Constituents of *Cannabis sativa* L. IV: Stability of Cannabinoids in Stored Plant Material

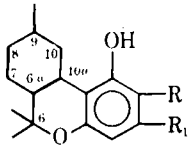
CARLTON E. TURNER▲, KATHY W. HADLEY, PATRICIA S. FETTERMAN, NORMAN J. DOORENBOS, MAYNARD W. QUIMBY, and COY WALLER

Abstract □ The (−)-Δ^9-*trans*-tetrahydrocannabinol content of *Cannabis sativa* L. stored at −18, 4, and 22 ± 1° decomposed at a rate of 3.83, 5.38, and 6.92%, respectively, per year, whereas the material stored at 37 and 50° showed considerable decomposition. *C. sativa* L. stored in the absence of direct light at −18, 4, and 22 ± 1° was more stable than cannabis stored under nitrogen. These data indicate that for normal research use, storage under nitrogen at 0° is not mandatory. Cannabinol is not the only decomposition product of (−)-Δ^9-*trans*-tetrahydrocannabinol. Tentative evidence supports the possible formation of hexahydrocannabinol as a decomposition product in stored *C. sativa* L.

Keyphrases □ *Cannabis sativa* L.—stability of cannabinoid constituents in stored plant material □ Cannabinoids—stability in stored *Cannabis sativa* plant material □ GLC—analysis, cannabinoid content in stored *Cannabis sativa* plant material

Eckler and Miller (1), using the dog ataxia assay, reported that certain preparations[1] of *Cannabis sativa* retained their biological activity after 5 years, whereas dry storage for the same period resulted in loss of biological activity. Moreover, it was reported (2) that a 43-year-old sample of cannabis fluidextract showed the presence of cannabidiol (II), (−)-Δ^9-*trans*-tetrahydrocannabinol (V), and cannabinol (VII) when analyzed by TLC and GLC. These two reports on fluidextracts are important but insufficient, since most cannabis currently used for research is stored dry under a myriad of conditions. Therefore, the limited data available on the effects of dry storage on a well-defined sample of *C. sativa* L. plant material prompted an investigation of II, V, and VII in stored plant material. GLC was employed as the principal analytical tool.

To duplicate as nearly as possible those conditions normally used by researchers for storage of cannabis and to determine the percent decomposition of V to VII or other products, the Mexican[2] variant normally supplied to researchers by the National Institute of Mental Health (NIMH) was selected. The study began in 1970 and terminated in 1972 after 104 weeks.



Number	Cannabinoid	Other	R	R_1
I	(−)-Δ^9-*trans*-tetrahydrocannabivarin	$\Delta^{9,10}$	H	C_3H_7
IV	(−)-Δ^8-*trans*-tetrahydrocannabinol	$\Delta^{8,9}$	H	C_5H_{11}
V	(−)-Δ^9-*trans*-tetrahydrocannabinol	$\Delta^{9,10}$	H	C_5H_{11}
VII	cannabinol	$\Delta^{8,9}$; $\Delta^{10,10a}$ $\Delta^{6a,7}$	H	C_5H_{11}
XI	(−)-Δ^9-*trans*-tetrahydrocannabinolic acid	$\Delta^{9,10}$	COOH	C_5H_{11}
XIII	hexahydrocannabinol		H	C_5H_{11}

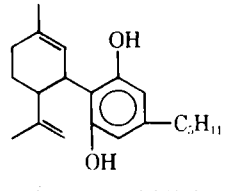
[1] Fluidextracts and granulated drug moistened with alcohol.

METHODS

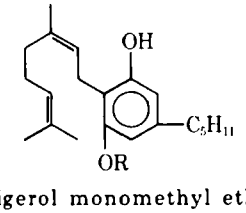
Mexican *C. sativa* L. grown from seed stock ME-A[3] was utilized. The plant material was grown in the Mississippi Delta in 1969 and was harvested at flowering[4]. Female plants were dried for 2 days at ambient temperature and humidity and subsequently manicured[5]; the plant material then was weighed and stored in 1-g. amber, large-mouth bottles. Each bottle was sealed with an aluminum cap containing an inserted paper lining, but the seal was not absolutely airtight. No sample was stored under nitrogen as proposed by Liskow (3). Five storage conditions were employed: (*a*) freezer at −18°; (*b*) refrigerator at 4°; (*c*) ambient temperature 22 ± 1°, with limited exposure to light; (*d*) oven at 37°; and (*e*) oven at 50°. Analyses were performed at 10-week intervals. Major cannabinoids were graphed as percent cannabinoids *versus* their neutral phenol and trimethylsilyl ethers or trimethylsilyl ester-ether derivatives.

GLC Analysis—The extraction procedure is basically that described by Lerner (4) and modified by Fetterman *et al.* (5) and Turner and Hadley (6). Two 1-g. samples were extracted simultaneously with 40 ml. of spectrograde chloroform. The resulting solutions were refrigerated at 4° and shaken for approximately 30 sec. at 10–12-min. intervals for 1 hr.

The plant material was removed by filtration, and the mother liquor was concentrated *in vacuo* at ambient temperature to a greenish paste void of solvent. This concentrate was dissolved in approximately 1 ml. of 95% ethanol. The ethanolic solution was subjected to continuous vibration from an ultrasonic vibrator until



II: cannabidiol



III: cannabigerol monomethyl ether, R = CH_3
VI: cannabigerol, R = H

[2] Seed Code ME-A.
[3] Origin: Acapulco, Guerrero, Mexico, 1968. Obtained through NIMH.
[4] Herbarium specimens of Mexican *C. sativa* L. are stored in the Herbarium, Department of Pharmacognosy, School of Pharmacy, University of Mississippi, University, MS 38677
[5] Manicured material is devoid of fruits and large stems. This was accomplished by passing the material through a 14-mesh sieve.

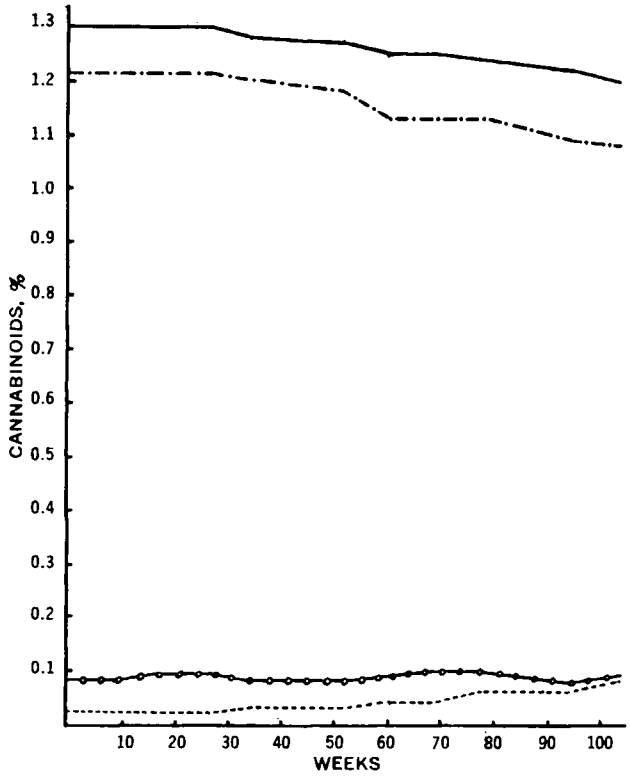


Figure 1—C. sativa *L. plant material stored at −18°. Key:* ——, (−)-Δ^9-trans-*tetrahydrocannabinol;* -·-, (−)-Δ^9-trans-*tetrahydrocannabinolic acid;* -O-, *cannabidiol; and* - - -, *cannabinol.*

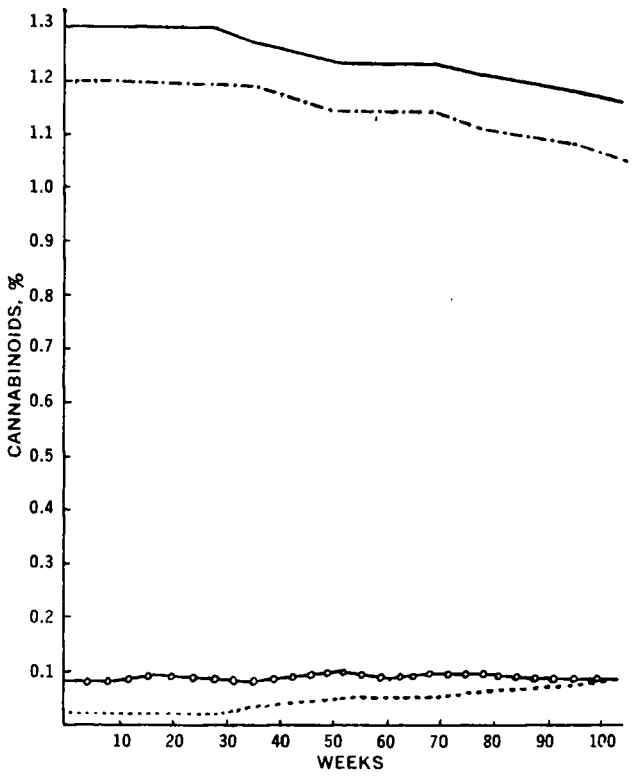
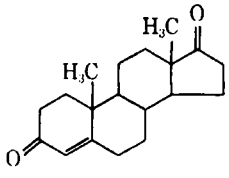


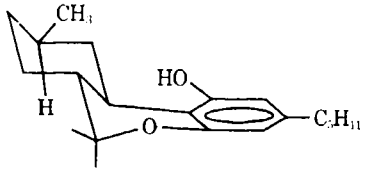
Figure 2—C. sativa *L. plant material stored at 4°. Key:* ——, (−)-Δ^9-trans-*tetrahydrocannabinol;* -·-, (−)-Δ^9-trans-*tetrahydrocannabinolic acid;* -O-, *cannabidiol; and* - - -, *cannabinol.*

all resin was in solution. At this point, an additional 0.5 ml. of an ethanolic solution containing a known concentration of 4-androstene-3,17-dione (VIII) as the internal standard was added. The concentration of VIII varies depending upon the plant sample. Usually from 0.05 to 0.5 μl. of this solution is injected into the chromatograph.

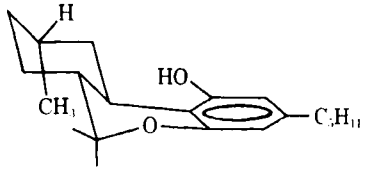
Analyses were performed using gas chromatographs[6] equipped with hydrogen flame-ionization detectors and operated isothermally at 210°. The inlet temperature was 240°, and the detector temperature was 260°. Glass and stainless steel columns [0.63 cm. (0.25 in.) o.d.; 2 mm. i.d. × 2.43 m. (8 ft.)] were packed[7] with 2% OV-17[8] on 100–120-mesh Chromosorb WHP or Gas Chrom Q. Nitrogen was used as the carrier gas at a flow rate of 10–30 ml./min., depending on the instrument used. Peak area measurements were made using the method of peak height times width at half height. Peak area of each cannabinoid was compared with the peak area of the internal standard and, using the appropriate relative response factors, the cannabinoid concentration was determined. Synthetic cannabinoids used in determining the relative response factors were obtained through NIMH.



VIII: 4-androstene-3,17-dione



XIV: C-9 equatorial hexahydrocannabinol



XV: C-9 axial hexahydrocannabinol

Silylation of Plant Extract—A 1-g. sample was extracted with 40 ml. of spectrograde chloroform. The resulting solution was refrigerated at 4° and shaken for approximately 30 sec. at 10-min. intervals for 1 hr. Then the plant material was removed by filtration, and the mother liquor was concentrated *in vacuo* at ambient temperature to a greenish paste void of solvent.

Anhydrous pyridine, 0.5 ml., was added followed by continuous vibration from an ultrasonic vibrator until all resin was in solution. The internal standard in a 10:1 pyridine to steroid solution was then added. The amount of internal standard varies with different plant samples. At this point, 0.5 ml. of *N,O*-bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane was added[9]. The resulting reaction mixture was then heated, using a heating mantle, for 10–12 min. at 80°, and 0.1 μl. was injected into the gas chromatograph.

RESULTS AND DISCUSSION

Lerner (4) reported that V converted to VII at the rate of approximately 5%/month. However, as Lerner pointed out, this was only an indication since no quantitative analysis was available of the original sample. Schou and Nielsen (7) reported that one sample of cannabis was examined twice with an interval of 4 years. The content of V had dropped to less than one-fourth of the original,

[6] Beckman GC-5, GC-45, and GC 72-5.

[7] By Beckman Instruments.

[8] High purity polar phenyl methyl silicone, mol. wt. approximately 30,000.

[9] BSTFA with 1% TMCS, Pierce Chemical.

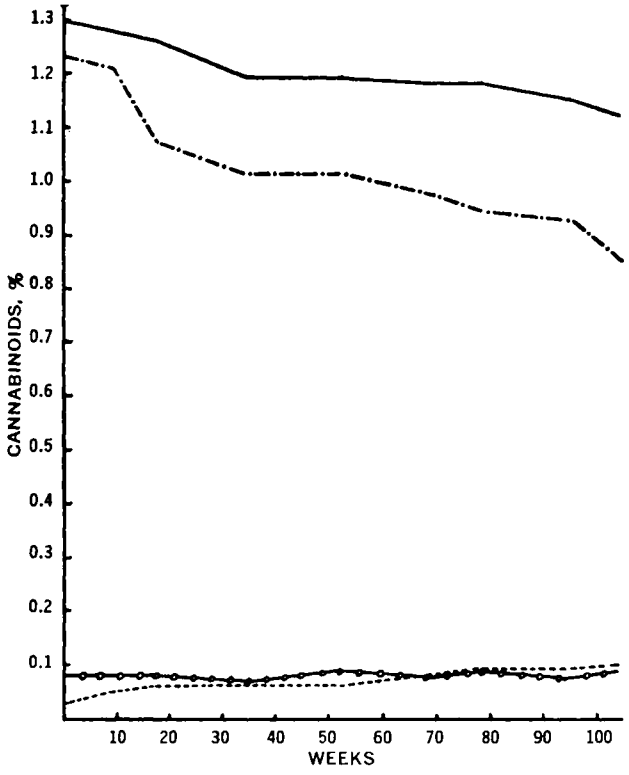


Figure 3—C. sativa *L. plant material stored at ambient temperature 22 ± 1°. Key:* ——, (−)-Δ^9-trans-*tetrahydrocannabinol;* --, (−)-Δ^9-trans-*tetrahydrocannabinolic acid;* -O-, *cannabidiol; and* - - -, *cannabinol.*

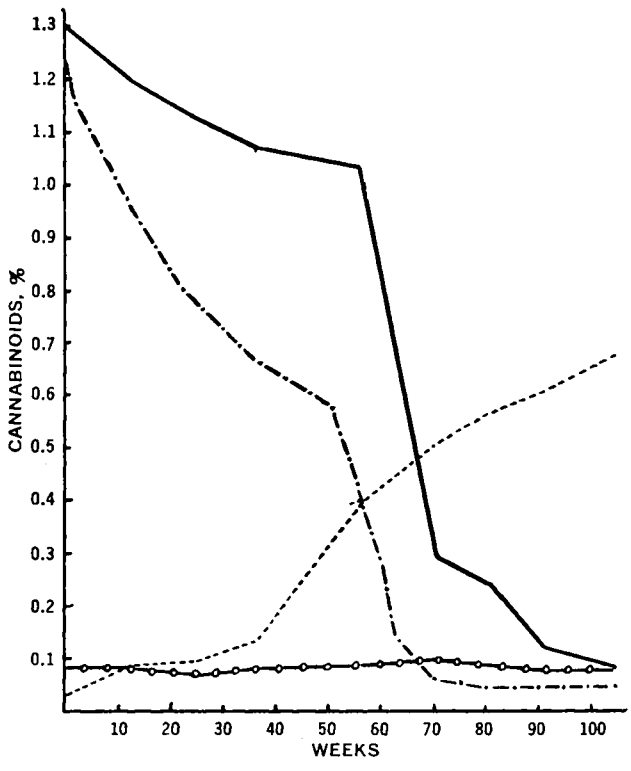


Figure 4—C. sativa *L. plant material stored at 37°. Key:* ——, (−)-Δ^9-trans-*tetrahydrocannabinol;* --, (−)-Δ^9-trans-*tetrahydrocannabinolic acid;* -O-, *cannabidiol; and* - - -, *cannabinol.*

but the contents of II and VII were relatively unchanged. This sample was stored under nitrogen at room temperature. This study indicated that V is not necessarily oxidized to VII under storage as previously proposed by Levine (8). Turk *et al.* (9) reported that a sample of 100% synthetic V decomposed to VII in the ratio of 97.1 : 2.9%, indicating that V is converted only to VII. Additionally, Razdan *et al.* (10) reported that synthetic V oxidized to VII at the rate of 10%/month at 25°. Thus, these reports seem to indicate that V in stored plant material probably decomposes by a different route than does stored synthetic V.

The Mexican variant chosen for this study contained 1.30% V, 0.02% VII, and 0.08% II. The percent of (−)-Δ^9-*trans*-tetrahydrocannabinolic acid (XI) as its trimethylsilyl ester–ether derivative (11) was 95%.

At the end of this study (104 weeks), the samples stored at 4 and −18° contained 1.16 and 1.20% of V, respectively; 0.08 and 0.07% of VII, respectively; 0.08 and 0.08% of II, respectively; and 91 and 90% of XI, respectively (Figs. 1 and 2). Although II was 0.08% at the start and 0.09% after 104 weeks in the material stored at −18°, this change is within experimental error for concentrations of such small percentages.

Figure 3 is a graph of plant material stored at 22 ± 1°. Percentages of the cannabinoids in the beginning were the same as those reported for 4 and −18°. However, after the end of 2 years, V was 1.12% and XI was 76%. The content of VII and II was 0.10 and 0.09%, respectively. These data do not correlate with data obtained previously (4, 7). However, it is obvious that conditions greatly determine the stability of cannabinoids in stored plant material. Under the conditions set forth in this experiment, the decomposition of V was 6.92%/year when stored at 22 ± 1°; material stored at −18 and 4° decomposed at 3.83 and 5.38%, respectively.

Plant material stored at 36° showed a steady decline in V and XI through Week 60 (Fig. 4). Between Weeks 60 and 70, there was a marked decrease in V from 1.03 to 0.29%. The increase of VII was from 0.40 to 0.50%. From Week 70, there was a steady decline in V and a steady rise in VII. At the termination date, the composition of V and VII was 0.08 and 0.67%, respectively. Thus, there was a material balance deficit of 0.57% between these two cannabinoids (1.30 + 0.02 = 1.32% at the start and 0.08 + 0.67 = 0.75% at termination).

The material deficit and decomposition rate between V and VII were more pronounced when the material was stored at 50°. Pars

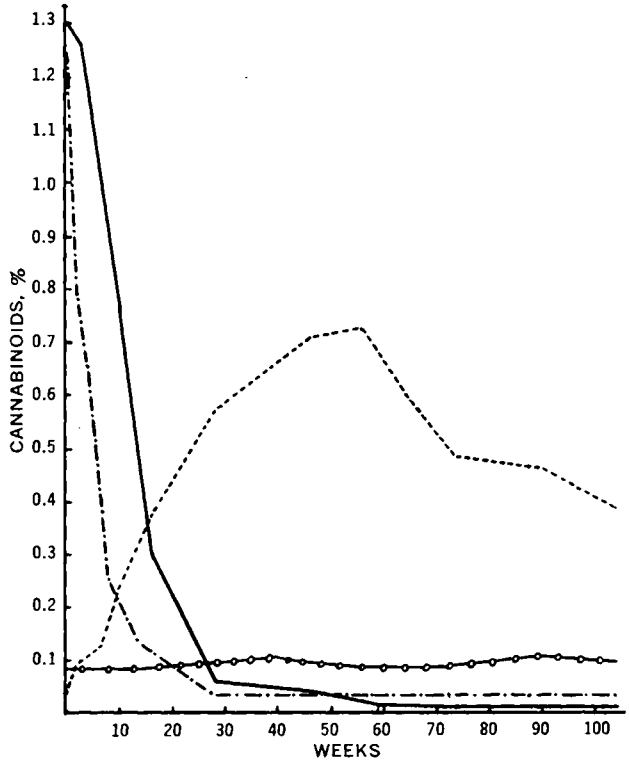


Figure 5—C. sativa *L. plant material stored at 50°. Key:* ——, (−)-Δ^9-trans-*tetrahydrocannabinol;* --, (−)-Δ^9-trans-*tetrahydrocannabinolic acid;* -O-, *cannabidiol; and* - - -, *cannabinol.*

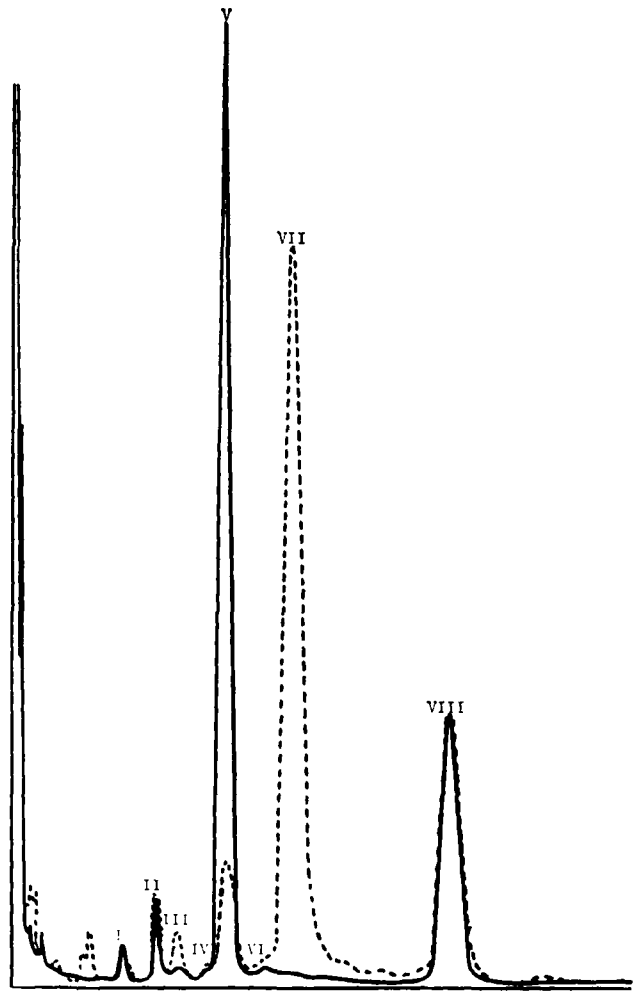


Figure 6—*Overlay of original plant sample* (——) *and plant sample stored at 37° for 2 years* (- - -). *Key: I,* (−)-Δ^9-trans-*tetrahydrocannabivarin; II, cannabidiol; III, cannabigerol monomethyl ether; IV,* (−)-Δ^8-trans-*tetrahydrocannabinol; V,* (−)-Δ^9-trans-*tetrahydrocannabinol; VI, cannabigerol; VII, cannabinol; and VIII, 4-androstene-3,17-dione.*

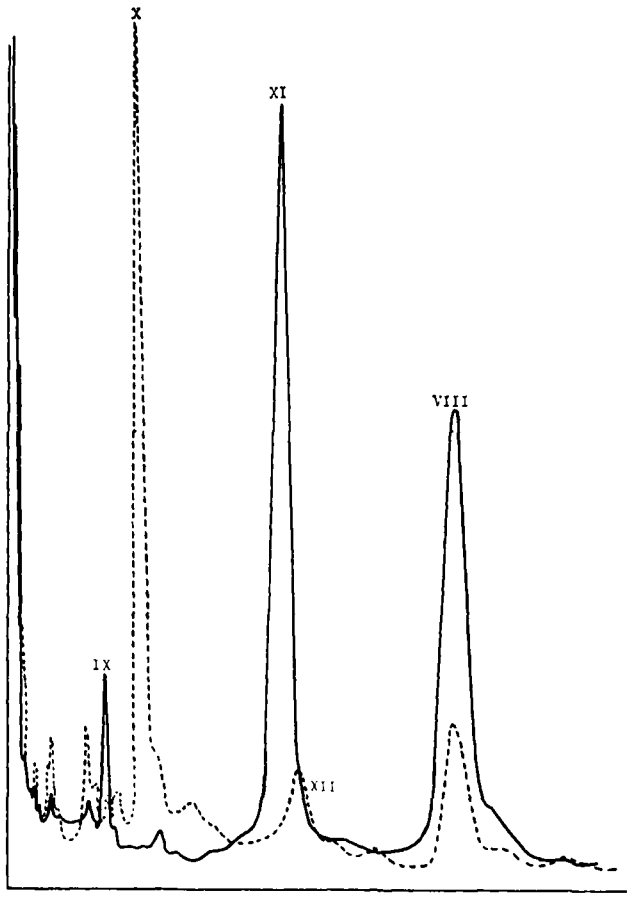


Figure 7—*Overlay of original plant material silylated* (——) *and silylated plant material stored at 37° for 2 years* (- - -). *Key: IX,* (−)-Δ^9-trans-*tetrahydrocannabinol trimethylsilyl ether; X, cannabinol trimethylsilyl ether; XI,* (−)-Δ^9-trans-*tetrahydrocannabinolic acid trimethyl ester-ether; XII, cannabinolic acid trimethylsilyl ester-ether; and VIII, 4-androstene-3,17-dione.*

and Razdan (12) observed that synthetic V heated at 80° for 7 days disappeared with concomitant formation of VII, whereas IV showed no change in composition. Data in Fig. 5 indicate that V in stored plant material under thermal conditions did not decompose entirely to VII as was observed with synthetic V. Moreover, the concentration of VII decreased after 50 weeks. The deterioration of V in plant material stored at 37 and 50° could possibly be initiated by an autocatalytic state.

Since V possesses a benzylic proton which is also allylic, the formation of a radical stabilized by resonance would be highly possible under thermal conditions; however, a radical formation would not be as stabilized by delocalization in IV. Thus, the concentration of IV would be expected to remain fairly constant even under thermal conditions, but the concentration of V would decrease precipitously (Fig. 6).

If, indeed, a radical is formed at carbon-10*a* in V, a disproportionation reaction would be possible. It is known that certain heterocyclic systems readily undergo disproportionation, with three molecules giving one hexahydro and two aromatic levels (13). The relative retention time for the aromatic cannabinoid level (VII) is well documented, but no relative retention time is available for hexahydrocannabinol (XIII) using VIII as the internal standard. Thus, XIII was prepared by catalytic hydrogenation of V as described by Archer *et al.* (14). GLC analysis of the reaction mixture produced one peak having a relative retention time of 0.37. This is close to cannabigerol monomethyl ether (III), which has a relative retention time of 0.38. Although the concentration of III increased slightly on storage (Fig. 6), it was impossible to determine if this increase was due exclusively to the formation of hexahydrocannabinol since the amount of plant material allocated for this study was limited. This will be the subject of a future article.

Since, to the authors' knowledge, no GLC data are available in the literature on the trimethylsilyl ether of XIII, this derivative was produced. Silylation of XIII followed by GLC analysis yielded relative retention times of 0.17 and 0.22, respectively. Two peaks were expected since there existed the possibility of a mixture of C-9 equatorial (XIV) and axial (XV) methyl isomers (Fig. 7). (See Fig. 7 for silylated plant material.)

Archer *et al.* (14) reported an axial-to-equatorial ratio of 3:1 as determined by PMR analysis. In these laboratories, the ratio was 3:2 as determined by GLC analysis of the trimethylsilyl ethers. Therefore, since the C-9 methyl axial isomer was the major product, it was assigned the relative retention time of 0.17; the equatorial isomer was assigned the relative retention time of 0.22.

Decomposition of V in *C. sativa* L. produces many products not yet identified; some are probably polymerization products. Moreover, biological data obtained on *C. sativa* L. will remain contradictory due to variations in chemical constituents. Although the cannabinol content of cannabis does, indeed, increase with storage, the suggestion that V plus VII would approximate the V content, irrespective of degradative changes (5), must be revised.

The cannabidiol content of all stored material remained relatively constant with no detectable decomposition. Additionally, a sample of *C. sativa* L. plant material of 1930 vintage[10] was analyzed in 1970 and 1972. The content of II (0.28%) was constant over this period, whereas V and VII changed from 0.05 and 0.65 to 0.04 and 0.66, respectively.

[10] From a pharmacy in Wesson, Miss.

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▲ To whom inquiries should be directed.

Kinetics and Mechanisms of Monolayer Interactions II: Effect of Chain Length of Alkyl Ionic Surfactants on Their Interaction with Dipalmitoyl Glycerol, Dipalmitoyl Phosphatidylethanolamine, and Dipalmitoyl Lecithin

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Abstract □ The energies of activation of the interaction of alkyl trimethylammonium surfactants with dipalmitoyl glycerol, dipalmitoyl phosphatidylethanolamine, and dipalmitoyl lecithin monolayers spread at the air–water interface were estimated from the increase of the surface pressure with the concentration of the injected surfactant. The energies of activation are a linear function of the chain length of the injected surfactant for the six-, eight-, 12-, and 16-carbon chains studied. The ionic groups of the polar hydrophilic moiety of dipalmitoyl lecithin were not equivalent in the perturbation that an attached surfactant ion produces in the surface pressure of the monolayer. The energies of activation per methylene group of the hydrocarbon chain and per polar ionic head of the injected surfactant were calculated and were compared with those that correspond to the energies of adsorption of these groups at hydrocarbon–water and air–water interfaces.

Keyphrases □ Alkyl sulfates and alkyl trimethylammonium ions—interactions with dipalmitoyl glycerol, dipalmitoyl phosphatidylethanolamine, and dipalmitoyl lecithin monolayers, kinetics, mechanisms □ Monolayers, dipalmitoyl glycerol, dipalmitoyl phosphatidylethanolamine, and dipalmitoyl glycerol—interactions with alkyl sulfate and alkyl trimethylammonium ions, kinetics, mechanisms □ Phospholipid monolayers—interactions with long-chain surfactants, kinetics, mechanisms □ Surfactants, long chain—interaction with phospholipid monolayers, kinetics, mechanisms □ Chain length effect—interaction of alkyl surfactants with phospholipid monolayers

The energies of interaction of cetyl sulfate and cetrimonium ions with dipalmitoyl glycerol and dipalmitoyl lecithin monolayers spread at the air–water interface were estimated recently (1) from the variation of the equilibrium surface pressure with varying concentrations of subphase-injected surfactants on the premise that the entropy factor calculated on the basis of collision theory was constant for all species.

The energies of adsorption at the oil–water and air–water interfaces of homologous series of alkyl surfactants are provided in the literature (2–11). These data permit the estimation of the contributions of the hydrocarbon moiety per methylene group and that of the polar head to the total energy of adsorption at a "clean" interface. A clean interface is defined here as a liquid–liquid or air–liquid interface without any spread monolayer.

These studies were designed to determine the dependence of the maximum obtainable changes in surface pressure and energies of interaction of monolayers of dipalmitoyl glycerol, dipalmitoyl phosphatidylethanolamine, and dipalmitoyl lecithin with subphase-injected surfactants on the numbers of methylene groups and on the nature of the charged polar head in the injected surfactant.

EXPERIMENTAL

Reagents—Dipalmitoyl glycerol[1], dipalmitoyl lecithin[1], cetyl sodium sulfate[1], and cetrimonium bromide[2] were the same samples

[1] Schwarz/Mann Research Laboratories, Orangeburg, N. Y.
[2] Eastman Kodak, Rochester, N. Y.