Stability of Tetrahydrocannabinols II

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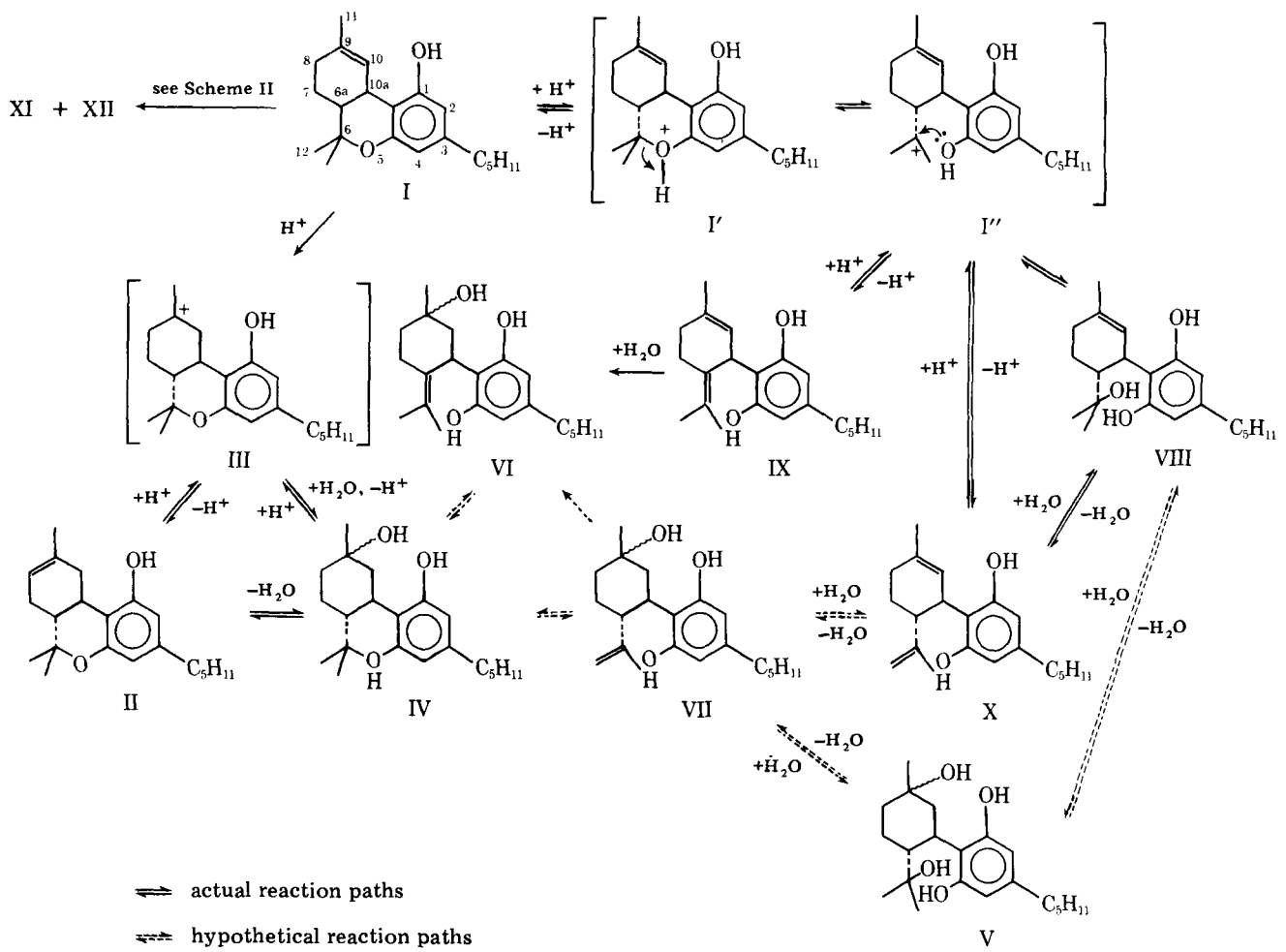
Abstract □ The biphasic degradation of Δ^9-tetrahydrocannabinol (I), as monitored by flame-ionization GLC, produced Δ^8-tetrahydrocannabinol (II), cannabidiol (X), 9-hydroxyhexahydrocannabinol (IV), 9,10-dihydro-9-hydroxyisocannabidiol (VI), and 6,12-dihydro-6-hydroxycannabidiol (VIII) in acidic solutions. Further identification was made by GLC, mass spectrometry, and comparison with authentic samples. Only II and IV were produced above pH 4 in the neutral region by first-order kinetics. The acidic degradation of cannabidiol (X) gave I and the products of the acidic degradation of I. The initial phase of acidic I degradation was assigned to the development of solvolytic equilibria among I, VIII, X, and, possibly, isocannabidiol (IX), with the concomitant production of II and IV. Compounds VIII, IX, and X did not appear in the neutral region since ether cleavage occurred only in strong mineral acids. Hydration of the Δ^9-double bond resulted only in acid-catalyzed equilibria of cleaved ethers with the Δ^8-configurations and characterized the second phase of acid degradation of I. Cannabinol and hexahydrocannabinol were found together in several cases due to the disproportionation of I as catalyzed by silicic acid, silica gel, and chloroform.

Keyphrases □ Δ^9-Tetrahydrocannabinol—kinetics of biphasic degradation in acidic solution □ Degradation—Δ^9-tetrahydrocannabinol in acidic solution, kinetics □ Cannabinoids—Δ^9-tetrahydrocannabinol, kinetics of biphasic degradation in acidic solution

Previous studies (1) on the stability of Δ^9-tetrahydrocannabinol (I) in acidic media below pH 4, monitored by GLC, demonstrated an apparent biphasic semilogarithmic plot of undegraded I against time. Two alternative rationalizations were possible. Either an intermediate was formed that had the same retention time as I and gave rise to the observed products, or there was a relatively rapid equilibration of I with other compound(s) and slower, further, irreversible degradation of one or all of these compounds. Both explanations were consistent with transformations (Scheme I) of I, by hydrogen-ion-catalyzed double bond migration to Δ^8-tetrahydrocannabinol (II) and by hydrogen-ion-catalyzed hydrolysis of the ether linkage, with possible dehydration and acid-catalyzed hydration of the isolated Δ^9-double bond.

The products observed on GLC and identified (1) were



Scheme I

II, 9,10-dihydro-9-hydroxyisocannabidiol (VI), 9-hydroxyhexahydrocannabinol (IV), and cannabinol (XII). Compounds I, II, IV, and VI, on acid degradation, gave final similar ratios of II:IV:VI under the same acidic conditions to indicate the final equilibria among them. The fact that XII resulted from I under anaerobic conditions was anomalous and difficult to explain.

These studies were conducted to rationalize the apparent biphasic nature of I degradation in acid and the anomalous appearance of XII. The purpose was also to identify other products in the acidic and nonacidic degradations. New high-pressure liquid chromatographic (HPLC) techniques for cannabinoid separation and purification were developed, GLC methods were modified, and additional kinetic studies were conducted.

EXPERIMENTAL

Materials—*trans*-Δ^9-Tetrahydrocannabinol[1] (I), *trans*-cannabidiol[2] (X), and cannabinol[3] (XII) were used only after TLC[4] or HPLC[5] purification. The various degradation products were isolated and purified (TLC or HPLC). The internal standards for the GLC studies were tetraphenylethylene[6] and 4-androsten-3,17-dione[7]. The solvents werechromatographic grade *n*-heptane fraction[8] (bp 95–99°), ether[8], and chloroform[8] or UV grade *n*-hexane[8] and tetrahydrofuran[8]; all were distilled in glass.

Isoamyl alcohol was analytical reagent grade[9], as were the other chemicals (mono- and dibasic sodium phosphates[10]). Hydrochloric acid was made from concentrates in plastic ampuls[11] and extracted with chloroform prior to use to remove the plasticizers (phthalate diesters revealed by mass spectrometry) or was taken from a glass bottle[10]. Pure absolute ethanol[12] was used. The double-distilled water was stored in glass containers or purified by means of HPLC[13]. All degradations were conducted in a dark hood.

HPLC Methods for Purification of I and X and Separation of Products on Acid Degradation—HPLC techniques were developed that improved on the previously described (1, 2) TLC purification techniques for cannabinoids. A flow rate of 0.5 ml/min of tetrahydrofuran–*n*-hexane (5:95) quantitatively separated I and II, with respective retention volumes on a 4-mm i.d. × 30-cm column[14] of 6.62 and 6.37 ml. In the same system, cannabidiol had a retention volume of 6.06 ml. The same column with a 3:7 ratio of solvents separated XII, I, and II at 4.5 ml from the products of acid degradation, VI and IV, with respective retention volumes of 8.5 and 11 ml. The collection fraction of the tetrahydrocannabinol could then be evaporated under nitrogen at 60° and separated and purified on HPLC with the 5:95 solvent ratio.

The stock solution of X originally analyzed by GLC[15] (column length of 1.8 m, 3% OV-17 on 100–120-mesh Gas Chrom Q, 240°) showed the following composition by percent areas[16] under a specific peak to the total area (compound, retention time in minutes, and percent area): unknown, 2.25, and 0.39%; unknown, 2.96, and 0.13%; unknown, 3.12, and 0.14%; X with traces of hexahydrocannabinol (XI), 3.85, and 86.4%; I, 5.28, and 12.28%; and XII, 6.70, and 0.65%. Two milliliters of this stock solution was evaporated to dryness and reconstituted in 0.5 ml of the HPLC solvent, 5% tetrahydrofuran in *n*-hexane, which was used to purify X on two columns[14] in series. The retention volumes in this system were 13.3, 14.0, and 14.6 ml for X, II, and I, respectively. GLC analysis of the X fractions showed greater than 99.5% purity with less than 0.3% contamination by I and negligible other peaks.

Table I—Comparison of Retention Times of a Mixture of HPLC-Purified Cannabinoids and Products of Acid Degradation on Different Columns

Compound	OV-225 Retention Time, min	OV-225 Retention Time Relative to IV	OV-17 Retention Time, min	OV-17 Retention Time Relative to IV
II	4.93	0.38	4.65	0.58
I	5.53	0.41	5.19	0.64
XII	8.45	0.64	6.43	0.80
VI	10.62	0.81	7.35	0.91
IV	13.14	1.00	8.07	1.00

GLC Procedures—Cannabinoid separation on GLC has been largely performed on a 3% OV-17 column (2, 3), although 3% OV-225 on 100–120-mesh Gas Chrom Q also was used (1, 4). The efficiencies of separation of the two columns were compared for a mixture of the HPLC-purified products of the acid degradation of I by flame-ionization detection under the following conditions: column length, 1.8 m; column temperature, 235°; detector and injector temperature, 260°; hydrogen flow, 30 ml/min; air flow, 300 ml/min; and helium flow, 35 ml/min. The retention times for both columns are given in Table I and clearly show the superiority of OV-225 in peak separation of these particular compounds. However, OV-17 columns do have the greater thermal stability, and their use is favored for repetitive studies at 235°.

Determination when Products of Acid Degradation Had Same Retention Time as I on GLC—An aqueous solution of I (1 mg/liter of 0.1 *N* HCl) was purged with nitrogen, reacted at 60° for 1 hr, and adjusted to pH 6 with 0.5 *M* phosphate buffer. Three 500-ml chloroform extracts were combined, evaporated to dryness at 50° under nitrogen, and reconstituted in 1 ml of chloroform. Half (500 μl) was streaked on TLC sheets and developed with cyclohexane–acetone (10:1) for 15 cm. The dried sheet was cut into 15 × 1-cm strips, and each was eluted with absolute ethanol. The dried eluates from each zone were reconstituted in chloroform and gas chromatographed (OV-17). Aliquots of the other half of the reconstituted chloroform extracts were taken to dryness and treated as discussed under HPLC methods. All fractions, including those that gave no UV signals, were gas chromatographed.

The TLC and HPLC fractions of the acid degradations corresponding to those from pure I were silylated (5) with bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane in pyridine[17] and gas chromatographed (3% OV-17).

Similar studies were conducted with the other HPLC- and TLC-separated products. The known degradation products, their retention times at 235°, and the retention times of their silylated derivatives at 220° in minutes were, respectively: II, 4.21 and 3.56; I, 4.64 and 3.93; XII, 5.90 and 4.82; VI, 6.49 and 5.54; and IV, 7.3 and 5.9. The peak areas of the nonsilylated compounds and the relative peak area ratios of nonsilylated compound to the silylated derivative were, respectively: II, 72,330 and 0.77; I, 171,456 and 0.79; XII, 10,448 and 0.42; VI, 115,228 and 0.57; and IV, 102,833 and 0.59.

Investigation of Degradation Intermediates in Equilibrium with I in Acid Degradation—Similar studies were conducted with ^{14}C-I. The material was degraded at 60° in 0.1 *N* HCl. The reaction was inhibited at timed intervals by adding 5 ml of a 0.5 *M* Na_2HPO_4–NaH_2PO_4 (1:1) buffer to 20-ml aliquots, which were then extracted with chloroform. The extracts were dried under nitrogen at 60°, and the reconstituted residues in 100 μl of chloroform were thin layered on sheets (previously activated at 100° for 2 hr) with cyclohexane–acetone (9:1).

The sheets were dried and then scanned with a radiochromatogram scanner[18], and the area of each peak was recorded as a percentage of the total for the aliquots taken at different times. The total radioactivity in an aliquot of the extract was also counted[19] and was the same for each sample taken with time; this finding implies equal extraction efficiency for the degradation products as for the parent compound. No detectable activity was observed in the aqueous sample after chloroform extraction. The extraction efficiency of radiolabeled material with the chloroform was determined to be 98%. Three pronounced peaks were found on the TLC sheets at R_f 0.07, 0.40, and 0.67. Elution of the first peak (R_f 0.07) with ethanol gave counts that increased with time over 240 min and contained IV and VI by GLC. The second peak (R_f 0.40) gave counts that

[1] Lot SSC 79124, National Institute of Mental Health, Bethesda, MD 20014.
[2] Lot NMH-IV-65C.
[3] Lot SSC 61565, National Institute of Mental Health, Bethesda, MD 20014.
[4] Eastman Chromatogram Sheet 6060, Eastman Kodak Co., Rochester, NY 14650.
[5] Model 440 liquid chromatograph (absorbance UV detector at 254 nm), Waters Associates, Milford, MA 01757.
[6] Aldrich Chemical Co., Milwaukee, WI 53233.
[7] Batch 10751, Applied Science Laboratories, State College, PA 16801.
[8] Burdick and Jackson Laboratories, Muskegon, MI 49442.
[9] Reagent ACS, Matheson, Coleman and Bell, Norwood, OH 45212.
[10] Fisher Scientific Co., Fair Lawn, NJ 07410.
[11] Dilut-it, J. T. Baker Chemical Co., Phillipsburg, NJ 08865.
[12] U.S. Industrial Chemicals Co., New York, NY 10016.
[13] Bondapak C_{18} column.
[14] μPorasil.
[15] Varian Aerograph 2400, Walnut Creek, CA 94598.
[16] HP 3380 A integrator, Hewlett-Packard, Palo Alto, CA 94303.
[17] Pierce Chemical Co., Rockford, IL 61105.
[18] Model 7201 C, Packard Instrument Co., Downers Grove, IL 60515.
[19] Beckman liquid scintillation, Beckman Instruments, Fullerton, CA 92634.

decreased with time over 240 min and contained XII, I, and II. The R_f 0.67 peak was not detectable under UV light on the plate and gave no significant GLC peaks on elution. Its radioactive content was reasonably constant over the studied time interval. The original solution showed material at the same R_f value, so this R_f may be assigned to a nondegradable impurity.

Studies to Seek Other Degradation Products of I—One liter of 20% ethanolic 0.1 *N* HCl, preextracted with chloroform, was purged with nitrogen and preequilibrated at 61°; 15 mg of purified I in 1.5 ml of ethanol was added. After 30 min at 60°, the solution was extracted with 3 × 500 ml of ether, and the ether extract was washed with 3 × 250 ml of water to remove any acid. The extract was taken to dryness at room temperature under nitrogen and reconstituted in 0.5 ml of ethanol. Aliquots were injected into the gas chromatograph. Room temperature instead of 50° was preferred since less cannabinol was formed.

Studies to Determine If X Solutions Gave Rise to I—HPLC purified X (20 μg) (chromatographic purity greater than 99.5%) was reacted in 20 ml of 0.1 *N* HCl and in 0.05 *M* phosphate buffers at pH 5.72, 7.03, and 9.12 for 3.3 hr at 60°. The solution was then adjusted to pH 5.5. A 20-ml ether extract, washed with 20 ml of distilled water, was taken to dryness under nitrogen at room temperature and reconstituted in 0.5 ml of ethanol. Aliquots were analyzed by GLC.

Production of XII in Acid Degradation of I—Purified (HPLC or TLC) I (50 μg) was degraded in 50 ml of nitrogen-purged 0.1 *N* HCl, preequilibrated at 60.0°, and then maintained for 30 min. The reaction was halted by adjustment to pH 5.5 with phosphate buffer, and the mixture was extracted with 3 × 10 ml of chloroform. The internal standard (4-androsten-3,17-dione solution) was added to the extract, which was evaporated to dryness and reconstituted in 20–50 μl of chloroform. Then 1 μl was injected on the gas chromatograph equipped with the OV-17 column, and the areas were determined under all peaks.

The reactions were made under different conditions and with differently treated reactants including: (*a*) distilled water cleaned on HPLC[13], (*b*) distilled water preextracted with chloroform, (*c*) concentrated hydrochloric acid[10], (*d*) hydrochloric acid concentrate[11], (*e*) TLC purified I, (*f*) HPLC-purified I, (*g*) unsilylated glassware, (*h*) glassware coated with a water-soluble silicone concentration[20], and (*i*) glassware presilylated with a trimethylsilyl compound[21]. Blank solutions without material were studied in the same manner.

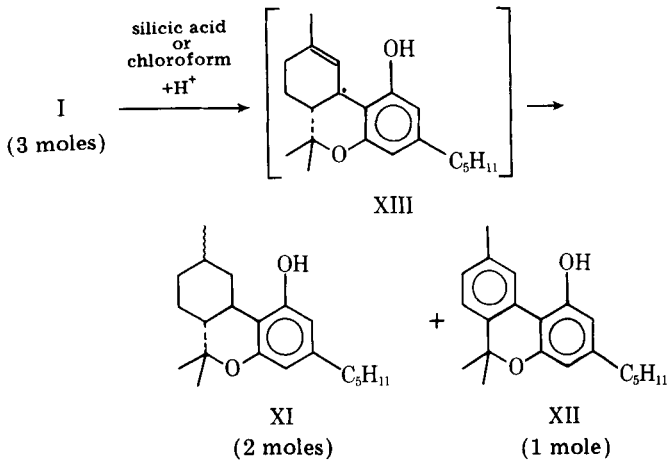
Kinetic Studies on Degradation of I—Aliquots of HPLC-purified I in aqueous hydrochloric acid (1.00 mg/liter) at pH 0.70 and 1.40, preequilibrated and reacted at 60°, were taken with time. Each 20-ml aliquot was neutralized with 5 ml of phosphate buffer and extracted with 3 × 10 ml of dichloromethane[8], which was evaporated to dryness under nitrogen at 50°. The internal standard, 200 μl of a tetraphenylethylene[6] solution (300 μg/ml), was added to the residue, which was reconstituted in 200 μl of chloroform at 0°. Then 1.0 μl was injected.

Studies in the neutral pH region at 5.28, 7.36, and 9.22 were performed by adding 1 mg/liter of HPLC-purified I to the appropriate nitrogen-purged, 60°-preequilibrated, 0.05 *M* phosphate buffer. Aliquots of 20 ml, taken with time, were extracted with 24 ml of *n*-heptane containing 1.5% isoamyl alcohol. The extraction efficiency was the same as chloroform. The organic layer was dried under nitrogen after the addition of the internal standard: 100 μl of a solution of 140 μg of 4-androsten-3,17-dione/ml of chloroform. This internal standard was preferred because there was a greater potential for XI formation and the retention times of the alternative internal standard, tetraphenylethylene (1), were too close to those of XI. The residue was reconstituted in 100 μl of absolute ethanol, and 1.0 μl was injected for GLC analysis (OV-17). At the higher pH values, adequate volumes of 1.0 *N* HCl were added to adjust the pH to 5.5 before extraction.

Kinetic Studies on Degradation of X at pH 1.12—A solution containing 100 ml of 1.0 *N* HCl, 200 ml of absolute ethanol, and 700 ml of distilled water (apparent pH 1.12) was purged with nitrogen and then preequilibrated at 60°. HPLC-purified X (6.35 mg in 1 ml of absolute ethanol) was added. Aliquots (24 ml) were taken with time and delivered in a 50-ml conical centrifuge tube containing 250 μl of the internal standard solution (140 μg of steroid/ml of chloroform) and 6.0 ml of 0.5 *M* Na_2HPO_4–NaH_2PO_4 (1:1) buffer. The cannabinoids were then extracted with 2 × 10 ml of ether[8].

The pH after extraction was in the 5.71–5.74 range. The ether extracts were dried under nitrogen, and the residues were reconstituted in 100 μl of absolute ethanol. Aliquots (1.0 μl) were analyzed by GLC (OV-17) under the following conditions: column temperature, 240°; helium flow rate, 40 ml/min; air flow, 300 ml/min; hydrogen flow, 30 ml/min; injector, 290°; and detector, 300°.

[20] Siliclad, Clay-Adams, Parsippany, NJ 07054.
[21] Regisil, Regis Chemical Co., Morton Grove, IL 60053.



Scheme II

RESULTS AND DISCUSSION

Production of XII in Acid Degradation of I—The formation of XII in nitrogen-purged acidic solutions is difficult to explain in the absence of oxidizing agents. The probable route is a disproportionation of I to XI and XII. GLC analyses of the contents of aqueous solutions (0.1 *N* HCl) of I, reacted at 60° for 30 min under various conditions, not only demonstrated the peaks assignable to I, II, IV, and VI but also significant areas under the peaks assignable to XI (relative retention time with respect to the steroid of 0.36) and XII (relative retention time of 0.63). Both peaks

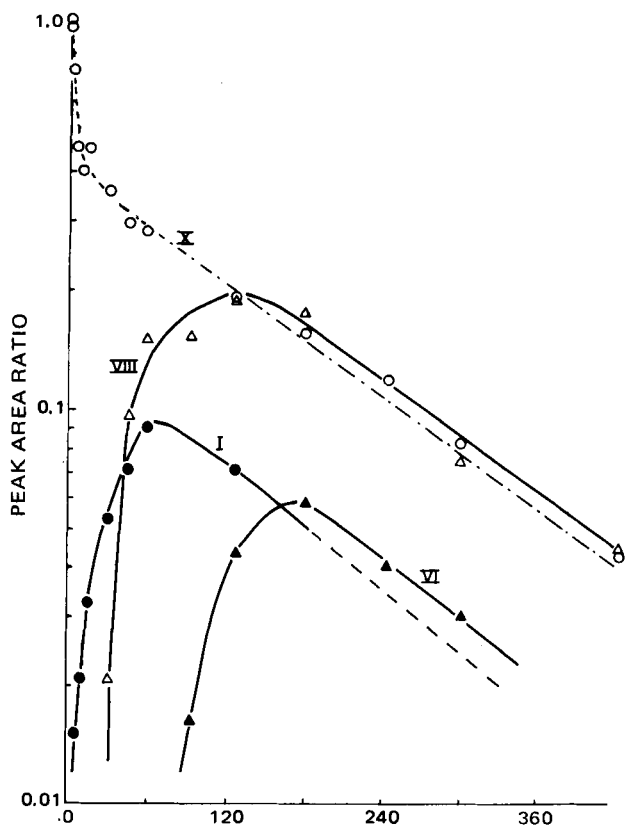


Figure 1—*Semilogarithmic plots against time of the ratios of the area of the GLC peaks for cannabinoids to the peak area of the internal standard 4-androsten-3,17-dione for the degradation of a 20% ethanolic solution (0.1 N HCl) of HPLC-purified X at 60°. The dashed curve for X was based on a nonlinear digital computer fitting.*

Table II—GLC[a] Characterization of Acid Degradation[b] of HPLC-Purified I in 20% Ethanol in 0.1 *N* HCl

Retention Time, min	Percent of Total Area[c]	Compound
4.00	0.66	X
4.90	1.49	II
5.44	81.53	I
6.66	9.62	XII
7.43	2.57	VI
8.34	1.55	IV
9.18	2.16	VIII

[a] Conditions: OV-17 column, 240°; injector, 290°; detector, 300°; helium, 35 ml/min; air, 300 ml/min; and hydrogen, 30 ml/min. [b] In 100 ml of 1.0 *N* HCl, 200 ml of ethanol, and 700 ml of water at 60° for 30 min. [c] The total is not 100% since there are some minor unidentified peaks.

were always present together. Both were absent when degradations occurred in HPLC-purified water with both sources of hydrochloric acid when the glassware was previously silylated. The use of TLC-purified, but not HPLC-purified, I showed both products. Also, when the water used was preextracted with chloroform for both untreated glassware or aqueous silicone-coated glassware, both of these products were present in high evidence.

Thus, it can be concluded that traces of chloroform in the reaction mixture and traces of silicic acid from TLC catalyze the formation of free radicals of I and lead to its disproportionation to XI and XII (Scheme II). Untreated and aqueous silicone-coated glassware also catalyze this reaction. It was shown previously that the latter greatly adsorbed I (3). The radical XIII can be stabilized by the benzene ring and the allylic activation of the C-10 benzylic hydrogen.

The peak for XI was not observed previously (1) since its small amount was obliterated by that of the internal standard (tetraphenylethylene) which, unfortunately, occurred at the same retention time [0.74 of that for I (1)] in the system used.

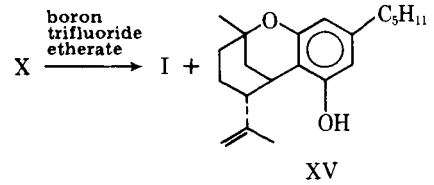
Determination If Products of Acid Degradation Had Same Retention Time as I—One possible explanation of the biphasic kinetics in acid solution, monitored by the loss of the peak assigned to I, on GLC was that an intermediate degradation product was formed with the same retention time that degraded more slowly. The maximum amount of this intermediate should occur at 60° in 0.1 *N* HCl at about 1 hr (Fig. 1). TLC analysis of the chloroform extracts showed that only the eluted material from TLC with the I R_f value gave a GLC peak at the retention time of I. The eluates of the zones with R_f values corresponding to the known degradation products gave only their respective GLC peaks. The eluates of other zones gave no significant peaks on GLC.

HPLC analysis of the chloroform extract showed no other peaks than those assigned to the known degradation products. The collected fractions, other than those encompassing the retention volume of I, did not show the GLC retention time of this compound. Silylation of fractions eluted from TLC and collected on HPLC from the chloroform extract gave silylated products that only showed one peak on GLC. Specifically, the HPLC collections and the TLC elutions at the retention volume and

Table III—GLC[a] Characterization of Degradation Products from Cannabidiol at Different pH Values[b]

Compound	Retention Time, min	Retention Time Relative to Steroid	Percent of Total Area[c] pH 0.3	pH 1.10	pH 5.72	pH 7.03	pH 9.12
X	3.19	0.36	72.33	81.71	94.71	99.30	97.11
XI	3.23	0.37	—	—	—	—	—
II	3.97	0.45	5.65	—	—	—	—
I	4.34	0.50	10.61	8.87	3.88	0.57	2.71
XII	5.48	0.63	—	1.48	1.19	—	—
VI	6.10	0.70	8.13	1.54	—	—	—
IV	6.95	0.80	0.25	—	—	—	—
Unknown (VII?)	7.27	0.83	0.30	—	—	—	—
VIII	7.68	0.88	2.02	5.16	—	—	—
4-Androsten-3,17-dione	8.73	1.00					

[a] Conditions: OV-17 column, 240°; injector, 290°; detector, 295°; helium, 40 ml/min; hydrogen, 30 ml/min; and air, 300 ml/min. [b] In aqueous hydrochloric acid (pH 0.3 for 20 hr; pH 1.1 for 3 hr) or in 0.05 *M* phosphate buffer for 200 min. [c] The total is not 100% since there are some minor unidentified peaks.



XV

Scheme III

R_f values, respectively, of I gave silylated products that had only the one peak given by silylated pure compound.

Thus, it can be concluded that the hypothesized degradation product could exist only if it had the same retention time on GLC, had the same R_f value on TLC, had the same retention volume on HPLC, and had a silyl ether with the same GLC retention time of the trimethylsilylated I. This is highly improbable.

Existence of Degradation Intermediates in Equilibrium with I on Acid Degradation—The alternative hypothesis to explain the biphasic kinetics in acid media is that I undergoes an initial relatively rapid loss due to its equilibration with other compounds and that the subsequent slower loss of assayed aliquots at its GLC retention time is due to further irreversible transformations. Attempts were made with 1 mg of radiolabeled substance/liter to discover additional spots by radiochromatogram scanning of TLC plates other than those assignable to previously reported compounds (*e.g.*, I, II, IV, VI, and XII). None was observed.

Other Degradation Products of I—Since a possible reason was that the specific activity or the concentration was too low, additional studies of 30 min of degradation in 0.1 *N* HCl with 20% ethanol at 10 mg of I/liter were conducted. The entire reaction mixture was extracted, taken to dryness, and reconstituted in 0.5 ml of ethanol. It was then analyzed on GLC (3% OV-17), and the results are given in Table II. These retention times were the same as for purified authentic materials.

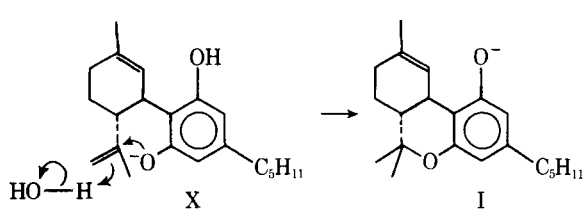
The identification of X [*m/e* (%) 314 (M^+) (21.3), 299 ($M - CH_3\cdot$) (5.6), 271 ($M - C_3H_7\cdot$) (5.4), 258 ($M - C_4H_8\cdot$) (4.6), 246 (19.7), 231 (100), and 193 (7.7)] at a retention time of 4.00 min and of 6,12-dihydro-6-hydroxycannabidiol (VIII) [*m/e* (%) 332 (M^+) (18.5), 314 ($M - H_2O$) (31.5), 299, (14.0), 273 ($M - 59$) (6.5), 271 (16.5), 258 (13.5), 231 (100), 193 (16.0), and 59 ($CH_3C^+(OH)CH_3$) (2.5)] as the trihydroxy derivative of I at 9.18 min was effected by GLC–mass spectrometry[22]. The presence of these two compounds had been predicted (1) as shown in Scheme I.

Thus, the postulated equilibria among I, X, VIII, and possibly isocannabidiol (IX) were confirmed.

Determination If X Solutions Gave Rise to I—These postulated equilibria were challenged by subjecting HPLC-purified X (with a I content less than 0.3%) to degradation and assaying the resultant products by GLC (Table III). The appearance of I and VIII and the appearance of the product VI of I degradation were confirmatory.

The acid-catalyzed cyclization of X to I was first reported by Adams *et al.* (6, 7) and, in the presence of boron trifluoride etherate (8), gave I in 60% yield and isotetrahydrocannabinol (XV) in 13% yield (Scheme III). Compound XV, at the GLC retention time of an authentic sample[23], was not found in the present acidic degradations. This result is consistent with the fact that boiling X with *p*-toluenesulfonic acid in benzene gave II in practically quantitative yield (9, 10). In *trans*-I, the olefinic C-10 hydrogen is very close to the free hydroxyl group (11) and the double bond migration would tend to relieve this nonbonded interaction.

The formation of I from X in the alkaline region can also be expected



X I

Scheme IV

[22] Varian 2700 gas chromatograph equipped with an OV-17 column (20 psi of helium); column temperature from 55 to 300° (10°/min); and du Pont 21-490F mass spectrometer, E. I. du Pont de Nemours and Co., Instrument Products Division, Monrovia, CA 91016, electron impact mode (70 ev).

[23] Gift of R. Mechoulam, Laboratory of Natural Products, School of Pharmacy, Hebrew University, Jerusalem, Israel.

Table IV—GLC[a] Characterization of Degradation Products from I at Different pH Values[b]

Compound	Retention Time, min	Retention Time Relative to Steroid	Percent of Total Area								
			pH 5.28			pH 7.36			pH 9.22		
			30 sec	6 hr	100 hr	30 sec	6 hr	192 hr	30 sec	6 hr	192 hr
XI	3.24	0.37	0.14	—	—	0.42	0.05	Traces	0.06	0.31	0.25
II	3.95	0.45	0.93	0.95	Traces	0.79	0.89	8.42	0.79	3.73	11.46
I	4.38	0.50	62.48	61.78	43.00	64.18	63.65	13.20	63.56	59.06	5.32
XII	5.52	0.63	Traces	Traces	4.23	3.44	2.36	3.55	1.66	1.86	1.38
VI	6.15	0.70	Traces	Traces	Traces	—	—	—	—	—	—
IV	7.02	0.80	0.56	0.55	—	0.79	0.50	0.02	0.61	0.65	1.15
4-Androsten-3,17-dione	8.77	1.00	35.65	36.62	52.76	30.36	32.53	74.80	33.26	34.36	80.42
Total area of cannabinoids/area of steroid			1.80	1.73	0.89	2.29	2.07	0.34	2.00	1.91	0.24

[a] Conditions: OV-17 column, 240°; injector, 290°; detector, 300°; helium, 40 ml/min; hydrogen, 30 ml/min; and air, 300 ml/min. [b] In 0.05 *M* phosphate buffers for the reported reaction times at 60°.

(Scheme IV), but it would be anticipated that the ether linkage would be affected only by concentrated acids and high temperatures. Negligible products of ether solvolysis (*e.g.*, VI) were observed on neutral degradation of I (Ref. 1 and Table IV) or of X (Table III), which gives rise to I, to confirm this hypothesis.

Degradation of X at pH 1.12—A kinetic study of X degradation in 0.1 *N* HCl with 20% ethanol, using 4-androsten-3,17-dione as an internal standard, showed a biphasic decline on semilogarithmic plotting against time (Fig. 1). Concomitant with the initial decline was the appearance of I, undoubtedly due to acid-catalyzed cyclization. The almost simultaneous appearance of the trihydroxy compound (VIII) could be due to the reversible acid-catalyzed hydration of the exocyclic double bond of X or the acidic cleavage of the ether linkage of I in the equilibrium (Scheme I). The retarded appearance of VI indicates that acid-catalyzed hydration of the endocyclic double bond may preferentially be effected only when the intact ether of I exists. The final equilibria must favor VI, IV, and II, where dehydration of IV favors the Δ^8-configuration (11).

Degradation of I—The kinetics of I degradation in an acidic medium, using an OV-17 column with 4-androsten-3,17-dione as the internal standard and peak area ratios, confirmed the prior studies (1) using peak height ratios.

Previously (1), it had been argued that the peak height ratios of each degradation product would be proportional to the number of molecules observed if each compound had the same retention time as II. On this basis, the final molar ratios of products II, IV, and VI were shown to be invariant under the same acidic conditions for peak height ratios corrected to the same retention time and were independent of which product was studied.

The premise for the peak height correction was that the ratio of the peak heights for equal amounts of I or II at their normal retention time, R_{t_n}, to their peak heights at another retention time, R_{t_i} (obtained by chromatography at other temperatures), gave factors that could be used for correction of product peak heights at their respective R_{t_i} retention times. Multiplication of the peak height of a product by the pertinent factor should give a corrected peak height for that compound that would be proportional to its amount in the gas chromatographed mixture. This premise would be valid if the detector response for each compound were the same, even though a compound of longer retention time but of the same quantity would demonstrate a lower peak height ratio without correction since the peak areas would be the same.

This method to correct peak heights is theoretically correct and reasonably accurate in practice. The relation between peak width, w, and its retention time, R_t, is (12):

$$w = 4R_t/\sqrt{N} \qquad \text{(Eq. 1)}$$

where N is the number of theoretical plates of a column and w is the length of a baseline cut by the two tangents drawn on either side of the peak at the inflection points. The area of the peak is directly related to the amount of the related compound and can be estimated with reasonable accuracy by multiplying this width by half of the height, h, of the peak:

$$\text{area} = \alpha(\text{amount of drug}) = hw/2 = 2hR_t/\sqrt{N} \qquad \text{(Eq. 2)}$$

Thus, the height of the peak against its retention time for the same amount of drug (as measured at different column temperatures) should give the curved line observed previously (Fig. 11 in Ref. 1).

The validity of the method can be checked by plotting the ratio of the area to the peak height for the same substance against the retention time in minutes in accordance with a rearrangement of Eq. 2:

$$\text{area}/h = 2R_t/\sqrt{N} \qquad \text{(Eq. 3)}$$

The linearity of such plots (Fig. 2) with an intercept of zero and the same slope of $2/\sqrt{N}$ for each of the tetrahydrocannabinol degradation products chromatographed at different temperatures confirms this premise and the reliability of the yield estimates given previously (1).

The log k–pH profile at 60° (Fig. 3) shows the results of both previous and present kinetic studies. The first phase of the biphasic acid degradation, characterized by k_1, can now be assigned (Scheme I) in its greater part to the equilibration of I with VIII, X, and, possibly, IX with slower production of IV and II. It appears that subsequent dehydration of IV favors the II configuration because of the previously mentioned steric hindrance between the Δ^9-double bond and the phenolic hydroxyl. The second phase, characterized by k_2 and encountered only in acidic regions, can now be largely assigned to products of ether solvolysis: VIII–X. This reverses the assignments of the hydrolysis in the neutral region given previously (1), which was taken as related to the k_2 phase rather than the k_1 phase in the acidic region.

The degradations of I in the more neutral regions were studied for as long as 192 hr (Table IV). The GLC-observed products had terminal areas (relative to the internal standard) that, in total, did not account for more than 49% (pH 5.28 for 100 hr), 15% (pH 7.36 for 192 hr), and 12% (pH 9.22 for 192 hr) of the original area of I (Table IV).

These results were confirmed with ^{14}C-radiolabeled I. The chloroform extract of I, degraded under the same conditions for the same time, was

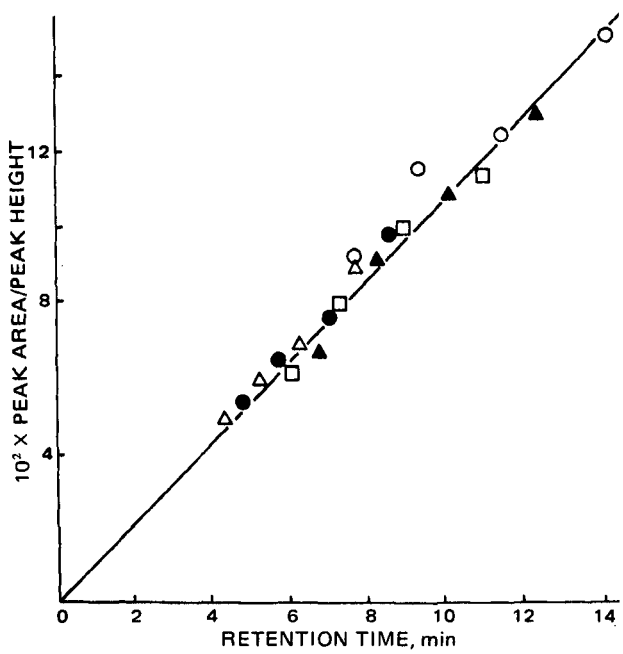


Figure 2—*Plots of the ratios of the peak area to the peak height for different cannabinoids against their respective retention times. The retention times were obtained by varying the column temperatures. Key:* ●, *I;* △, *II;* ○, *IV;* ▲, *VI; and* □, *XII.*

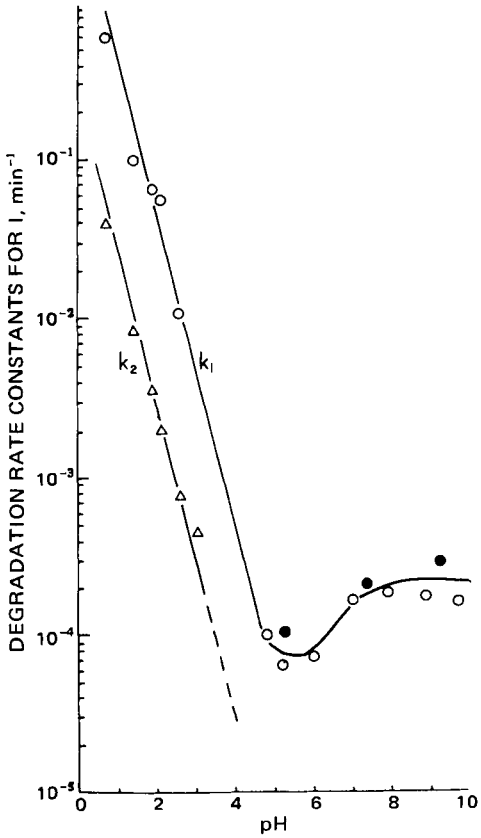


Figure 3—*Log* k–*pH profile for the degradation of I at 60.8°, where the concentration could be expressed as* $C = C_1e^{-k_1t} + C_2e^{-k_2t}$ *as a function of time, t, in minutes. The open symbols are previous data (1) and the closed symbols are additional data. The rate constants for monophasic degradation above pH 4 are taken as related to* k_1 *(see text) in contrast to the previous assignment (1).*

evaporated to dryness and reconstituted in 200 μl of chloroform. The solution was streaked on TLC sheets, developed for 15 cm in cyclohexane–acetone (9:1), and the sheets were analyzed by radiochromatogram scanner. No additional R_f values were observed other than those for GLC-identified products and a large amount of radioactivity at the origin. This result implies polar or polymeric degradation products that gave no pertinent signals under the GLC conditions, even after treatment of the methanolic extract of the origin with bis(trimethylsilyl)trifluoroacetamide containing 1% trimethylchlorosilane[21].

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Direct Spectrophotometric Determination of Thebaine in Arya II Population Capsules of *Papaver bracteatum* Lindl

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Abstract □ A simple spectrophotometric determination of thebaine based on the complexation reaction with bromcresol green was developed. The yellow complex was extracted with chloroform over the pH 1.5–4.5 range. The solution of the complex in chloroform showed the maximum absorption at 415 nm and obeyed Beer's law over the concentration range of 4.0–14.0 μg/ml. The molar absorptivity of the complex was 1.9460×10^4. The ratio of thebaine to bromcresol green in the complex was 1:1. The method was applied successfully to the direct determination of thebaine in the Arya II population capsules of *Papaver bracteatum* Lindl. The thebaine content of the dried capsules was 3.14%.

Keyphrases □ Thebaine—spectrophotometric analysis in Arya II population capsules of *Papaver bracteatum* □ *Papaver bracteatum*—Arya II population capsules, spectrophotometric analysis of thebaine □ Spectrophotometry—analysis, thebaine in Arya II population capsules of *Papaver bracteatum* □ Narcotics—thebaine, spectrophotometric analysis in Arya II population capsules of *Papaver bracteatum*

Most methods for the quantitative determination of thebaine (I) were based on chromatographic techniques (1–9). Other procedures include ion-exchange separation (10, 11), IR spectrophotometric determination (12, 13), and nonaqueous titration (14).

Several spectrophotometric methods have been reported (15–23). The UV spectrophotometric method (18) determines thebaine, when present alone, in completely pure solutions. Sulfuric acid methods (19–22) are specific but complicated and with low extinction values. A selective but