MICROGRAM

BUREAU OF NARCOTICS AND DANGEROUS DRUGS / U.S. DEPARTMENT OF JUSTICE

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INDEX ISSUE

CORRECTION

"Structure Elucidation of 'LBJ' ", by Sander W. Bellman, John W. Turczan, James Heagy and Ted M. Hopes, Micro-Gram 1, 3, 6-13 (Dec. 1968)

Page 7, third and fourth sentences under Discussion:

Change to read: "The melting point of the acid moiety found in step (g) was 148-150°C., compared to the literature value of 151°C for the melting point of benzilic acid (2); thus the benzilic acid melting point gives support to the proposed structure for 'LBJ'. Spectral evidence also supports the proposed structure".

MICRO-GRAM REVISION

Please re-number the pages of your copies of Micro-Gram, Volume I. Re-number pages bearing printing only. Volume I will then be numbered from page 1, the front page of issue No. 1, through page 189 the last page of issue No. 12. To help with this task, pages contained within each issue are as follows:

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CANNABIS

Attached is a copy of "A Short Rapid Method for the Identification of Cannabis." The method was developed by Mr. H.D. Beckstead and Dr. W.N. French of the Pharmaceutical Chemistry Division, Food and Drug Directorate Research Laboratories, Ottawa, Canada. It was furnished to us by Mr. R.A. Graham of the Research Laboratories, who writes that it has been in use for some time in their field laboratories.

LSD

BNDD agents in the East recently purchased LSD tablets which were small, polished and were consistent in size. These have been obtained in blue, red and aqua, or green, in color. It is believed that colors are used by the clandestine operator to identify batches.

Other LSD tablets were also recently purchased by BNDD agents, and were new to our laboratory. These tablets were round, biconvex, approximately 7/16 inch in diameter, and were white, speckled with red, green, blue and purple fragments embedded in the tablet. Average LSD content was 388 micrograms per tablet.

LSD continues to come in many colors. Recent powder has been lavender colored. Tablets have been charcoal grey, raspberry, purple, pink and blue, among other colors. Recently a poorly made, pink LSD tablet appeared in various parts of the country bearing the so-called peace symbol as a monogram.

HAWAIIAN BABY WOOD ROSE (Argyreia nervosa)

BNDD agents in the East recently purchased capsules containing a light brown powder alledged to be mescaline. Analysis showed it to be Argyreia nervosa. It is reportedly available in pound quantities at $2500 per pound.

METHYLPHENIDATE HCl

Methylphenidate hydrochloride (Ritalin HCl), as well as the parent compound and its other salts, became a controlled drug under the "DACA" amendments on April 6, 1969.

PHENCYCLIDINE

Phencyclidine and its salts became a controlled drug under the "DACA" amendments on April 6, 1969.

PHENCYCLIDINE BASE

Our Research and Special Testing Laboratory recently identified phencyclidine base submitted by a West Coast police department. The drug was reportedly involved in a non-fatal shooting, in which one member of the "Hell's Angels" shot another member fifteen times with a 45-caliber weapon.

The compound is known as "Dead on Arrival" or "Dust of Angels," and may be known as "DOA". It is reportedly used by placing about fifty milligrams on a cigarette. The sample submitted was a white powder in a plastic bag, with a paper marked "1 Gram $125.00, 20 cigarettes ..."

Joseph Koles, BNDD forensic chemist, reports that the melting point of the base is 46 to 46.5 degrees. For the hydrochloride, the melting point ranges from 222 to 228 degrees C. (1) The base must be dissolved in a little acid before making the crystal test, according to Koles.

Albert R. Sperling, Ph.D., BNDD Research chemist, reports that the base is chloroform soluble and the ultraviolet spectrum and color tests are the same as for the hydrochloride salt. (2, 3)

Dr. Sperling furnished the attached infra-red spectrogram, obtained from a film between salt plates. A potassium bromide disc of the powdered free bases gives an identical spectrum.

Ref: 1. The Merck Index, 8th Edition, page 806

2. Microgram, Vol. I, No. 3, 30-32 (Jan. 1968)

3. Microgram, Vol. I, No. 12, 173 and 184-188 (Dec. 1968)

FORENSIC CHEMIST'S SEMINAR

The next chemist's seminar is schedule for May 19-23, 1969, in Washington, D.C. For application forms or information about the seminars write to:

Director
Bureau of Narcotics and Dangerous Drugs
Washington, D. C. 20537

ATTN: John Doyle, Chief
Special Programs Division
Office of Training

A SHORT RAPID METHOD FOR THE IDENTIFICATION OF CANNABIS

H.D. Beckstead and W.N. French
Food and Drug Directorate
Research Laboratories
Ottawa, Canada

Definition: This is a rapid, simple and reliable method for the identification of cannabis (marihuana) in exhibits. The procedure permits the differentiation of cannabis from other plant materials such as oregano, khat, ragweed, etc. which frequently are used as diluents.

Principle: The sample is subjected to visual macroscopic and microscopic examination as well as to the Duquenois colour test and acid chloral test. The identity of cannabis is confirmed by a thin-layer chromatographic test on a n-hexane extract of the sample material (Note 1). Additional evidence of identity may be acquired by the use of gas chromatography.

Apparatus:

1. Low power microscope.
2. TLC equipment.
3. Impregnation chamber (Note 2).
4. Gas-Liquid chromatograph equipped with flame ionization detector (Note 3).

Reference Materials:

1. Mixed hashish resin standard (10 mg per ml n-hexane) (Note 4).
2. Cannabidiol (5 mg per ml n-hexane) (Note 4).
3. Cannabinol (5 mg per ml n-hexane) (Note 4).

Reagents and Materials:

1. Acid chloral reagent - Dissolve 10 g chloral hydrate in 100 ml of 20% v/v aqueous HCl.
2. Duquenois reagent - Dissolve 2 g vanillin in 100 ml of 95% ethanol. This stock solution keeps indefinitely if refrigerated. Immediately before use, mix 1 ml of solution with 3 drops of acetaldehyde.

3. Thin-Layer Chromatoplates

(a) Preparation of layers - Using standard thin-layer chromatographic apparatus, coat 8 x 8 inch glass plates to a thickness of 0.50 mm using a slurry of 55 g of Kieselguhr G, 100 ml H_2O and 10 ml of 1% w/v aqueous sodium carboxymethylcellulose for 5 plates (Note 5). Allow layers to air dry in a dust-free atmosphere. Activation is not necessary.

(b) Impregnation with N-methylformamide - Mark the chromatoplate with a sharp stylus to indicate the spotting positions with 15 cm solvent-developing distance. Immerse the plate vertically, with spotting positions uppermost, in a solution of 100 ml of N-methylformamide and 325 ml of acetone (Note 2). After 5 minutes, remove the plate, allowing to drain, and air dry for 5 minutes. The impregnated plate is not susceptible to moisture pick-up, even over-night, so the sample need not be spotted immediately (Note 6).

4. Thin-Layer Chromatographic Solvent - Shake 100 ml of cyclohexane with 10 ml of N-methylformamide. Use the upper layer (Note 7).

5. Thin-Layer Chromatographic Detection Reagent - Dissolve 200 mg Fast Blue Salt B or Fast Blue Salt RR in 100 ml 50% ethanol. This reagent must be prepared fresh each day.

Procedure: 1. Visual Examination of Sample Material

(a) Macroscopic - This examination is used only to establish the presence of plant material in the exhibit. The colour, odour and form of cannabis is usually quite distinctive and different from materials used as diluents or substitutes. Examine the material under a hand lens or low power stereoscopic microscope, and separate suspected marihuana plant parts from diluents.

(b) Microscopic - This examination is used to observe the detailed physical characteristics of the plant material using a microscope. The cannabis plant has typical cystolith hairs on the stems and underside of leaves. Extracts of cannabis (i.e. cannabis resin) usually contain considerable amounts of plant material including cystolith hairs. The latter may be readily detected by mixing a small quantity of resin with n-hexane (as in 3(b) below), transferring a drop of suspension to a microscopic slide and observing under the microscope when solvent evaporates.

2. Acid Chloral Test

This test is used to detect the presence of carbonate deposits on the suspected plant material. Add a small drop of acid chloral reagent to a portion of the suspected plant material while observing under the microscope. Note any effervescence which may occur. The cystolith hair of cannabis contains a calcium carbonate deposit which liberates CO_2 on treatment with acid giving rise to effervescence.

3. Preparation of Sample

(a) Green Plant Material - Place approximately 50 mg of sample in a 10 x 75 mm test tube, and add sufficient n-hexane (0.5 ml) to just cover the sample. Allow the tube to stand for approximately 10 minutes with occasional shaking.

(b) Cannabis Resin (hashish, charas, bhang etc.) - Place approximately 2 mg of sample in a 10 x 75 mm test tube and add 0.25 ml n-hexane. Allow the tube to stand for approximately 10 minutes with occasional shaking.

(c) Smoking Devices (hookah pipes, pipes, cigarette holders) - Scrape the inside of the device and examine scrapings under the microscope for evidence of cannabis plant material by the presence of leaf material as well as cystolith hairs. Wash the exhibit, including the scrapings,

with hot methanol (3 - 5 ml), and evaporate the extract to dryness under a stream of nitrogen. Dissolve the residue in a small amount of n-hexane - usually 0.25 ml is sufficient. With water-cooled pipes (hookah pipes), wash the water resevoir with methanol and combine with the pipe washings. Any water present in the pipe will contain only traces of resin, and need not be extracted.

(d) Cigarettes Containing Other Material Mixed with Marihuana - The ordinary diluents of marihuana - tobacco, oregano, green tea, and many common weeds - do not interfere with the identification tests. Since marihuana will be present in much smaller amounts in these samples, material suspected to be marihuana may be physically separated and then treated as directed for green plant material.

4. Duquenois Spot Test

Place one drop of sample extract solution on a white porcelain spot plate and allow the solvent to evaporate. Add one drop of Duquenois reagent followed by one drop of concentrated hydrochloric acid. A deep blue colour develops if cannabis is present. The colour developed is stable at least for several days. Some species of oregano give a grey-blue colour with this reagent but a differentiation from cannabis can readily be obtained by adding a few drops of chloroform to the blue solution in the plate followed by a few drops of water. With cannabis, a blue-violet colour develops in the chloroform layer whereas with oregano, the blue colour disappears completely.

5. Thin-Layer Chromatography

(a) At the separate pre-marked locations on the impregnated chromatoplate, spot 10 µl of the sample extract solution and 2 µl of the standard cannabis resin reference solution. If deemed necessary spot 2 µl each of cannabinol solution and a cannibidiol solution.

(b) Place the chromatoplate in a suitable chromatographic chamber (lined with filter paper and pre-equilibrated with solvent) and allow the solvent front to advance 15 cm beyond the spotting points - developing time: 45-55 minutes. Remove the plate and dry the layer with a stream of warm air.

(c) Spray the plate lightly with the thin-layer chromatographic detection reagent. Allow the colour to develop for one minute, then place the plate in an oven at 110°C for 3 minutes. This heating step removes excess solvents and prevents the spots from diffusing. The colours developed are stable indefinitely (Note 8).

6. Gas-Liquid Chromatography (Note 9)

i. Columns

(a) 3% SE-30 on Chromsorb W - HMDS treated, 60/80 mesh

(b) 3% QF-1 on Chromsorb W - HMDS treated, 60/80 mesh

ii. Operating Conditions

(a) Column temperature: 180°C isothermol or programmed from 125 to 225°C at the rate of 10° per minute.

(b) Carrier gas flow: 30 ml per minute

(c) Hydrogen gas flow: 20 ml per minute

(d) Injector temperature: 275°C

(e) Detector temperature: 275°C

iii. Injection Volumes

(a) Standard solutions - 0.2 µl

(b) Sample extract - 4 µl (as prepared for thin-layer chromatography)

Note 1: Adequate cannabis proof of identity is usually given by the visual examination, acid chloral test, Duquenois test and thin-layer chromatography. Gas chromatographic examination may be used as an optional means to provide additional proof of identity.

Note 2: A convenient device for impregnating the plates is a stainless steel chamber approximately 1 x 21 cm inside cross-section by 26 cm in depth and a stainless steel frame, fitted with handle, which holds the chromatoplate and of suitable size to slide easily into the chamber. When not in use, a tight fitting stainless steel cover reduces solvent evaporation. (See Figure 1). After each plate immersion, the original volume of solvent should be restored by the addition of acetone. In this manner, 100 ml of N-methylformamide will impregnate approximately 100 plates before depletion.

Note 3: Most gas chromatographs equipped with a flame ionization detector are suitable for this work. Both 1/8" and 1/4" columns (6' in length) have been used to give equally good results.

Note 4: The following method was used to extract and purify the reference materials:

Lebanese hashish was exhaustively extracted with methanol and the methanol removed at 40° under reduced pressure. The residual oil was taken up in cyclohexane, the mixture filtered, and the filtrate evaporated to dryness. The resulting oil amounted to approximately 1/3 of the weight of the hashish.

This residue was then taken up in methanol and the suspension cooled to -78° to precipitate plant waxes. Filtration and evaporation of the filtrate gave a residual oil having approximately 1/4 of the original weight of the hashish.

This crude preparation was dissolved in benzene, and the benzene solution extracted with aqueous NaOH to remove any acids present. The extracted benzene solution was then concentrated by evaporation and chromatographed on a Florisil column using benzene as eluant. The solvent was removed under reduced pressure from the eluate, and the residual resin, of mixed phenolic content, used as the mixed hashish resin standard.

The components of this resin were separated by chromatography on a large partition column of DMF on Kieselguhr, using cyclohexane as the mobile phase. The mixed fractions thus obtained were

rechromatographed to effect complete separation. Pure cannabidiol obtained by this method melts at 65-66° and shows a tendency to discolour on prolonged storage. Pure cannabinol, isolated as described, melts at 75-76°.

Note 5: With Kieselguhr G alone, flaking sometimes occurs in humid weather during impregnation - especially on older chromatoplates. The use of a small amount of sodium carboxymethylcellulose as an additional binder to that already present in Kieselguhr G prevents this flaking. Freshly prepared chromatoplates may also be oven-dried if urgently required. The chromatographic behaviour of the components of Cannabis is not affected.

Note 6: The use of N-methylformamide (NMF) causes no significant change in the Rf-values of the cannabis constituents from that observed for the use of N, N-dimethylformamide (DMF). NMF has the major advantage over DMF in that it is less prone to moisture absorption and therefore plates impregnated with NMF can be stored for a period of time before use. In addition, the spots are better defined using NMF than with DMF.

Note 7: The developing solvent may be prepared in quantity and used as required. No change in Rf-values has been noted after three months storage.

Note 8: Typical Rf-values observed for the main components of cannabis using NMF as impregnating material are the following: (see figures 2 and 3 for structural formulae)

	Rf	Colour
1. Cannabigerol	0.05	orange
2. Cannabidiol	0.20	orange-brown
3. Cannabinol	0.35	blue
4. Δ^1-THC	0.55	blue
5. Δ^6-THC	0.58	pink
6. Cannabichromene	0.69	light-orange

The ratios of these components may vary widely depending upon the source and/or growing conditions of the cannabis plant, the age of the material being examined, the form of the sample (plant

material, hashish, resin, pipe extract, etc.) as well as other factors(see also Note 9). In most samples, several unknown spots appear on the chromatogram. The corresponding carboxylic acid derivatives of the phenolic compound appear as a purple spot elongated from the origin. Plant waxes are variable, and appear near the leading edge of the solvent front and near the midway point. These fluoresce bright red under long-wave UV light. They do not interfere with the chromatographic separation.

The Rf-values quoted are approximate only and may vary to some extent with the age and condition of the chromatoplate. Nevertheless, samples of cannabis will afford the same pattern of spots and degree of resolution as the standard reference resin thus offering convincing evidence of the identity of the sample.

Note 9: As pointed out in Note 8, the phenolic content of material from different sources varies considerably. Most of the recent samples received at Ottawa have had a fairly high proportion of $\triangle^1$-tetrahydrocannabinol in comparison to the amounts of cannabinol and cannabidiol. In Canadian grown samples examined a number of years ago, cannabidiol was the main component observed, with only traces of the other two phenols. Under normal operating conditions with low attenuation, only the three major components, cannibinol, cannabidiol and $\triangle^1$-tetrahydrocannabinol, show significant peaks on the gas chromatogram.

Typical retention times using the Varian Aerograph A700 Autoprep with the specified operating conditions (3% SE-30, 1/4" x 6' column, isothermal) are the following:

Compound	Retention Time (min)
Cannabidiol	4.2
$\triangle^1$-tetrahydrocannabinol	5.1
$\triangle^6$-tetrahydrocannabinol	5.4
Cannabinol	6.6

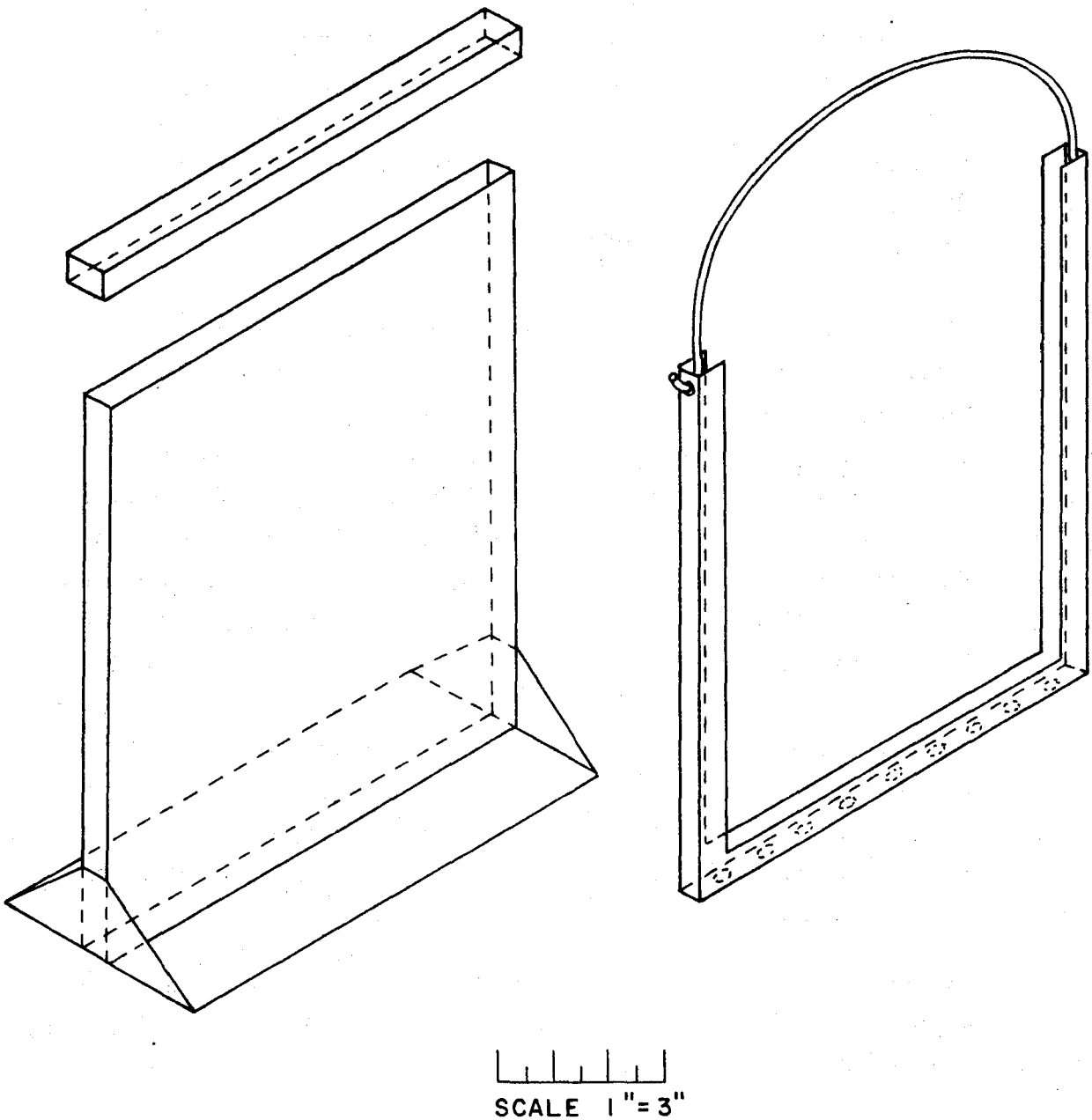
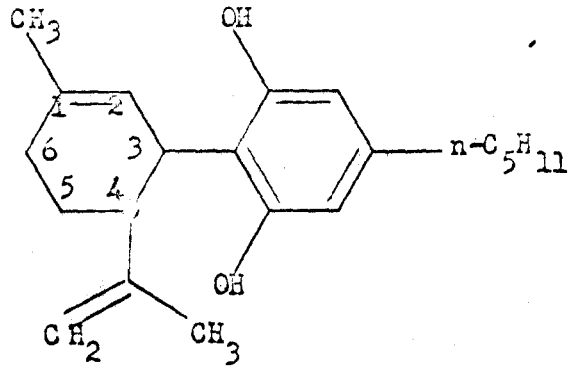
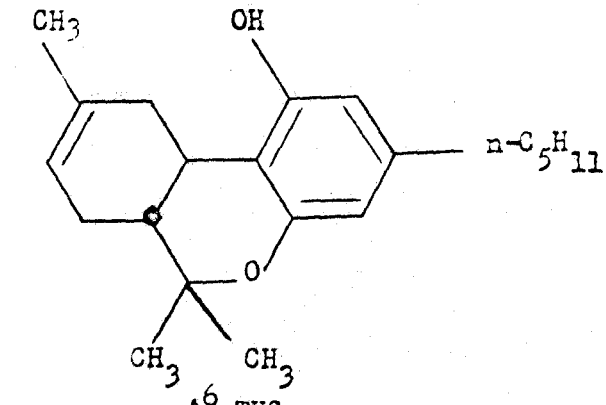


Figure 1 - Chamber for Impregnating Thin-layer Chromatoplates



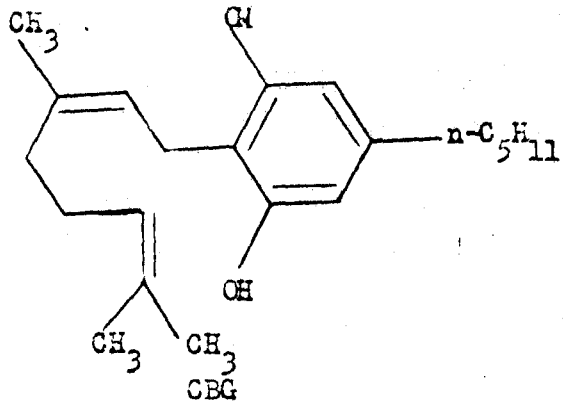
CBD

Cannabidiol



Δ^6-THC

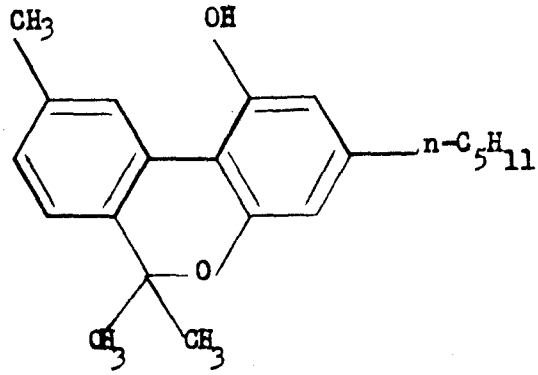
Δ^6-3,4-trans-Tetrahydrocannabinol



CBG

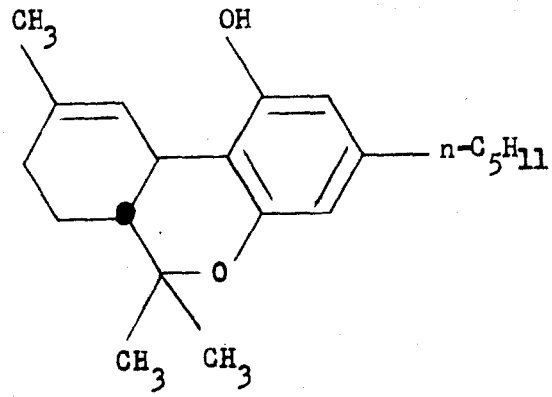
Cannabigerol

Figure 2 - Structures of Known Components of Cannabis



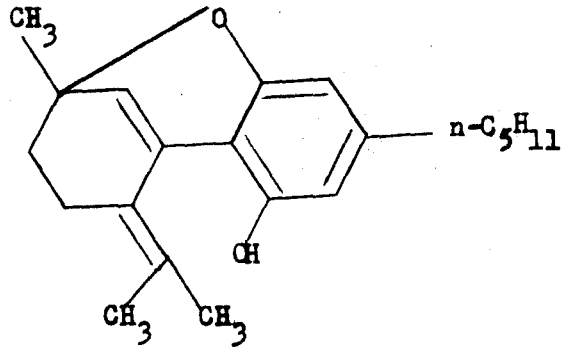
CBN

Cannabinol



Δ^1-3,4-trans-Tetrahydrocannabinol

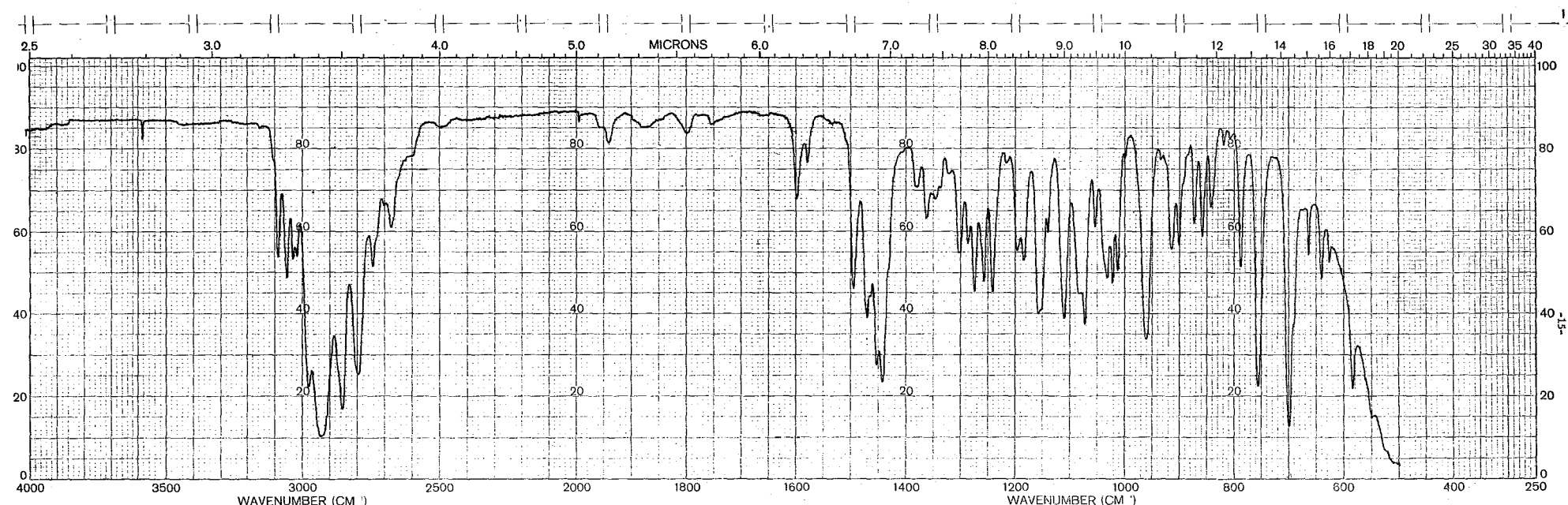
Δ^1- THC



CBC

Cannabichromene

Figure 3 - Structures of Known Components of Cannabis



SAMPLE PHENCYCLIDINE FREE BASE FILM BETWEEN SALT PLATES	SOLVENT	REMARKS	SCAN SPEED	OPERATOR A. SPERLING
	CONCENTRATION		SLIT	DATE
	CELL PATH		PERKIN ELMER	
ORIGIN	REFERENCE		PART NO. 457 - 5001	REF. No.

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IT-290 See alpha-methyltryptamine or 3-(2-aminopropyl) indole			
IT-430 See alpha-methyltryptamine			
JB-318 N-Ethyl-3-piperidyl benzilate	10	142	September
JB-329 N-Ethyl-3-piperidyl (cyclo-pentyl Mandelate) (Ditran)	12	172	December
JB-336 N-Methyl-3-piperidyl benzilate HCl) LBJ	8	122	May
JB-336	9	129,134-136	June
JB-336	10	140-144	September
JB-336	12	173	December
KAT See Khat, Catha	4	39	January
Kinortine Tablets	5	70	February
Khat See Kat			
Kif See Marihuana			
Kinortine	5	70	February
LSD (lysergic acid diethylamide)	1	1	November
LSD (lysergic acid diethylamide)	2	15, 24-29	December
LSD (lysergic acid diethylamide)	4	34, 42-44	January
LSD (lysergic acid diethylamide)	4	46-49,50-59	January

LSD (lysergic acid diethylamide)	4	63-66	January
LSD (lysergic acid diethylamide)	5	67	February
LSD (lysergic acid diethylamide)	8	123	May
LSD (lysergic acid diethylamide)	9	130	June
Laboratory Examination, How To Request From BNDD	5	68	February
Laboratory Operations	11	158-159	November
LBJ See JB-336			
Lophophora	4	39,41-42	January
Lysergamide	4	38	January
Marihuana See Cannabis			
MDA See Methylenedioxyamphetamine			
Mecloqualone	5	70	February
Mephentermine Sulfate Tablets (Wyamine)	7	114	April
Mescal Buttons See Peyote			
Mescaline	4	42	January
Mescaline	4	60-62	January
Mescaline	6	81,88	March
Mescaline	7	108,118-120	April
Methamphetamine	5	79	February
Methamphetamine	6	81,90-91	March
Methamphetamine	7	115-117	April
Methylcyclopentylphenylglycolate	12	171	December
4-Methyl-2,5-dimethoxyamphetamine (DOM) (STP)	1	1,4-8	November
4-Methyl-2,5-dimethoxyamphetamine	2	15	December
4-Methyl-2,5-dimethoxyamphetamine	7	99	April
4-Methyl-2,5-dimethoxyamphetamine	8	124-125	May
4-Methyl-2,5-dimethoxyamphetamine	10	139	September
1-Methyl-lysergic acid butanolamide	6	81	March
Methylphenidate	5	69	February
Methylphenidate	6	82	March
3,4-Methylenedioxyamphetamine (MDA)	5	70-71,72-78	February
N-Methyl-3-Piperidyl benzilate HCl (JB-336)	9	129,134-136	June
N-Methyl-3-Piperidyl cyclopentyl-glycolate	4	42	January
7-[2-(1"-Methyl-2"-Phenylethylamino)-ethyl]- theophyllin HCl tablets	5	70	February
alpha-Methyltryptamine (IT-290)	4	42	January
alpha-Methyltryptamine (IT-290)	6	80	March
Morning Glory	4	38	January
Morning Glory	10	137	September
New Address, BNDD	12	173-174	September
Nitrous Oxide	8	121	May
Obedrin-LA	8	122	May
Ololiuqui	4	38	January
PAM	8	122	May
PCP See Phencyclidine HCl			

Peace Pill See Phencyclidine HCl			
Peganum harmala	4	38	January
Peyote	4	41-42,60-61	January
Phalaris	2	10	December
Phencyclidine HCl (Peace Pill, PCP, Hog)	3	30-32	January
Phencyclidine HCl	6	81,84	March
Phencyclidine HCl	9	130	June
Phencyclidine HCl	12	173	December
Phencyclidine HCl	12	184-188	December
Phenmetrazine, Analysis	7	118-120	April
Phenylpropanolamine, Analysis	7	118-120	April
Piperidyl Benzilate HCl (JB-336)	9	129,134-136	June
Piperidyl Benzilate HCl (JB-336)	12	172-173,176-183	December
Piperidyl Glycolates	12	171-172	December
Piptadenia	2	10	December
Piptadenia	4	37,41	January
Psilocybe	4	38	January
Psilocybe	6	81,88-89	March
Psilocybe	8	121,126-128	May
Publications of Interest	4	33	January
Publications of Interest	9	131-132	June
Police Chemist School	1	2	November
Police Chemist School	4	33	January
Police Chemist School	9	131-132	June
Queen Anne's Lace	5	70	February
References	4	39	January
References	6	80-81	March
References	7	117	April
References	8	121,125,128	May
References	10	137,156	September
References	12	183	December
Rilatin See Methylphenidate			
Ritalin See Methylphenidate			
Rivea	4	38,41	January
Romilar Tablets	5	70	February
Safety	12	175	December
Salvia	4	41	January
Sansert (Methysergide) Sandoz 1-methyl-lysergic acid butanolamide	6	81	March
Seminar, Chemist	1	2	November
Seminar, Chemist	4	33	January
Seminar, Chemist	5	68	February
Serynyl, Serylan See Phencyclidine HCl	9	130	June
Smash	4	33	January
Sominex	2	15	December
STP See 4-Methyl-2,5-dimethoxyamphetamine)			

STP Tablets	8	123	May
STP Tablets	9	130	June
Stramonium Preparations	2	15	December
Stramonium Preparations	4	42	January
Stropharia	4	38,42	January
Sympathomimetics Analysis	7	115-117	April
Tablets			
Amphetamine	5	67	February
Captagon	5	70	February
7-[2-(1-"Methyl-2"-Phenyl-ethylamino) ethyl]	5	70	February
LSD, Blue	8	123	May
LSD, Blue	9	130	June
Romilar (Dextromethorphan HBr)	5	70	February
STP	8	123	May
STP	9	130	June
Talwin	11	159-160	November
Telepathine	4	38	January
Tetrahydrocannabinol (THC)	4	39	January
Tetrahydrocannabinol (THC)	10	138	September
Tetrahydrocannabinol (THC)	10	157	September
Tetryl	5	71	February
Tetryl	6	83	March
THC			
See Tetrahydrocannabinol			
TLC of Sympathomimetics	7	115-117	April
TMA			
See Trimethoxyamphetamine			
Tranquinal	2	14	December
Trichocereus	4	42	January
Trimethoxyamphetamine (TMA)	4	39	January
TWA			
See JB-336			
U-14,164E			
See IT-290			
Ultraviolet Absorption Date	10	145-156	September
Wheat Flour, LSD Capsule	1	1	November
Wild Carrot			
See Queen Anne's Lace			
Wild Rue	4	38	January
Wyamine Tablets	7	114	April
Yage	4	38	January
Yageine	4	38	January