aromatic); mass spectroscopy: fragments at *m/e* 385, 265, 238, 219, 181 (base peak), and 135.

*Anal.*—Calc. for $C_{22}H_{24}O_7$: C, 66.00; H, 6.00; mol. wt. 400. Found: C, 66.85; H, 6.25; $CH_3O$, 22.02; *m/e* 400.

*Lignan II*—This was identified as (−)-*trans*-2-(3″,4″-dimethoxybenzyl)-3-(3′,4′-methylenedioxybenzyl)butyrolactone; $[\alpha]_{546\ nm.}^{23}$ −45.26° ($CHCl_3$, c 3.8%); $\lambda_{max}^{CHCl_3}$: 286 and 246 nm. (log ε 3.78 and 3.76); $\nu_{max}^{CHCl_3}$: 1770 (γ-lactone), 1600, 1582, 1450 (aromatic), 3010, 1460, 1340, 1250, 1140, and 1020 ($CH_3O$ and $CH_2O$) cm.$^{-1}$; NMR: 6.18 (6H, $CH_3O$), 4.12 (2H, methylenedioxy), 7.5 (4H, benzylic), 5.8–6.0 (2H, $CH_2$ adjacent to the lactone oxygen), a doublet at 7.06–7.12 (*trans*-2H, C-2 and C-3), and 3.2–3.6 (6H, aromatic); mass spectroscopy: fragments at *m/e* 235, 219, 208, 151 (base peak), 135, 123, 95, and 77.

*Anal.*—Calc. for $C_{21}H_{22}O_6$: C, 68.10, H, 5.94; mol. wt. 370. Found: C, 68.15; H, 6.07; $CH_3O$, 16.36; *m/e* 370.

**Deuterium Exchange of Compound II**—A small piece of sodium, about the size of a pinhead, was placed cautiously into 1 ml. of $D_2O$; 70 mg. of Compound II was dissolved in a minimal amount of tetrahydrofuran and this was added to the sodium–$D_2O$ reaction mixture. Additional tetrahydrofuran was added until the reaction mixture was homogeneous. The mixture was allowed to stand overnight at room temperature; excess $D_2O$ was added to the reaction mixture, followed by extraction with chloroform. Drying of the chloroform extract over anhydrous magnesium sulfate and evaporation yielded 50 mg. of II deuterated alpha to the carbonyl carbon.

**Preparation of (±)-*trans*-2-(3″,4″,5″-Trimethoxybenzyl)-3-(3′,4′-dimethoxybenzyl)butyrolactone from I**—Compound I (100 mg.) was placed in a Pyrex tube, sealed at one end (8 mm. in diameter), along with 0.8 ml. of methanol and 200 mg. of potassium hydroxide. The tube was sealed and placed in an oil bath maintained at 175° for 7 hr. Upon completion of heating, the tube was allowed to cool and was opened. After adding excess water, the reaction mixture was extracted with three 5-ml. portions of chloroform, and the chloroform extract was discarded. The aqueous layer was acidified with 5% aqueous hydrochloric acid and again extracted repeatedly with 5-ml. portions of chloroform. The combined chloroform extracts were dried over anhydrous magnesium sulfate, and the solvent was removed under vacuum (4). The residue was taken up in methanol and methylated with diazomethane prepared from *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (1 g.) and 40% aqueous potassium hydroxide (5 ml.) covered with ether (19.7 ml.)[4]. The product of this reaction was applied to TLC along with the authentic sample; the portion of the reaction product that corresponded to the authentic sample was purified by preparative silica gel G (0.5 mm.) TLC, yielding 30 mg. of product. The solvent system used was dichloromethane–benzene–ethyl acetate (3:6:1).

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## ACKNOWLEDGMENTS AND ADDRESSES


Received March 1, 1972, from the *Division of Pharmaceutical Chemistry, College of Pharmacy, University of Arizona, Tucson, AZ 85721*

Accepted for publication July 5, 1972.

Supported by Contract PH 43-67-1484 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health.



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# Photochemical Studies of Marijuana (Cannabis) Constituents


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**Abstract** □ The marijuana (Cannabis) constituents, cannabidiol, (−)-$\Delta^9$-*trans*-tetrahydrocannabinol, and (−)-$\Delta^8$-*trans*-tetrahydrocannabinol were found to be photoreactive. The only interconversion of these cannabinoids detected by GLC, however, was the conversion of cannabidiol to (−)-$\Delta^9$-*trans*-tetrahydrocannabinol. From a photoreaction mixture obtained by the irradiation of cannabidiol, a sample of (−)-$\Delta^9$-*trans*-tetrahydrocannabinol was isolated and identified by GLC, optical rotation, NMR, and mass spectrometry. A yield of 16% was obtained. The activating energy for the conversion appears to be in the 235–285-nm. wavelength area.



**Keyphrases** □ Marijuana constituents—photochemical study □ Cannabis constituents—photochemical study □ Cannabidiol—photoreactions □ Tetrahydrocannabinols—photoreactions


Although a number of reports have dealt with the photoreactivity of various cannabinoid substances, the first definitive work in this area was done by Shani and Mechoulam (1, 2). Those authors showed clearly that cannabidiolic acid undergoes an intramolecular photooxidative cyclization when irradiated with UV light in the presence of oxygen. These authors also observed the photoreactivity of cannabidiol in the absence of oxygen (1). In the latter study, various transformation products, including (−)-$\Delta^9$-*trans*-tetrahydrocannabinol, were shown to form when solutions of cannabidiol in different solvents were exposed to UV radiation for rather

**Table I**—Typical Irradiation Experiment

| Irradiation Time, min. | Cannabinoid Irradiated[a] — $\Delta^8$-Isomer[b] Amount[d,e] Remaining, mg. | $\Delta^9$-Isomer[c] Amount[d,f] Remaining, mg. | Cannabidiol — Amount[d] Remaining, mg. | Amount $\Delta^9$-Isomer Present, mg. |
|---|---|---|---|---|
| 0 | 160.0 | 160.0 | 160.0 | 0.0 |
| 1 | 137.5 | 137.4 | 152.2 | 10.0 |
| 2 | 122.7 | 127.6 | 140.5 | 21.5 |
| 5 | 117.5 | 113.1 | 128.1 | 22.4 |
| 10 | 98.5 | 101.5 | 91.3 | 24.0 |
| 15 | 87.8 | 89.0 | 68.6 | 25.4 |
| 20 | 83.6 | 84.1 | 53.3 | 26.5 |
| 25 | 75.1 | 75.4 | 44.2 | 22.4 |
| 30 | 65.4 | 69.4 | 38.9 | 22.6 |
| 60 | 35.0 | 31.9 | 21.2 | 11.5 |
| 90 | 21.1 | 19.5 | 19.7 | 9.2 |
| 120 | 12.9 | 15.3 | 11.5 | 0.0 |

[a] Concentration of cannabinoid was 160 mg. in 320 ml. *n*-hexane. A Vycor filter sleeve was used at 29°. [b] (−)-$\Delta^8$-*trans*-Tetrahydrocannabinol. [c] (−)-$\Delta^9$-*trans*-Tetrahydrocannabinol. [d] Amount of cannabinoid remaining at time listed. [e] No (−)-$\Delta^9$-*trans*-tetrahydrocannabinol, cannabidiol, or cannabinol was formed. [f] No (−)-$\Delta^8$-*trans*-tetrahydrocannabinol, cannabidiol, or cannabinol was formed.

extended periods. For example, (−)-$\Delta^9$-*trans*-tetrahydrocannabinol could only be isolated from a cyclohexane solution of cannabidiol following a photoreaction time of 22 hr. Turk *et al.* (3) recently presented evidence that approximately 95% of (−)-$\Delta^9$-*trans*-tetrahydrocannabinol was converted into cannabinol following storage at room temperature and exposure to light for 5 months. Crombie *et al.* (4) showed that cannabichromene can be converted into cannabicyclol under irradiation with a 450-w. lamp for 4.5 hr.

Thus far, no work has been reported that deals specifically with the effect of UV radiation on the biologically active cannabinoids, namely, (−)-$\Delta^9$-*trans*-tetrahydrocannabinol and (−)-$\Delta^8$-*trans*-tetrahydrocannabinol. Consequently, it was considered important to study the photoreactivity of these two substances. Furthermore, preliminary experiments in these laboratories indicated that (−)-$\Delta^9$-*trans*-tetrahydrocannabinol was formed very rapidly from cannabidiol subsequent to UV irradiation. On this basis, it was of interest to reinvestigate the photoreactivity of cannabidiol using short-term exposures to UV radiation.

## EXPERIMENTAL AND RESULTS

**Reagents and Standard Solutions**—All reagents used were of analytical grade purity. Cannabidiol, cannabinol, (−)-$\Delta^8$-*trans*-tetrahydrocannabinol, and (−)-$\Delta^9$-*trans*-tetrahydrocannabinol were used[1]. Separate standard solutions of these substances were prepared in *n*-hexane to furnish a final concentration of 0.05 g./100 ml. in each case. These solutions were used to obtain the standard tracings for GLC analyses and were stored at 4° in the dark prior to use.

**Irradiation**—A solution of a particular cannabinoid was irradiated in a water-jacketed reaction vessel equipped with a high pressure quartz mercury vapor lamp[2]. Dry nitrogen was bubbled through the reaction mixture to exclude air and to stir the mixture. The temperature was maintained at 29° throughout the experiment.

At predetermined intervals, 100-μl. aliquots were withdrawn and analyzed by GLC.

[1] Obtained through the courtesy of Dr. John H. Scigliano, National Institute of Mental Health, Rockville, MD 20852

[2] Hanovia L679A, Ace Glass Inc., Vineland, NJ 08360

**Table II**—Column Chromatographic Separation of Irradiated Cannabidiol Reaction Mixture

| Fraction Number[b] | Cannabinoid Detected, mg.[a] — $\Delta^9$-Isomer[c] | Cannabidiol |
|---|---|---|
| 1–27 | 0.0 | 0.0 |
| 18–22 | 0.0 | 27.4 |
| 23 | 1.0 | 7.4 |
| 24 | 2.9 | 8.1 |
| 25 | 5.3 | 9.5 |
| 26 | 5.3 | 9.0 |
| 27 | 5.5 | 8.2 |
| 28 | 5.8 | 7.7 |
| 29 | 6.2 | 5.9 |
| 30 | 7.0 | 4.0 |
| 31 | 7.7 | 1.3 |
| 32–36 | 35.9 | 0.0 |
| 37–43 | 0.0 | 0.0 |

[a] As determined by GLC. [b] All fractions were 20 ml. [c] (−)-$\Delta^9$-*trans*-Tetrahydrocannabinol.

**GLC**—All determinations were carried out on a gas chromatograph[3] equipped with a hydrogen flame-ionization detector and a 0.32-cm. × 1.83-m. (0.125-in. × 6-ft.) stainless steel coiled column packed with 60–80-mesh Chromosorb W (solid phase) and coated with 10% silicon rubber (SE-30) (liquid phase). The injector and detector temperatures were maintained at 265°, while the column was operated isothermally at 240°. Helium was used as the carrier gas at a flow rate of 100 ml./min. Under these conditions, the order of emergence from the column was cannabidiol (14 min.), (−)-$\Delta^8$-*trans*-tetrahydrocannabinol (16 min.), (−)-$\Delta^9$-*trans*-tetrahydrocannabinol (17 min.), and cannabinol (19 min.). The peak areas recorded for the analyzed samples were calculated by the triangulation method (area = height × width at half-height) and compared with standard curves prepared by plotting the areas against known concentrations of standard cannabinoids analyzed in a similar manner. The conditions for the combined GLC–mass spectrometric analyses were similar, except that an all-glass coiled column [0.32 cm. × 1.83 m. (0.125 in. × 6 ft.)] packed with 80–100-mesh Gas Chrom Q (stationary phase) and coated with 3% OV-101 (liquid phase) was used. The injector and detector temperatures were kept at 260°, while the column was operated isothermally at 220°. The order of emergence from this column was cannabidiol (14 min.), (−)-$\Delta^8$-*trans*-tetrahydrocannabinol (10.5 min.), (−)-$\Delta^9$-*trans*-tetrahydrocannabinol (11.2 min.), and cannabinol (17.0 min.).

**Mass Spectrometric Analyses**—Mass spectra were determined with a mass spectrometer[4] using either a direct insertion probe (probe temperature 75–100°) or the column effluent from the attached gas chromatograph. All spectra were recorded at an ionizing beam energy of 70 ev., and the ion source was 270°.

**NMR Analyses**—NMR measurements were performed with a Varian A-60 instrument operating at 60 MHz. The spectra were recorded in deuteriochloroform using tetramethylsilane as an internal standard. Chemical shifts were relative to tetramethylsilane in parts per million on the delta scale.

**Optical Rotations**—Optical rotations were taken in absolute alcohol using a polarimeter[5].

**Preliminary Irradiation Experiments**—A solution of cannabidiol in *n*-hexane (0.480 g./960 ml.) was divided into three parts. One part was irradiated with a Vycor filter sleeve, one part with a Corex filter sleeve, and the third part with a Kimax filter sleeve[6], all at 29°. At predetermined intervals, aliquots of each reaction mixture were analyzed by GLC as previously described. The results with the Vycor filter sleeve and the Corex filter sleeve were identical; (−)-$\Delta^9$-*trans*-tetrahydrocannabinol was formed in each case. With the Kimax filter sleeve, no reaction occurred.

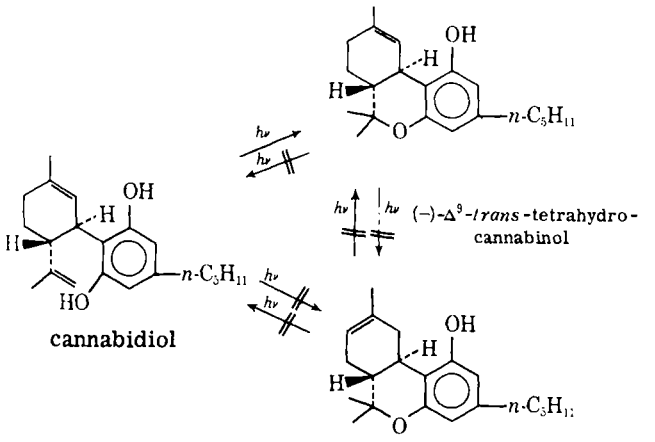
When separate solutions of (−)-$\Delta^8$-*trans*-tetrahydrocannabinol and (−)-$\Delta^9$-*trans*-tetrahydrocannabinol were irradiated in the manner described, each decomposed to products unknown when the Corex or Vycor filter sleeve was used. As was the case for cannabidiol,

[3] F&M model 810.

[4] LKB-9000.

[5] Rudolph model 80.

[6] Vycor allows the transmission of wavelengths down to 205 nm., Corex to 235 nm., and Kimax to 285 nm.




*Scheme I—Photochemical reactions of some cannabinoids*

however, no change in the starting material was observed when the Kimax filter sleeve was used.

The results of a typical experiment using a Vycor filter sleeve are shown in Table I.

**Preparation of (−)-Δ⁹-*trans*-Tetrahydrocannabinol from Cannabidiol Irradiation**– A solution of 0.500 g. of cannabidiol in 500 ml. of *n*-hexane was irradiated for 20 min. at 29° in a vessel equipped with a Vycor filter sleeve. The solvent was removed under reduced pressure. The oily residue was analyzed by mass spectrometry in two ways. Analysis by direct probe showed the presence of significant peaks at *m/e* 314, 299, 271, 258, 246, 243, and 193. These observed peaks are characteristic for certain cannabinoids, particularly (−)-Δ⁹-*trans*-tetrahydrocannabinol and cannabidiol (5). In addition to these peaks, other peaks in the high mass region of the spectrum were observed at *m/e* 505, 548, 626, and 710. Combined GLC–mass spectrometry showed only two GLC peaks. These GLC peaks were identified as cannabidiol and (−)-Δ⁹-*trans*-tetrahydrocannabinol on the basis of retention time (GLC) and mass spectral data.

**Column Chromatographic Separation of Irradiation Mixture** – A total of 150 g. of diatomaceous earth[7] was thoroughly mixed with 75 ml. of dimethylformamide saturated with cyclohexane. This mixture was packed into 3.7 × 60-cm. glass column according to De Ropp (6). The oily residue from the irradiation mixture was taken up in 1 ml. of cyclohexane saturated with dimethylformamide and applied to the top of the column, and elution was initiated with the same solvent mixture. The flow rate was adjusted to deliver 2 ml./min. Fractions of 20 ml. each were collected and monitored for the presence of cannabinoids by spotting on filter paper, followed by spraying with Fast Blue B indicator (7). Those fractions showing positive color reactions were analyzed by GLC. These data are shown in Table II.

**Isolation and Identification of (−)-Δ⁹-*trans*-Tetrahydrocannabinol**—Those fractions indicating only the presence of (−)-Δ⁹-*trans*-tetrahydrocannabinol (32–36 in Table II) were pooled and reduced to a volume of about 50 ml. This concentrated extract was washed with an aliquot of water (3 × 50 ml.) to remove dimethylformamide. The washed extract was dried (anhydrous sodium sulfate), and the solvent was removed to give a pale-yellow oil (31 mg.), which proved to be (−)-Δ⁹-*trans*-tetrahydrocannabinol by GLC, optical rotation, NMR (8), and mass spectrometry (5).

[7] Celite 545.

## DISCUSSION

It was found that under UV irradiation the three cannabinoids [cannabidiol, (−)-Δ⁸-*trans*-tetrahydrocannabinol, and (−)-Δ⁹-*trans*-tetrahydrocannabinol] were photoreactive. Examination of the reaction mixture after irradiation of (−)-Δ⁸-*trans*-tetrahydrocannabinol and (−)-Δ⁹-*trans*-tetrahydrocannabinol showed that no interconversion of these substances occurred (by GLC). No further attempt was made to identify the decomposition products in these cases (Table I). In the case of cannabidiol, however, it was clearly shown that (−)-Δ⁹-*trans*-tetrahydrocannabinol, as well as other decomposition products, was formed (Table I). These photoreactions are summarized in Scheme I.

Since the maximum amount of (−)-Δ⁹-*trans*-tetrahydrocannabinol was formed in 20 min., the preparation and identification of this compound were carried out by irradiation of cannabidiol in *n*-hexane for 20 min. Mass spectral analyses (direct probe and combined GLC–mass spectrometry) showed the presence of at least two high molecular weight species (*m/e* 626 and 710) as well as peaks corresponding to cannabidiol and (−)-Δ⁹-*trans*-tetrahydrocannabinol. One of these peaks (*m/e* 626) may be the same dimer isolated by Shani and Mechoulam (1) when they irradiated cannabidiol in methanol.

The yield of (−)-Δ⁹-*trans*-tetrahydrocannabinol (16% by GLC) in *n*-hexane was similar to that reported by Shani and Mechoulam (1) who isolated 13% (−)-Δ⁹-*trans*-tetrahydrocannabinol after irradiation of cannabidiol in cyclohexane. The fact that both Vycor and Corex filter sleeves permitted the reaction to proceed while the Kimex filter sleeve did not leads us to believe that the activating energy for the conversion of cannabidiol to (−)-Δ⁹-*trans*-tetrahydrocannabinol lies in the 235–285-nm. area. Attempts to isolate the specific wavelength(s) by the method of Discher *et al.* (9) and Felmeister and Discher (10) have not thus far been successful.

Studies are currently in progress to determine whether the photoproduction of (−)-Δ⁹-*trans*-tetrahydrocannabinol is practical.

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## ACKNOWLEDGMENTS AND ADDRESSES

Received December 17, 1971, from the *Department of Pharmaceutical Chemistry and the †Department of Pharmacognosy, Rutgers—The State University, New Brunswick, NJ 08903

Accepted for publication July 5, 1972.

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