P_2 has a strong mass peak at *m/e* 332, which can be assigned to the molecular weight of P_2 which is consistent with the NMR evidence for water addition to the isolated double bond. The mass spectrum[11] pattern of P_2 is similar to that of Δ^8-tetrahydrocannabinol since both compounds have

[9] Beckman Acculab 3.
[10] Varian A60 NMR spectrometer.
[11] AEI MS 30 mass spectrometer combined with a DS10 data system, Scientific Apparatus Corp., Manchester, England.

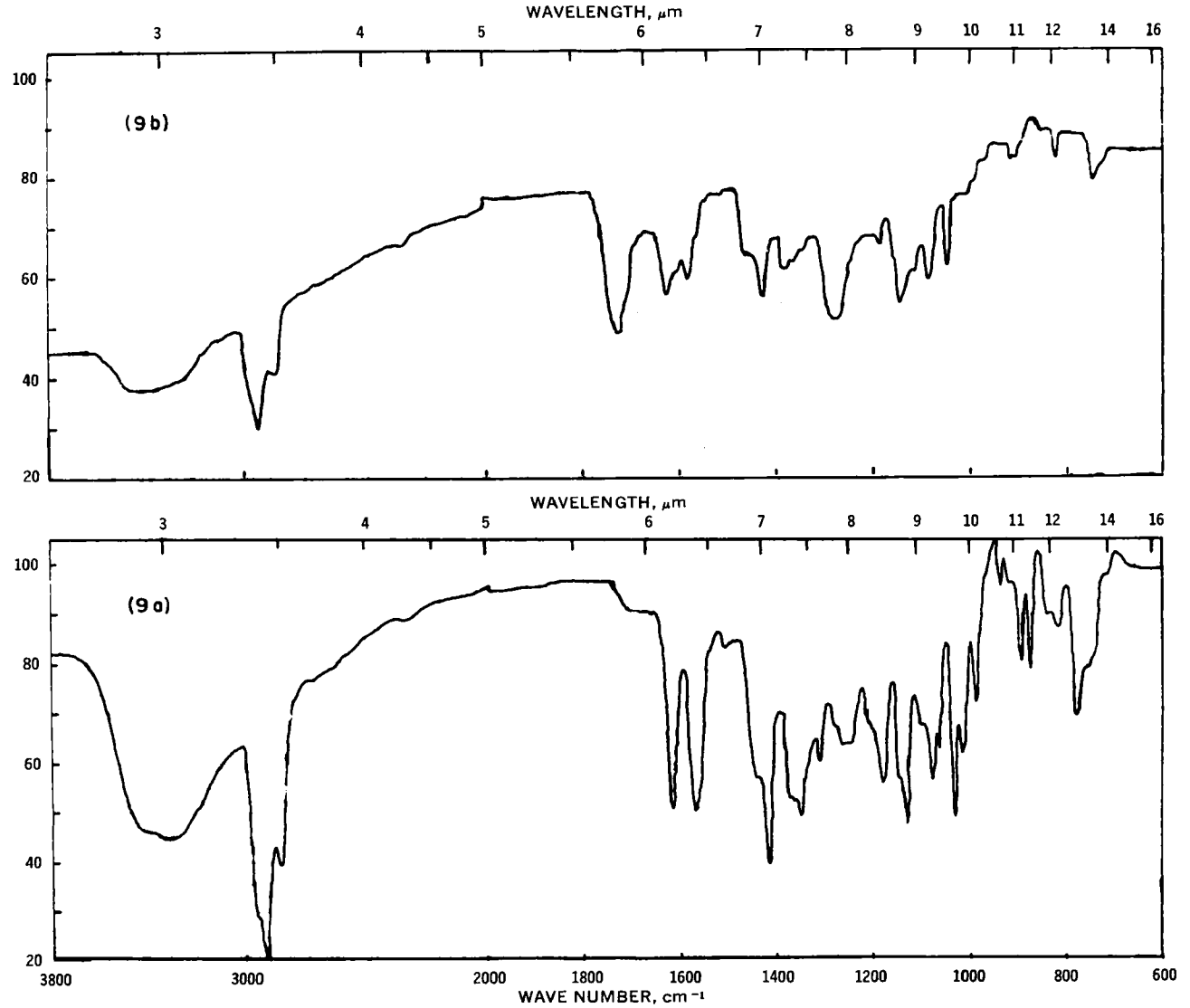


Figure 9—*IR spectra in KBr pellets of* (a) P_2 *and* (b) P_3, *isolated by and extracted from thin-layer chromatograms.*

strong fragment peaks at 314, 299, 271, 258, 243, 231, and 193.

The evidence strongly suggests that P_2 results from: *(a)* the specific hydrogen-ion-catalyzed addition of a water molecule to the nonconjugated double bond of Δ^9- and Δ^8-tetrahydrocannabinols, and *(b)* the opening of the internal ether as shown in Scheme III. The hydrolysis of the ether linkage with subsequent dehydration may result in two possible molecular structures (Scheme IV). Since no $=CH_2$ hydrogens were indicated in the NMR spectrum (about δ 4.7) (5), Structure VI can be assigned to P_2.

Tentative Identification of 9-Hydroxytetracannabinol as P_3—The NMR[10] spectrum of P_3 (Fig. 10*b*) also suggests that the nonconjugated double bond of tetrahydrocannabinol has been saturated since peaks assigned to the vinylic hydrogen and to the CH_3 group adjacent to the double bond (δ 1.68) are missing. The mass spectrum[11] (332 peak) also suggests the addition of a water molecule to tetrahydrocannabinol. The UV[8] and IR[9] (Fig. 9*b*) spectra of P_3 are similar to those of Δ^9-tetrahydrocannabinol and imply that P_3 results from the mechanisms proposed in Scheme III for the production of IV. It is well known that addition to the isolated double bond occurs when cannabidiol is heated in ethanol with dilute hydrochloric acid (5).

Possible Relationship among Yields of P_1, P_2, P_3, and Δ^8-Tetrahydrocannabinol from Δ^9-Tetrahydrocannabinol—The use in GLC of a ratio of the peak height given by a specific compound at its retention time to the peak height of a constant amount of internal standard at its retention time is a standard procedure to establish an analytical calibration curve for the assay of amounts of the compound. A peak height ratio of another compound to the internal standard that is the same as that for the initial compound does not necessarily imply that the amounts of both compounds assayed are the same. In general, a compound of a longer retention time will demonstrate a lower peak height and ratio than a compound with a shorter retention time and of the same quantity since the distribution or spreading of the band of material emerging from the column will be greater. Also, even with the same retention times under a given set of chromatographic conditions, the magnitudes of the detector response for each unit amount of compound may vary. In the specific case of flame-ionization detection, the detector response per unit amount of compound will vary with the burning efficiency of each compound.

If the chemical structures and burning efficiencies of Δ^8- and Δ^9-tetrahydrocannabinols and their GLC-observed products, P_1, P_2, and P_3, are assumed to be similar, and if the same peak heights at the same retention time are assumed to be measures of equivalent amounts of different compounds, it may be possible to correct the peak heights of different retention times to the same retention time to compare yields of GLC-observed products from a given reactant.

Equal amounts of Δ^9- and Δ^8-tetrahydrocannabinols were injected into the gas chromatograph at different column temperatures, and the relative peak heights were obtained as a function of retention time (Fig. 11). The ratios of the relative peak heights of Δ^9- and Δ^8-tetrahydrocannabinols at their normal retention times to their relative peak heights at the retention time of Δ^9- and Δ^8-

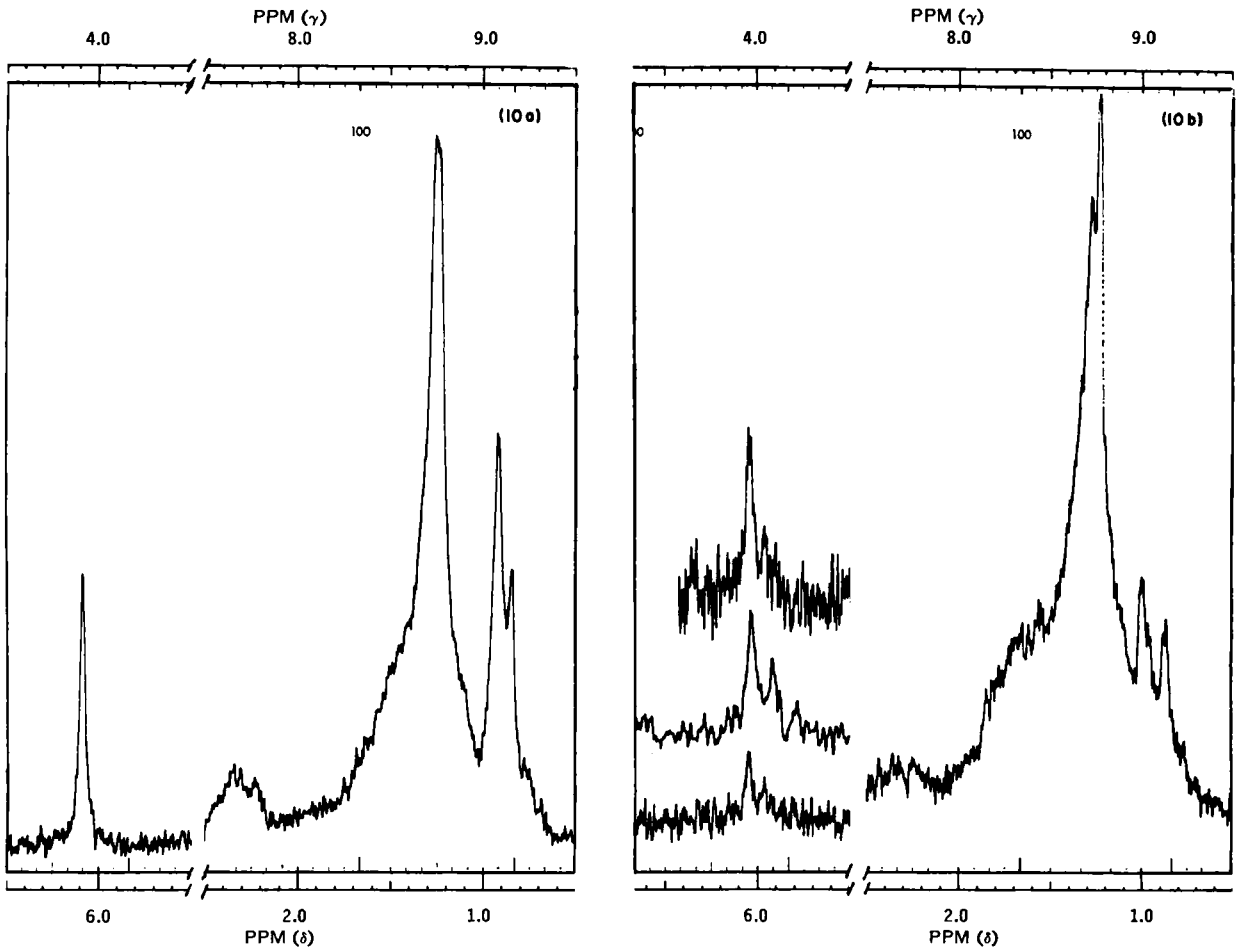


Figure 10—*NMR spectra of* (a) P_2 *and* (b) P_3, *isolated by and extracted from thin-layer chromatograms.*

tetrahydrocannabinols, P_1, P_2, and P_3 were calculated. If the assumptions underlying this approach are correct, the ratios in parentheses are the values necessary for multiplying the peak heights or peak height ratios of Δ^8-tetrahydrocannabinol (0.73), P_1 (1.6), P_2 (2.0), and P_3 (2.6) to relate their amounts to equivalent amounts of Δ^9-tetrahydrocannabinol in the reacting mixture. The ratios in parentheses are the values necessary to multiply the peak heights or peak height ratios of Δ^9-tetrahydrocannabinol (1.4), P_1 (2.2), P_2 (2.8), and P_3 (3.7) to relate their amounts to equivalents of Δ^8-tetrahydrocannabinol reactant. The peak height/amount values obtained from the GLC calibration curves of Δ^9- and Δ^8-tetrahydrocannabinols were 0.155 and 0.182/μg, respectively, which gives a ratio of 0.85 which is in the vicinity of the 0.73 factor obtained by the described procedure.

Production of GLC-Observed Δ^8-Tetrahydrocannabinol, P_1, P_2, and P_3 from Degradation of Purified Δ^9-Tetrahydrocannabinol in Aqueous Solution—The appearance of the peak heights at the GLC retention times of Δ^8-tetrahydrocannabinol, P_1, P_2, and P_3 and the loss of peak height of Δ^9-tetrahydrocannabinol of the chloroform-extracted material (Fig. 7) were observed with time when Δ^9-tetrahydrocannabinol was maintained in aqueous solution at constant temperature. Typical graphs are given in Figs. 12–14 where the actual peak height ratios (to the internal standard tetraphenylethylene) of Δ^9-tetrahydrocannabinol, P_1, P_2, and P_3 were multiplied by the appropriate factors that would correct the diminutions of their peak height ratios at higher retention times on the presumption that equivalent peak height ratios at the same retention time as Δ^9-tetrahydrocannabinol would give reliable estimates of relative amounts of products and precursor.

The ratios of the amounts of products and their relative yields from Δ^9-tetrahydrocannabinol were reasonably the same within the errors of measurement at all pH values below 4 and their rates of appearance were proportional to the hydrogen-ion concentration. This was confirmed by the negative linear slopes of approximately unity of the logarithms of initial rates of peak height appearance of products plotted against pH up to pH 3.0.

In general, the rates of appearance of P_1 and the Δ^8 compound paralleled the initial fast phase (k_1) of loss of the peak for Δ^9-tetrahydrocannabinol and seemed to be greater than the rates of appearance of P_2 and P_3. In several cases, there may have been an anomalous discontinuity in the rate of appearance of the P_1 peak (dashed lines in Figs. 12–14) which could only be explained by the fact that two products have the same retention time where the more rapidly appearing compound is farther degraded or equilibrated to some other at that or some other retention time.

No significant formation of Δ^8-tetrahydrocannabinol, P_1, P_2, and P_3 was observed above pH 4 until pH 8.9, beyond which pH the production of small amounts of Δ^8-tetrahydrocannabinol was again observed.

Patterns of Acidic Degradation of P_2, P_3, and Δ^8-Tetrahydrocannabinol—Pertinent spots obtained from TLC of acid-degraded aqueous solutions of Δ^9-tetrahydrocannabinol were extracted, and the isolated compounds had the GLC retention times of P_2 and P_3. These materials, as well as Δ^8-tetrahydrocannabinol, were reacted in aqueous solutions of pH 0.79 at 60.8°, and the corrected peak height ratios of each compound with time are plotted in Fig. 15. The premise for such plots is that if each compound had the same retention time as Δ^8-tetrahydrocannabinol, the corrected peak height ratios would be proportional to the number of molecules observed. When starting with P_2 and P_3, the sum of the corrected peak height ratios for all those materials at any time was reasonably close to the corrected peak height ratio for P_2 and P_3 at zero time. This was not true when Δ^8-tetrahydrocannabinol was the starting material.

The final ratios of the corrected peak height ratios with time at 60.8° and pH 0.79 (Fig. 15) for the various starting materials are for P_3: $P_2/\Delta^8 = 5.3$, $P_3/\Delta^8 = 2.7$, and $P_2/P_3 = 2.0$; for P_2: $P_2/\Delta^8 = 6.2$, $P_3/\Delta^8 = 2.0$, and $P_2/P_3 = 2.0$; and for Δ^8: $P_2/\Delta^8 = 5.3$, $P_3/\Delta^8 =$

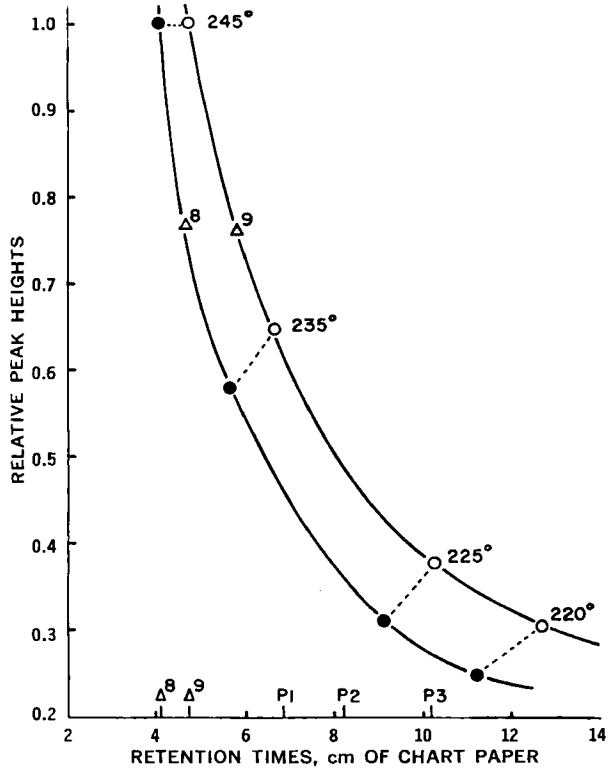


Figure 11—*Plots of peak heights of Δ⁸- and Δ⁹-tetrahydrocannabinols at various column temperatures relative to their respective peak heights at a column temperature of 245° against observed retention times. It can be assumed that the factor necessary to multiply the peak height of a product at its normal retention time with a 245° column temperature to equate it to an equivalent amount of Δ⁹- (or Δ⁸-) tetrahydrocannabinol is determined by the reciprocal of the relative peak height of Δ⁹- (or Δ⁸-) tetrahydrocannabinol at the retention time of the product under the conditions of a 245° column temperature. The labelings for the marks above the abscissa give the retention times for the stated product.*

2.5, and P_2/P_3 = 2.1. At pH 0.56 and 60.8° for P_3, P_2/Δ^8 = 5.0, P_3/Δ^8 = 2.2, and P_2/P_3 = 2.3. At pH 0.26 and 60.8° for Δ^8, P_2/Δ^8 = 3.9, P_3/Δ^8 = 1.6, and P_2/P_3 = 2.4. At pH 1.49 and 60.8° for Δ^8, P_2/Δ^8 = 5.1, P_3/Δ^8 = 2.4, and P_2/P_3 = 2.1. The ratios were remarkably consistent for the studies at these several pH values, well within the errors of measurement, and gave averages of P_2/Δ^8 = 5.1, P_3/Δ^8 = 2.1, and P_2/P_3 = 2.0. Therefore, it is reasonable to conclude that these values represent the final relationships of the equilibria that occur among Δ⁸-tetrahydrocannabinol, P_2, and P_3 in acidic solutions.

The degradation of Δ⁸-tetrahydrocannabinol in acidic solutions appears to be first order, although there may have been an initial lag at some higher pH values such as at pH 2.1 (Fig. 16). The apparent first-order rate constants are plotted semilogarithmically against pH in the inset of Fig. 16 and demonstrate a linear slope of unity in accordance with Eqs. 5 and 6, indicative of specific hydrogen-ion catalysis where $k_{H^+} = 3.6 \times 10^{-3}$ liter/mole/min at 60.8°, which is 0.0007 of the $k_{H_1^+}$ and 0.0135 of the $k_{H_2^+}$ for Δ⁹-tetrahydrocannabinol under the same conditions.

When Δ⁹-tetrahydrocannabinol was reacted in acidified solutions at 60.8°, the observed final P_2/Δ^8 ratios were for several pH values (pH, P_2/Δ^8): 0.68, 5.1 (Fig. 12); 1.40, 4.5 (Fig. 13); and 2.12, 6.0 (Fig. 14). These values are consistent with the results of the Δ⁸-tetrahydrocannabinol studies and indicate that the Δ⁸ product from Δ⁹ degradation forms similar equilibria among P_2 and P_3. The production of P_3 was increasing beyond the time the studies were terminated, which would be consistent with the transformation rates of Δ⁸-tetrahydrocannabinol, so the final P_3/Δ^8 ratio was difficult to estimate. The available data were consistent with a final value of 2.0–3.0 for this ratio. For example, at pH 2.12 at 60.8°, where P_3 was analyzed for longer times than are given in Fig. 14, the corrected peak height ratios for P_3 were 0.102 at 3300 min and 0.144 at 5800 min, which are P_3/Δ^8 final ratios of 1.7 and 2.4, respectively.

Available Evidence and Possible Transformations of Δ⁹-Tetrahydrocannabinol in Aqueous Solutions—The fact that an apparent final equilibrium exists among Δ⁸-tetrahydrocannabinol, P_2, and P_3 in acid solutions, coupled with the evidence (Schemes III and IV) for the structures of P_2 and P_3, indicates that there is an equilibration in the presence of acid among compounds with an intact and hydrolyzed ether linkage and among compounds with a hydrated and isolated double bond.

The fact that Δ⁹-tetrahydrocannabinol degrades in acid solution to an additional product P_1 as well as to Δ⁸ (Figs. 12–14) seems indisputable, but it is difficult on mechanistic grounds to assign P_1 to cannabinol even though chromatographic evidence so indicates.

In one 60.8° kinetic study of Δ⁹-tetrahydrocannabinol in aqueous 0.12 *N* HCl, there was no significant increase in the UV absorbance of the ethanolic reconstitutions of the dried chloroform extracts with time up to 930 min, when significant P_1 was observed by GLC (Figs. 12–14). Since (5) cannabinol has a molar absorptivity (ϵ_{285} = 18,000) more than 10 times that of Δ⁹-tetrahydrocannabinol (ϵ_{280} = 1050), there should have been an enormous increase in absorbance if cannabinol had formed directly from Δ⁹-tetrahydrocannabinol.

A possible rationalization of these contradictions would be that Δ⁹-tetrahydrocannabinol does transform into molecules with solvolyzed ether linkages and/or hydrated double bonds analogous to those prepared with Δ⁸-tetrahydrocannabinol. One of these, P_1, may have the same GLC retention time as cannabinol or is trans-

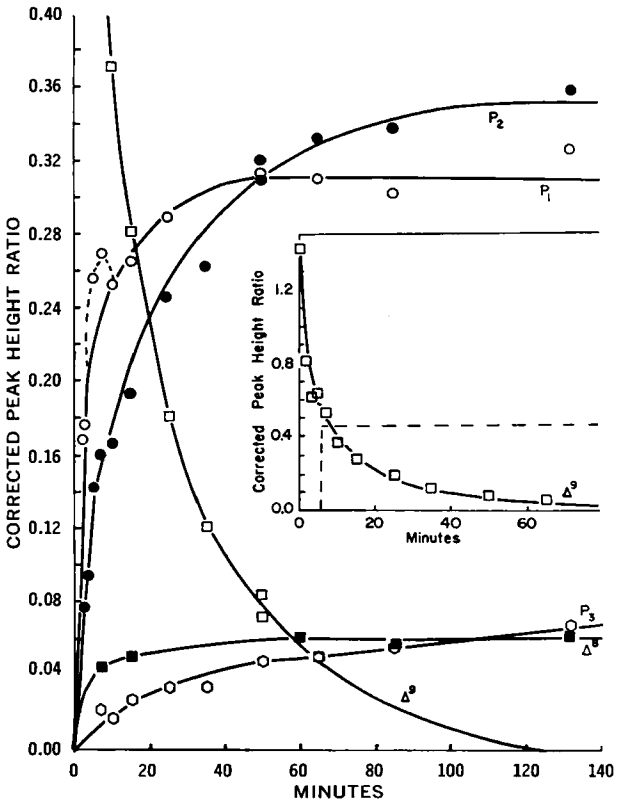


Figure 12—*Plots of the peak height ratio to that of the internal standard tetraphenylethylene against time of Δ⁹-tetrahydrocannabinol reacted at 60.8° in aqueous solutions at pH 0.68 and the corrected peak height ratios of the products of Δ⁸-tetrahydrocannabinol, P_1, P_2, and P_3 observed by GLC. The observed peak height ratios of these latter products were multiplied by 0.73, 1.6, 2.0, and 2.6, respectively, on the presumption that the resultant values would be equivalent to the peak height ratio of Δ⁹-tetrahydrocannabinol. The insets are condensed plots for Δ⁹-tetrahydrocannabinol, and the dashed lines encompass those values plotted in the larger graphs.*

Table III—Percent of Total Radioactivity of Original Δ^9-Tetrahydrocannabinol at Various R_f Values Corresponding to Products of Degradation in 0.06 *N* HCl at 60.8°[a]

Reaction Time, min	$\Delta^8 + \Delta^9$	P_1	$P_2 + P_3$	Subtotal	Origin	Total	Residual
5	64		11	75	6	81	19
15	42	10	22	76	14	86	14
40	26	19	22	67	19	86	14
120	24	18	36	78	13	91	9
1130	11	18	50	79	10	89	11
5760	13	21	44	78	11	88	12

[a] The thin-layer chromatograms of chloroform-extracted samples taken with time were monitored by a radio chromatogram scanner. The areas of the pertinent peaks of the chromatogram were cut and weighed.

formed to cannabinol after injection and travel through the column. Similarly, TLC separation and elution may permit its oxidation to cannabinol. The apparent reproducible maximum on the side of the curve (dashed lines in Figs. 12–14) for the production of material with the retention time characterized as P_1 from Δ^9-tetrahydrocannabinol could indicate the rapid formation of several products of the same retention time where one of them degrades further. The problem of proper characterization of the compounds that give rise to the P_1 retention time needs further elucidation.

The biphasic nature of the semilogarithmic plots of Δ^9-tetrahydrocannabinol degrading in acid solutions against time (Figs. 3 and 4) either may implicate the rapid production of a compound, *Y* (Scheme I), that has the same GLC retention time as Δ^9-tetrahydrocannabinol and that may be slowly transformed further or may implicate the rapid equilibration of Δ^9-tetrahydrocannabinol with a compound, *X* (Scheme II), and the slower further irreversible degradation of one or both compounds. Both explanations are consistent with transformations of Δ^9-tetrahydrocannabinol by hydrogen-ion-catalyzed double-bond migration to Δ^8-tetrahydrocannabinol, hydrogen-ion-catalyzed hydrolysis of the ether linkage (IV → V) with possible subsequent dehydration (Scheme IV), and acid-catalyzed hydration of the isolated Δ^9 double bond (Scheme III).

For both Schemes I and II, *Y* or *X* could be products of Δ^9-tetrahydrocannabinol resulting from a hydrolyzed ether linkage, VIII (Scheme V), or its dehydrated analogs, IX or X. Any one of these could undergo specific hydrogen-ion-catalyzed attack to form an activated complex, III, III′, III″, or III‴, which may further produce the 9-hydroxy analogs, IV, V, VI, or VII, on hydroxylation. Δ^8-Tetrahydrocannabinol does not produce Δ^9-tetrahydrocannabinol or P_1 but produces equilibrating P_2 and P_3, tentatively

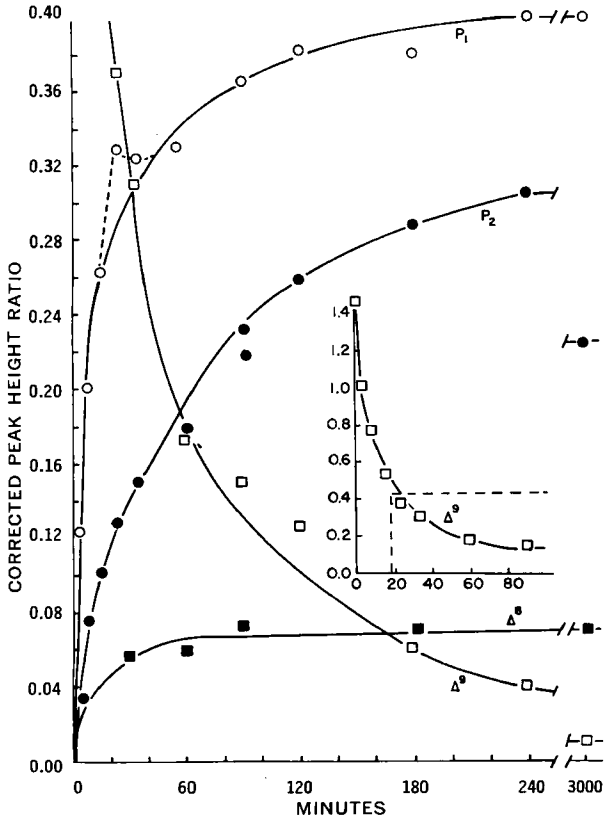


Figure 13—*Plots of the peak height ratio to that of the internal standard tetraphenylethylene against time of Δ^9-tetrahydrocannabinol reacted at 60.8° in aqueous solutions at pH 1.40 and the corrected peak height ratios of the products of Δ^8-tetrahydrocannabinol, P_1, P_2, and P_3 observed by GLC. The observed peak height ratios of these latter products were multiplied by 0.73, 1.6, 2.0, and 2.6, respectively, on the presumption that the resultant values would be equivalent to the peak height ratio of Δ^9-tetrahydrocannabinol. The insets are condensed plots for Δ^9-tetrahydrocannabinol, and the dashed lines encompass those values plotted in the larger graphs.*

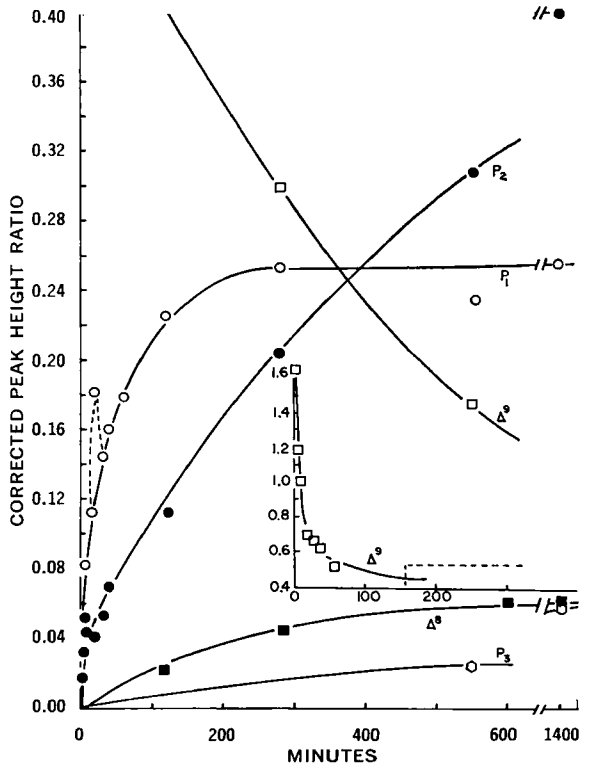


Figure 14—*Plots of the peak height ratio to that of the internal standard tetraphenylethylene against time of Δ^9-tetrahydrocannabinol reacted at 60.8° in aqueous solutions at pH 2.12 and the corrected peak height ratios of the products of Δ^8-tetrahydrocannabinol, P_1, P_2, and P_3 observed by GLC. The observed peak height ratios of these latter products were multiplied by 0.73, 1.6, 2.0, and 2.6, respectively, on the presumption that the resultant values would be equivalent to the peak height ratio of Δ^9-tetrahydrocannabinol. The insets are condensed plots for Δ^9-tetrahydrocannabinol, and the dashed lines encompass those values plotted in the larger graphs.*

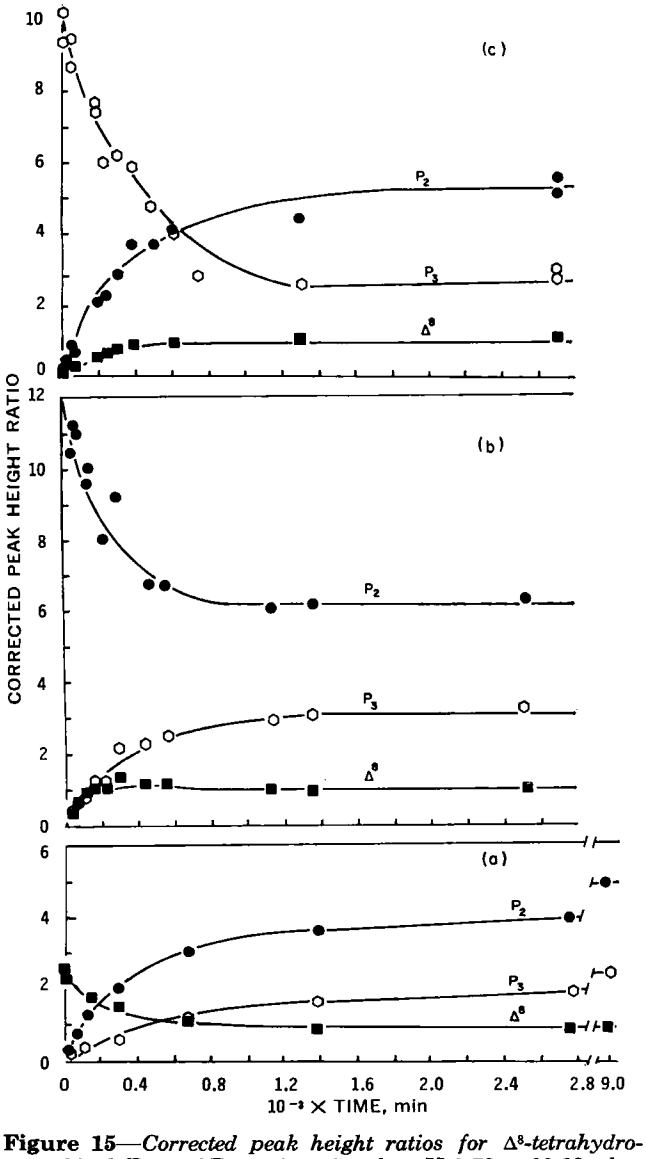


Figure 15—*Corrected peak height ratios for Δ⁸-tetrahydrocannabinol, P_2, and P_3 against time for pH 0.79 at 60.8° when the initial reactant, as material isolated by TLC, was* (a) *Δ⁸-tetrahydrocannabinol,* (b) *P_2, and* (c) *P_3 and their observed initial peak height ratios were 1.0, 1.0, and 1.8, respectively. The corrected peak height ratios were calculated on the premise that a unit peak height ratio of P_2 and P_3 would represent the same number of molecules as one unit of peak height ratio of Δ⁸-tetrahydrocannabinol when all three compounds had the same retention times. This would necessitate multiplying the observed peak height ratios at the observed retention times by 1.0 for Δ⁸-tetrahydrocannabinol, 2.8 for P_2, and 3.7 for P_3 in each graph. Each resultant ordinate value for each graph was actually multiplied by an appropriate factor so that the final value of the corrected peak height ratio for Δ⁸-tetrahydrocannabinol with time would be unity and the other corrected peak height ratios in each graph would be plotted relative to this value for proper comparison among the various graphs when either Δ⁸-tetrahydrocannabinol, P_2, or P_3 was used as the starting material. Thus, if Y is the actual final value of the peak height ratio of Δ⁸-tetrahydrocannabinol, the peak height values of P_2 at a given time in a particular graph were multiplied by 2.8/Y, those of P_3 were multiplied by 3.7/Y, and those of Δ⁸-tetrahydrocannabinol were multiplied by 1.0/Y. The actual values of Y were:* (a) *0.37,* (b) *0.24, and* (c) *0.64.*

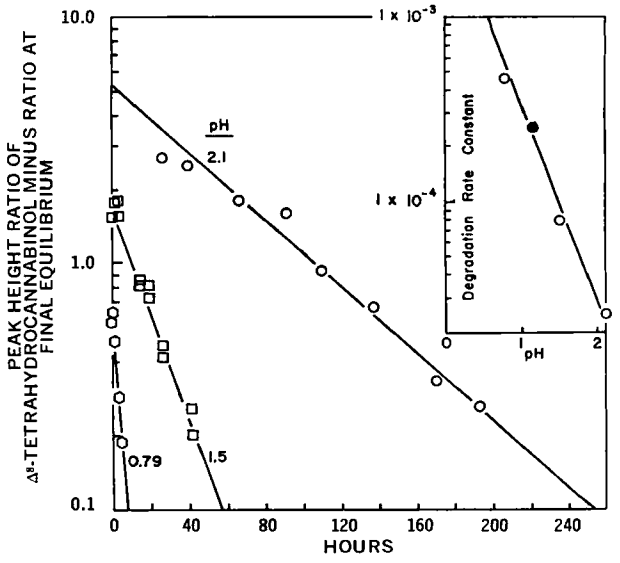


Figure 16—*Semilogarithmic plots with time of the difference between observed peak height ratios of Δ⁸-tetrahydrocannabinol at any time and the equilibrium value for degradations at various pH values. The inset is the log k–pH profile for these degradations plus a synthetic mixture (solid symbol) of Δ⁸- and Δ⁹-tetrahydrocannabinols.*

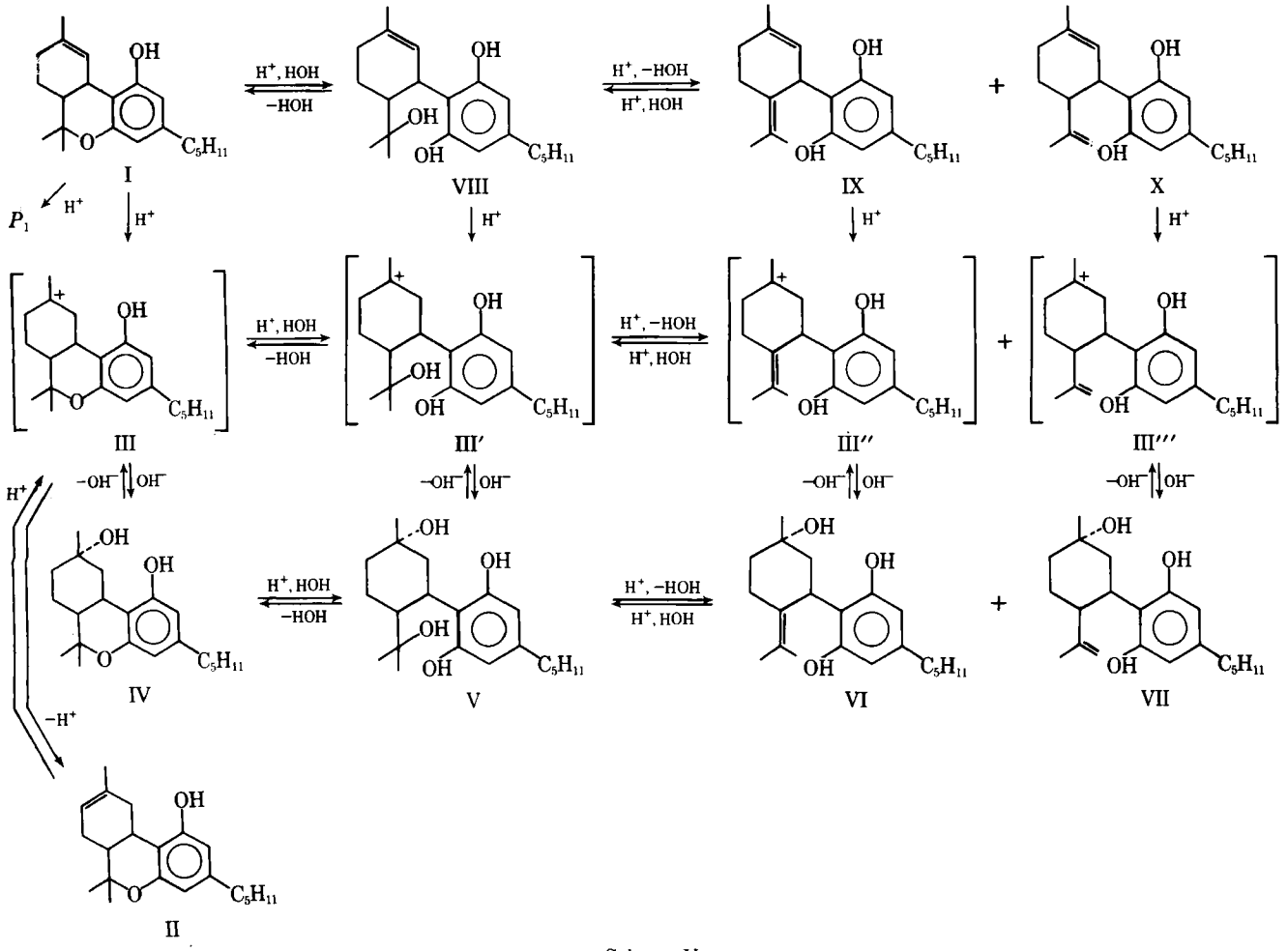
assigned to VI and IV, respectively; Δ⁹-tetrahydrocannabinol produces P_1, P_2, P_3, and Δ⁸-tetrahydrocannabinol. Thus, it is reasonable to postulate that the final product at the P_1 retention time is only a product of Δ⁹-tetrahydrocannabinol and not of Δ⁸-tetrahydrocannabinol and that P_1 and the equilibrating P_2, P_3, and Δ⁸-tetrahydrocannabinol are formed irreversibly from Δ⁹-tetrahydrocannabinol (Scheme V). Therefore, it may be concluded that the hydrogen-ion-catalyzed migration of the double bond favors the Δ⁸-isomer thermodynamically and that P_1 is not in equilibrium with it, P_2, or P_3. This does not exclude a preliminary equilibration among I, VIII, IX, and X where any one of these latter three may initially appear at the retention time of P_1.

The fact that Δ⁹- or Δ⁸-tetrahydrocannabinol in the more neutral pH regions does not produce Δ⁸-tetrahydrocannabinol, P_1, P_2, or P_3 implies that these products are results of hydrogen-ion attack and can be assigned to the k_1 and/or k_2 processes, where pH dependency is characterized by Eqs. 5 and 6. The pH-independent degradation characterization of Δ⁹-tetrahydrocannabinol by k of Eq. 4 must produce a product that was not observed by these GLC procedures.

The transformations of Scheme V readily explain why the major product of Δ⁹-tetrahydrocannabinol in acidified organic solvents or in self-emulsions is Δ⁸-tetrahydrocannabinol since the ether solvolyses and isolated double bond additions require water. In aqueous organic solutions, the activity of water would be lessened and tetrahydrocannabinol would also be favored over the products of hydrolysis or hydration (Fig. 7).

The sums of the radioactivities of the TLC spots assigned to acid-degrading Δ⁹- and Δ⁸-tetrahydrocannabinols, P_1, P_2, and P_3 (Table III) account for approximately 75% of the initial radioactivity of Δ⁹-tetrahydrocannabinol so that it can be concluded that the compounds at these R_f values comprise the most significant products of acid degradation. This does not necessarily mean that the peaks observed from the elution of these spots include the total number of radiolabeled molecules in the spots, since a compound of similar R_f value may be nondetectable by GLC. In fact, the inability of the sums of the observable corrected peak heights to remain invariant with time of degradation indicates that such may be the case. Approximately 12% of the total radioactivity remained at the origin and 12% could not be accounted for by the designated spots and the origin for all times of degradation. However, the fact that 86–90% of the total radioactivity (Table III) was extracted by chloroform from the reacting solution shows that there is a high extraction efficiency of all possible products.

Degradations of Δ⁹-Tetrahydrocannabinol in Alkaline Solutions—A few studies on Δ⁹-tetrahydrocannabinol degradation were conducted at highly alkaline pH values (Fig. 5) under nitrogen. Significant time lags were found at pH 10.5, 11.2, and 12.2 be-



Scheme V

fore the loss of compound became truly first order. In the last case there was little significant change in Δ^9-tetrahydrocannabinol for as long as 200 hr at 60.8°.

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