Chromatographic Separation of the Phenolic Compounds of *Cannabis sativa**

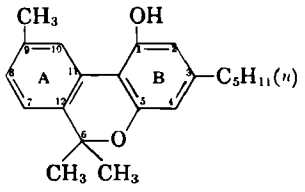
By ROBERT S. DE ROPP

A method is given for partially purifying the phenolic components of *Cannabis sativa* resin and for separating these components by means of paper chromatography. The application of this method for the preparation of pure cannabinol and tetrahydrocannabinol is described.

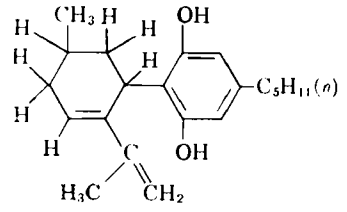
THE ACTIVE principle of the resin from *Cannabis sativa*, commonly known as red oil of hemp, resisted for many years the efforts of chemists to purify it and determine its structure. It was not until 1941 that Wollner, Matchett, Levine, and Loewe (1) described the isolation from Indian charas of tetrahydrocannabinol which was very potent physiologically, as manifested by its effect on dogs. This material, a colorless, viscous, optically active oil, was isolated as the acetate.

Adams and co-workers had already, at this time, determined the structure of cannabinol (2), isolated cannabidiol (3) and isomerized it to two isomeric, physiologically active tetrahydrocannabinols (4). Subsequently Adams and coworkers synthesized a series of analogs of tetrahydrocannabinol. One of the compounds they made had a potency considerably greater than that of natural tetrahydrocannabinol.

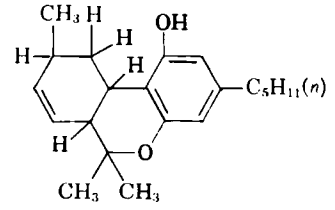
These compounds belong in a class by themselves, being completely unlike any other agents affecting the central nervous system. Chemically, they are all dibenzpyrans, their pharmacological activity being affected by the nature of the substituents and the location of double bonds. Some, like the tetrahydrocannabinols, are viscous, colorless oils. Others, (cannabinol and cannabidiol) can be crystallized. The chemical structures of some of the naturally occuring and synthetic substances belonging to this group are shown below.



I Cannabinol



II Cannabidiol



III Tetrahydrocannabinol

Stereo and optical isomerism make possible the existence of several different tetrahydrocannabinols. Of the synthetic compounds assayed by the dog ataxia test, potency was shown to be affected by the length of the side chain in position 3, the substituent $-CH(CH_3)C_7H_{15}$ conferring a potency 32.6 times that of the $C_5H_{11}(n)$ standard and about twice that of natural tetrahydrocannabinol acetate (5).

Despite the recent upsurge of interest in chemopsychiatric agents little work has been done on the mode of action of tetrahydrocannabinol. It produces in man a variety of reactions ranging from euphoria to depression, but the extreme unpredictability of its action has discouraged its use though synthetic preparations of the resin (Synhexyl or Pyrahexyl) have been available for several years. The pharmacology of these substances has been reviewed by Loewe (6).

EXPERIMENTAL

Preparation of Phenolic Fraction.—Forty pounds of Mexican marihuana[1] was reduced to a coarse powder in a mill. The powdered material was processed in batches of about 6 Kg., each batch stirred with 6 L. of methanol, packed in a 12 × 155-cm. column, and allowed to stand for twenty-four hours. The mass was percolated with methanol for a further twenty-four hours, 12 L. of percolate

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[1] The author is indebted to Mr. H. J. Anslinger, Commissioner of Narcotics, and Dr. Nathan B. Eddy of the National Institutes of Health for making this material available.

being recovered. The solvent was removed *in vacuo* and the residue was suspended in 4 L. of water which was extracted three times with 600 ml. of petroleum ether. Solvent was removed from the petroleum ether fraction which yielded 212.22 Gm. of solids. The aqueous fraction was discarded.

Solids from the petroleum ether fraction were dissolved in fresh petroleum ether and passed through a 90 × 600-mm. column of 1,720 Gm. Florisil (activated magnesium silicate) 100–200 mesh. The column was eluted with a further 2 L. of petroleum ether. The first 600 ml. containing a colorless oil was rejected. The second fraction containing a red oil was collected and freed from solvent. The yield was 10.286 Gm. of red oil. This red oil fraction produced ataxia in a dog at 25 mg./Kg.[2]

Colored impurities were removed from the red oil fraction by passing the material in petroleum ether through a second 40 × 600-mm. column packed with 160 Gm. Florisil, eluting with 500 ml. petroleum ether which removed an inert oil, followed by 750 ml. benzene which removed 1.123 Gm. of a mixture of phenolic compounds in the form of an almost colorless resin (referred to as the phenolic fraction). In other preparations of red oil, large amounts of inert oil from the hemp seeds first had to be removed by passing the material in chloroform through a column of alumina. The inert oil was eluted by chloroform; the phenolic fraction was removed with methanol.

Paper Chromatography of the Phenolic Fraction.—As the phenolic fraction was almost completely insoluble in water, a nonaqueous solvent system had to be found capable of separating its components. The system, cyclohexane: N,N-dimethylformamide, 10:1, gave satisfactory results. Sheets of Whatman No. 1 paper were first spotted with the material to be chromatographed. The sheets were then passed quickly through a bath containing the dimethylformamide phase of the above system until the line of solvent was about 1 cm. below the starting line. The sheets were then blotted, placed in the chromatography chamber, and left for one hour to come to equilibrium with the solvent vapors in the jar. The mobile phase (cyclohexane) was then added and the strips allowed to develop (descending) for eight hours. After drying, the sheets were examined in the light transmitted from a germicidal lamp passed through filter No. 9863 (Corning). Absorbing and fluorescent spots were marked. The sheets were then sprayed with freshly made diazotized sulfanilic acid, with which reagent the phenolic components of the material gave yellow or orange chromophores.

Table I shows the results obtained by means of this type of chromatography.

Eight spots developed on the strips after spraying with diazotized sulfanilic acid. Three of these (R_f 0.22, 0.36, 0.55) absorbed in the U. V. sufficiently strongly to be visible when the sheet was laid over a U. V. source. Both the color and the rate at which it developed with diazotized sulfanilic acid varied with different compounds. Crystalline cannabidiol produced a lemon-yellow spot almost instantly which had an R_f value of 0.12 and corresponded to spot No. 2 in the phenolic fraction. Cannabinol[3] gave an orange spot which did not become visible until thirty minutes after spraying. It corresponded to spot No. 4 (R_f 0.36) in the phenolic fraction and was the spot which absorbed most strongly in the U. V. spot No. 5 in the phenolic fraction overlapped with No. 4 and gave a yellow color which developed more rapidly than did the orange spot of cannabinol. Spot No. 6 (R_f 0.55) corresponded to the pharmacologically active fraction of this material. This was presumed to be tetrahydrocannabinol. Compound No. 7 gave a spot which partly overlapped with that of No. 6. It was present in some, but not all, of the preparations of the phenolic fraction. Compounds Nos. 8 and 9 (R_f 0.73 and 0.93) gave faint lemon-yellow spots and no visible absorption in the U. V.

TABLE I.—*Rf* VALUES, U. V. ABSORPTION, AND REACTIONS TO DIAZOTIZED SULFANILIC ACID (DSA) OF COMPONENTS OF THE PHENOLIC FRACTION OF *Cannabis sativa*

R_f of Spot	Intensity of U. V. Absorption	Color With DSA	Probable Identity
0.07	−	Pale yellow	?
0.12	−	Pale yellow	Cannabidiol
0.22	+	Brown	?
0.36	++++	Orange	Cannabinol
0.41	−	Yellow	?
0.55	+++	Yellow	Tetrahydrocannabinol
0.61	−	Yellow	?
0.73	−	Yellow	?
0.93	−	Yellow	?

Partition Chromatography on Celite of the Phenolic Fraction.—Celite 545 (diatomaceous earth) (150 Gm.) was thoroughly mixed with 75 ml. of the lower phase prepared by shaking together 1,500 ml. cyclohexane with 200 ml. of dimethylformamide. The Celite was packed in a column (37 × 580 mm.) the hold-back volume of which was 300 ml. The phenolic fraction, 1.448 Gm., was dissolved in the upper phase of the above solvent system and placed on the column. The upper (moving) phase of the solvent system was allowed to flow through the column at the rate of 2 ml. per minute. Cuts (25 ml.) were collected and washed twice with 75 ml. of water to remove the dimethylformamide. Three-tenths milliliter from each cut was diluted with 2.7 ml. of methanol and its absorption at 280 mμ was determined.

The two main peaks were well separated; the first, eluted in the second hold-back volume contained compound No. 6 with traces of No. 4. The second main peak, eluted in the third and fourth hold-back volumes, contained almost pure compound No. 4. Cuts were pooled as shown in Table II and the solvents removed *in vacuo*. The distribution of solids indicates that compound No. 6 (? tetrahydrocannabinol) was present in more than double the concentration of compound No. 4 (cannabinol) and constituted about 26% of the total solids in this preparation of the phenolic fraction.

In this preparation the amount of inert oil was high (cuts 1–5 total solids 494 mg.). To reduce the amount of inert material, 1.094 Gm. of phenolic fraction was dissolved in 25 ml. of the upper (cyclohexane) phase of the solvent system and extracted twice with 25 ml. of the lower (dimethylformamide)

[2] Assays of activity were carried out by Dr. G. Tonelli of the Experimental Therapeutics Research Section.

[3] The cannabidiol was kindly supplied by Dr. J. H. Clark and the cannabinol by Dr. Roger Adams.

TABLE II.—PARTITION OF PHENOLIC COMPONENTS OF *Cannabis sativa* ON CELITE BY MEANS OF CYCLOHEXANE/DIMETHYLFORMAMIDE[a]

Cuts	Solids, mg.	R_f of DSA Spot
1–5	494	No color
6–7	23	No color
8–9	12	No color
11–19	382	0.56
23–32	182	0.37
35–38	Trace	0.16

[a] Cuts 25 ml., hold-back volume 300 ml., paper strips sprayed with diazotized sulfanilic acid (DSA).

phase. The lower phase contained 840 mg. of the solids which, after passage through a partition column as described above, yielded 360 mg. of compound No. 6 (43%). Other batches of the phenolic fraction prepared in this way gave similar yields of compound No. 6. The cannabinol content of such fractions was approximately 10%. The amount of cannabidiol in these preparations was less than 1%.

Compound No. 6 (? tetrahydrocannabinol) as obtained in this way was active by the ataxia test in dogs. It was a colorless resin which rapidly acquired a purple tinge on exposure to air, later becoming yellow, and finally dark brown. Prevention of these color changes proved extremely difficult and even samples sealed under nitrogen became altered in this manner to some extent. The material also frequently contained traces of dimethylformamide, enough to give as much as 1% nitrogen on analysis.

Sublimation, Analysis, and Physical Characteristics of Active Fraction.—Further purification of the active fraction by sublimation at 97° at a pressure of $<10^{-4}$ mm. Hg yielded a nitrogen-free product giving a single spot (R_f 0.54) with the cyclohexane/dimethylformamide system. This colorless resin was solid at room temperature, had a specific rotation of $[\alpha]_D^{25}$ — 161°, U. V. absorption min. 251, max. 275, 282 mμ (log ε 3.26, 3.28) shifting in 0.1 *N* sodium hydroxide to min. 269, max. 292 mμ (log ε 3.53) with a second peak at 325 mμ.

Anal.—Calcd. for $C_{21}H_{30}O_2$ (tetrahydrocannabinol): C, 80.21; H, 9.62. Found: C, 79.13, 79.69; H. 9.89. 9.67.

The discrepancy between the found values for carbon and those calculated for tetrahydrocannabinol is rather large. Data for the U. V. absorption agree closely with that obtained by Wollner, *et al.*, for a preparation of tetrahydrocannabinol prepared from Indian charas via the acetate (peaks at 276 log ε = 3.42 and 280 log ε = 3.43).

Compound No. 4 crystallized on standing and proved, on comparison with a synthetic sample of cannabinol from Dr. Roger Adams, to have an almost identical U. V. absorption spectrum. Natural cannabinol max. 283, min. 248 mμ (log ε 4.22). Synthetic cannabinol max. 283, min. 248 mμ (log ε 4.25). This compound in 0.1 *N* sodium hydroxide (methanolic) gave double maxima and minima.

Natural cannabinol max. 283, min. 263 mμ (log ε 4.04, 328, log ε 3.91, 310). Synthetic cannabinol max. 284, min. 263 mμ (log ε 4.05, 328, log ε 3.92, 310). The I. R. absorption spectra of natural tetrahydrocannabinol and cannabinol are shown in Fig. 1.

The synthetic cannabinol from Dr. Adams, which

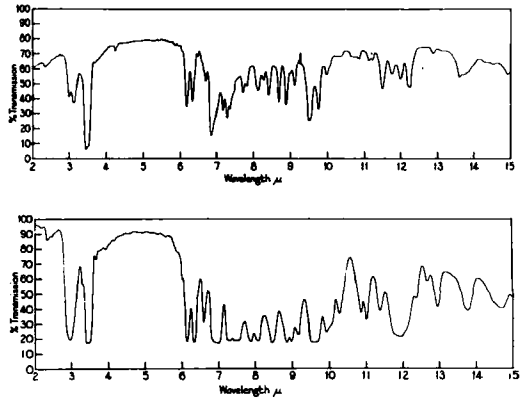

Fig. 1.—Infrared absorption spectra of natural cannabinol (above) and tetrahydrocannabinol (below).

was the color and consistency of pitch when received after ten years' storage, was purified by passage through a cyclohexane/dimethylformamide column and yielded five impurities of various shades of crimson, orange, and purple. About 88% of the sample was still cannabinol which indicates that this coumpound does not deteriorate as much as its change in color might suggest.

SUMMARY

1. The solids from a methanolic extract of the flowering tops of *Cannabis sativa* were adsorbed on Florisil and eluted with benzene to yield a red oil producing ataxia in dogs at 10 to 25 mg./Kg.

2. Paper chromatography of the red oil using the system N,N-dimethylformamide and cyclohexane separated eight phenolic components giving a yellow or orange color with diazotized sulfanilic acid.

3. By partition chromatography on Celite using the above system the active fraction (tetrahydrocannabinol) was separated from the other components (cannabinol, cannabidiol).

4. Further purification of the active fraction was achieved by high vacuum distillation.

5. The unstable colorless resin produced in this way could not be crystallized. Analyses were in fair agreement with the theoretical requirements for tetrahydrocannabinol ($C_{21}H_{30}O_2$). The other components isolated from this sample of cannabis did not appear to have pharmacological activity.

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