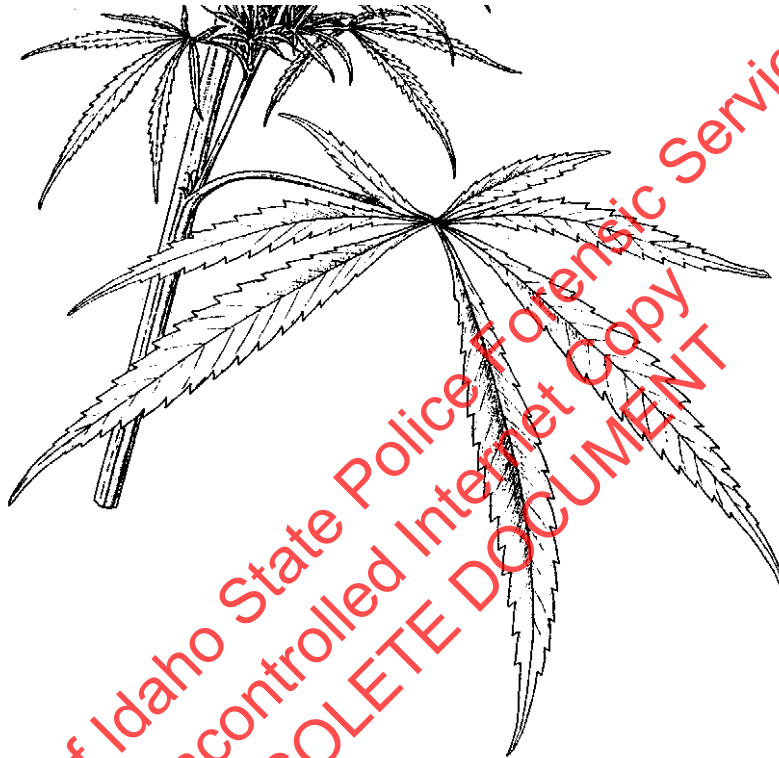


Idaho State Police

Forensic Laboratory Training Manual



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Marijuana Analysis

MARIJUANA ANALYSIS TRAINING PROGRAM

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1.0.0 INTRODUCTION AND BACKGROUND

- 1.1.0 Marijuana, also known as hemp and cannabis, is one of the more commonly abused controlled substance in Idaho. Possession and sale of marijuana has been prohibited by law in the United States since 1937.

Idaho Statute (37-2701(s)) defines marijuana as follows: "Marijuana" means all parts of the plant genus Cannabis, regardless of species, whether growing or not; the seeds thereof; the resin extracted from any part of such plant; and every compound, manufacture, salt, derivative, mixture or preparation of such plant, its seeds or resin. It does not include the mature stalks of the plant unless the same are intermixed with prohibited parts thereof, fiber produced from the stalks, oil or cake made from the seeds of the plant or the achene of such plant, any other compound, manufacture, salt, derivative, mixture or preparation of the mature stalks (except the resin extracted therefrom or where the same are intermixed with prohibited parts of such plant), fiber, oil, or cake or the sterilized seed of such plant which is incapable of germination. Evidence that any plant material or the resin or any derivative thereof, regardless of form, contains any of the chemical substances classified as tetrahydrocannabinols shall create a presumption that such material is "marijuana" as defined and prohibited herein.

The exceptions in the legal definition are present because there are some legitimate uses for certain parts of the marijuana plant. Fibers from the stalk are used to make rope, twine, canvas, cloth and hats. A rapid drying oil that is used in the arts and as a commercial substitute for linseed oil can be extracted from the seeds. This oil is also valuable in soap making. The oil cake is used as cattle feed. The seeds are sterilized and used as a constituent in commercial birdseed mixtures. Dilute solutions of the pollen extracts are occasionally used to develop antigens in those persons who exhibit allergic manifestations to the plant or pollen.

The main psychoactive ingredient of the marijuana plant, Δ^9 -Tetrahydrocannabinol (pronounced delta-9-Tetrahydrocannabinol) or (THC), is contained in all parts of the plant except for the roots and possibly the seeds. Some literature reports that there is no THC in the seeds - other literature reports finding THC in the seeds in very minute amounts. THC is most abundant in the resin that is secreted by the plant. Very young marijuana plants often contain little or no THC. Tetrahydrocannabinol is controlled separately from marijuana. A synthetic form of Tetrahydrocannabinol called Dronabinol is used to treat nausea associated with chemotherapy. This form is sold under the trade name Marinol and is packaged as gelatin capsules containing the THC suspended in sesame oil. Dronabinol is controlled separately from tetrahydrocannabinol and marijuana.

Marijuana is cultivated the world over. Its culture is presumed to have originated in China from whence it spread. It presently grows wild or is cultivated in North and South America, Asia, India, Africa and small quantities are produced in some European

countries. Although the plant is indigenous to many areas of the world, environmental factors govern the extent of growth and are responsible for many morphological modifications of the plants.

In northern climates, hemp usually grows to a considerable height and produces more fiber than that grown in southern latitudes where the plant is usually of the dwarf variety. The shorter summers of the northern latitudes can sometimes prevent the seeds from ripening fully. During World War II when our supplies of Manila hemp were aborted, the Treasury Department licensed several farmers in both southern and northern tier states to produce hemp, which was so vital to the war effort. While the longer growing season of the southern states permitted the harvest of a seed crop, the quality of the fiber was such as to render it unsuitable for making rope. Thus, the southern seed crop was sold to farmers in the north where the colder climate was conducive to a hardier and firmer fiber.

Hemp was grown in the New England Colonies for fiber used in making homespun. It was also grown in the Virginia and Pennsylvania colonies and cultivated at a very early date in the settlements of Kentucky and Tennessee from whence it spread to Missouri and westward with the settlers. It is not known when the plant was introduced to the Southwest and Mexico, but probably along with the early Spanish settlers.

Formerly, the majority, if not all, the imports of cannabis into the United States were from India where hemp was largely cultivated for smoking purposes. The menace of the habit, which its culture made possible, led the Indian authorities to impose drastic restrictions on its production, hence the supply of hemp required by the United States had to be sought elsewhere. Thus, the domestic industry mostly in Kentucky and the Illinois River valleys came into being. The early cultivation of hemp in the United States was of the small European variety but this was replaced around 1850 by the larger Chinese variety. A great deal of hemp was also produced in Russia, formerly a principal source for American importation. The use of hemp fiber in the manufacture of rope in this country has been replaced almost entirely by Abacca or Manila fiber derived from a species of the banana plant. With the development of synthetics, especially nylon, the use of Abacca has declined.

The glandular hairs of the plant produce a sticky and somewhat viscous resin. This resin is found on many areas of the plant, however, it is most abundant on the reproductive parts. Moreover, certain plants seem to produce more resin than others. Whether this high resin content is due in part to ecological conditions or a built-in defensive mechanism or a genetic characteristic of the plant has not been determined. However, there seems to be a definite correlation between altitude, moisture and temperature, and the quantity of resin produced. Some botanists maintain that plants grown in hot, dry climates exude more resin as a protection against moisture loss, particularly where it involves female flowers; thus insuring favorable conditions for the propagation of the species. There tends to be some credence in this theory since the hemp grown in the hot

and dry climates of the Himalayas, certain parts of Africa and the arid slopes of the Andean mountains of South America are noted for their relatively high resin production.

The true origin of the name marijuana is lost in antiquity. Gray attributes a Greek derivation of the word "Cannabis" to be from the Persian name "Kanab". Other authors cite many words from many languages as the possible genesis of the word "marijuana". History tells us that the murderous frenzy of the Malays, characterized by running "amok" was the result of the habitual use of hashish. Hashish is the unadulterated resin collected from the flowering tops of the marijuana plants. It is also reported that the Mohammadan leaders, opposing the Crusaders, utilized the services of individuals while under the influence of hashish to commit secret murders. The frenzy produced by the drug led these persons to be called "haschischin", "hashihash" or "hashishi" from which the modern English word "assassin" is derived.

The flowering tops, leaves, and small stems are gathered, dried, and usually smoked in a pipe or as a cigarette. Its use in cigarettes is the method most often chosen. Sometimes the resin is expressed or obtained by rolling the pods between the hands or "carpets" and then eaten. It has been reported that the Egyptians gathered the resin by donning leather jackets and walking through a field of shoulder high plants. The sticky resin that adhered to the jacket was then scraped off and utilized in the usual manner. However, the credibility of this tedious method is lacking in standard references.

In the United States, Canada, and Mexico, the dried crushed tops and leaves are rolled into cigarettes and smoked. In India and Central Asia, the raw resin is extracted from the tops and kneaded into sticks or mixed with various spices and called Charos or Dawamesk which is either smoked or eaten. The leaves are also powdered and mixed with spices, honey or water and the concoction, referred to as Bhang is eaten or drunk. In North Africa, the dried crushed tops are mixed with tobacco and smoked in pipes. The user in Tunis refers to this as "takroui" while the Moroccans call it "Kif". In the Eastern Mediterranean and around the Gulf of Arabia the raw resin from the flowering tops is reduced to powder for smoking (called Chira) or is expressed and kneaded into sticks for eating (hashish). While in the same area the flowering tops are soaked in butter and water and mixed with almonds and honey, then eaten in the form of cakes. The Turks call the mixture "Madjun" "Magoun" or "Esrar". In South Africa smoking "Djamba" or "Dagga", a mixture of crushed leaves and flowering tops seems to be the method of choice. In modern times, hash oil is produced by soaking the plant material in solvents to extract the THC. The solvent is removed from the plant material and evaporated, leaving behind an oil that is usually very high in THC content.

Δ⁹ - THC Content in Different Forms of Marijuana

<u>Form</u>	<u>Δ⁹ - THC Range</u>
Marijuana Leaves	0.2 - 3 %
Marijuana Flowering Tops	3 - 4 %
Sinsemilla	3.5 - 4.5 %

Compressed Marijuana (pressed cake)	2.5 - 4 %
Hashish	0.1 - 14 %
Hash Oil	0.5 - 45%

From the early 1900's to 1937, many pharmaceutical preparations containing resin extracts of cannabis were readily available and were promoted extensively as analgesics and sedatives. Clinicians, however, soon learned that these preparations, rather than contributing to the treatment of clinical disorders, actually manifested their symptoms and caused such untoward side effects as to preclude their use. Shortly after the passage of the Marijuana Tax Act of 1937, the Food and Drug Administration declared these preparations to be without medical utility and they were removed from the market place.

Scattered stands of wild hemp are reported each year throughout the United States. It is abundant as a wild plant in many localities, often growing along hedgerows, riverbanks and roadsides. The plants are indigenous to many areas and are adaptable to almost every type of soil and climatic conditions except those in the extreme northern latitudes. The heaviest infestation tends to follow the Corn Belt in the states of Iowa, Kansas, Nebraska and Missouri while the lowest level of infestation occupies an area from Indiana eastward through New England with the exception of Maine where very little growth occurs. Moderate growth occurs in the Virginias, Tennessee, Kentucky and Ohio while scattered growth occurs along the southern tier of states.

There are many problems with controlling the wild growth including; (1) the lack of recognition of the plants by the land owners, (2) the tendency of the plant to grow in small widely scattered stands and its ability to adapt to many types of habitats, (3) the resistance of the mature plants to herbicides and (4) the production of viable seeds over a ten to twelve week period from mid July to mid October.

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1.2.0 Exercises:

- E1-1. Obtain a copy of Idaho Drug Statutes and find where marijuana, THC and Dronabinol are listed.
- E1-2. Find, copy and read the listing for Marinol (Dronabinol) in the Physician's Desk Reference (PDR).

2.0.0 TAXONOMY

2.1.0 The discipline concerned with the classification and naming of living things is called taxonomy. The table below lists the taxonomic classification of marijuana. (NOTE: An alternate group of classification categories may be encountered. That group consists of Kingdom; Phylum or Division; Subphylum; Class; Order; Family; Genus; and Specific Name.)

<u>Category</u>	<u>Taxon</u>
Kingdom	Plant
Division	Spermatophyta (seed plant)
Class	Angiospermae (flowering plants)
Subclass	Dicotyledons (dicots) 31,874 species
Order	Urticales (elms, mulberries, nettles, and hems) 1,753 species
Family	Cannabinae (hops and marijuana) 3 species
Genus	Cannabis
Species	Sativa

Individual plants are identified by giving genus and species names. Hence, the full botanical name for marijuana is "Cannabis sativa L". The "L" refers to the botanist who first classified marijuana (Carlus Linnaeus).

Because there is only one species in the genus, Cannabis is known as a monotypic genus. There is debate among botanists as to whether there is more than one species of cannabis. A botany professor at Harvard University by the name of Dr. Schultes believes that there are several species including Cannabis sativa, Cannabis indica and Cannabis ruderalis (which is sometimes spelled ruberalis). He bases his belief on physical inconsistencies between plants. These inconsistencies include branching differences, broadness and thickness of leaves and overall plant shape and appearance. However, most experts believe that most of the variations between plants are neither adequate nor specific and are actually brought on by growing conditions. These botanists believe that indica, ruderalis, as well as americana are all agronomic varieties of Cannabis sativa. Most references agree that the different kinds of Cannabis interbreed and produce viable offspring, which is an indication that they are from the same species.

The early federal and state statutes that controlled marijuana used the name Cannabis Sativa L. Because of the species disagreement among botanists, several defendants were acquitted because the court felt that the prosecution did not prove the substance in question was actually controlled. Below is a list of three early rulings on the Federal level supporting the single species view.

1. U.S. vs. John Moore (E.D.Pa. No 69-137) 330 Fed. Supp. 684 (1970)
2. U.S. vs. Eric Honeyman, et al., (71-1035-RHS) Northern District, California, (1972)
3. U.S. vs. Mitchell Rothberg, et al., (7-CR-164) 351 Fed. Supp. 1115, Eastern District, New York (1972)

This legal argument is now mute because the statutes have been rewritten to define marijuana as plants in the genus Cannabis. It is important that the examiner be aware of this argument however, because even though it is now groundless, it may still be brought up in court.

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3.0.0 PHYSICAL CHARACTERISTICS

- 3.1.0 Despite much variation among individual plants, marijuana is so distinct from all others that it can be recognized at all stages of growth by its botanical characteristics. Marijuana plants grow from 1 to 5 meters high. When planted for the production of hemp fiber, the stalks are crowded and without foliage except near the top. Wild growing plants on the contrary have numerous branches. The size of individual plants is primarily determined by growing conditions.

The marijuana plant is an annual - it dies after seed production and is dependent upon seed for survival of the species. The life cycle can be as little as two months or as long as eight months. Marijuana is a full sun plant that requires both the proper duration and wavelengths of light for photosynthesis and reproduction to occur. Marijuana is adaptable however, and will grow in the shade, although more slowly. High temperatures cause the plant to wilt due to water loss and, as a result, resin production usually increases to prevent dehydration. While marijuana can adapt to heat, exposure to a hard frost or several days of repeated light frost could kill the plant.

3.2.0 LEAVES:

The leaf of any plant consists of a blade and a leaf stem or petiole. The leaf is classified by the characteristics of the blade. If the leaf blade is undivided, the leaf is classified as simple. If the leaf blade is divided into distinct parts called leaflets, the leaf is classified as compound. There are two types of compound leaves, palmately compound in which the leaflets are all attached at the tip of the petiole, or pinnately compound in which the leaflets arise along the sides of a central stalk. The edges of the leaf or leaflet can either be serrated (toothed) or without serrations.

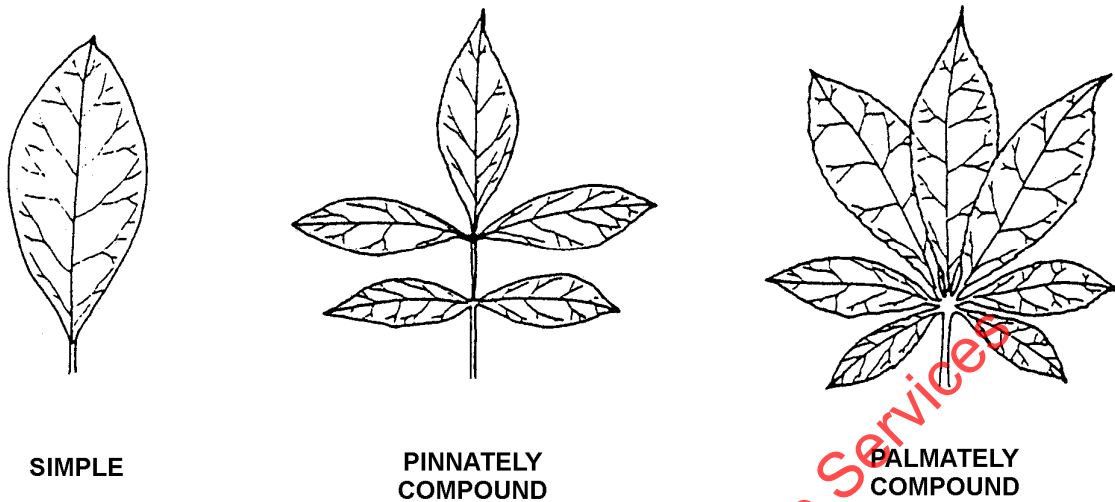


Figure 1 - Leaf Forms

Leaves can either be opposite or alternate in terms of their point of attachment to the stem. This point of attachment is known as the node. If only one leaf is attached to a node, the leaves are said to be alternate. If two leaves are found on a node, the leaves are oppositely arranged.

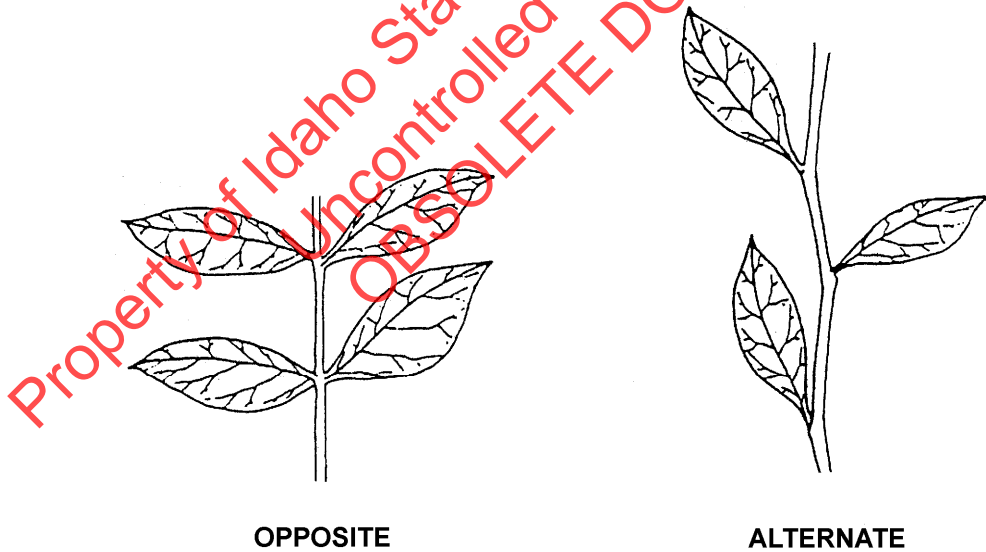


Figure 2 - Leaf Arrangements

Finally, leaves and leaflets are described in terms of their shape.

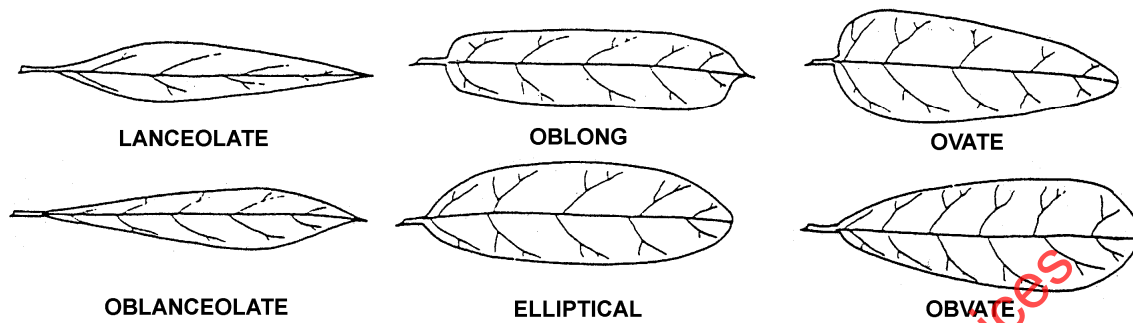


Figure 3 - Leaf Shapes

The leaves of the marijuana plant are its most distinctive feature and are easily recognizable. Marijuana leaves are palmately compound and usually have 3 to 11 finger-like leaflets. The leaf is usually composed of an odd number of leaflets. The number of leaflets that a leaf possesses generally increases up the stem while the size of the leaves becomes progressively smaller toward the top of the plant. In other words, the lower leaves have fewer leaflets but are larger. The leaf attachments of marijuana are generally opposite near the bottom and alternate near the top. The leaflets are lanceolate in shape (i.e. 6 or so times as long as they are wide and widest below the middle) with a narrow wedge shaped base and a drawn out pointed tip. The leaflets are serrated and the teeth are sharp and pointed toward the tip of the leaflet. On large leaflets, the serrations can have serrations. The petiole or leaf stem has a groove and can be described as u-shaped.

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Figure 4 - Marijuana Leaf

The veins of the leaf are best seen on the lower surface. There is a major vein (the midrib) that runs from the petiole to the tip of the leaflet. There are other veins extending from the midrib to the point of a serration. A tiny vein branches from these veins and leads to the deepest indentation of the adjacent notch. The upper side of each leaflet is deep green in color and the lower side is lighter green.

3.3.0 HAIRS

Many plants have hairs or trichomes over various parts of the plant. Trichomes have several functions, such as protection against dehydration or predation. Two basic types of trichomes are found on plants. Those that secrete substances (referred to as glandular) and those, which don't secrete substances (referred to as non-glandular). Both glandular and non-glandular trichomes can be composed of one cell (unicellular or monocellular) or have more than one cell (multicellular).

Cannabis plants have non-glandular, single-celled cystolithic hairs. These hairs, which are shaped somewhat like a bear claw, are short and fat due to calcium carbonate deposits called cystoliths in the base of the hair. Cystolithic hairs are found on the upper surface of the leaf pointing to the tip of the leaflet and most other parts of the plant. Dilute hydrochloric acid will cause bubbles of carbon dioxide to be freed from these hairs. Non-glandular, single-celled covering hairs are found on the lower surface of the leaf pointing

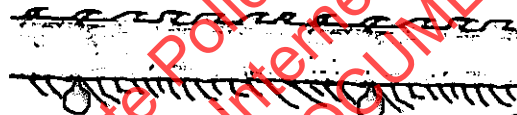
to the tip of the leaf. They are longer and more profuse than the cystolithic hairs. **This combination of cystolithic trichomes on the upper leaf surface and covering trichomes on the lower leaf surface is a characteristic that is unique to marijuana.** A plant that does not show cystolithic hairs is not marijuana.

Multicellular glandular hairs are also found on the lower surface of the leaf. These glandular hairs secrete a resin that spreads over the surface of the leaf and also on various parts of the plant's flowers. This resin is thought to reduce moisture loss and protect the plant against predators. This resin contains the active ingredient tetrahydrocannabinol (THC). The glandular hairs look like glistening globules on the surface of the leaf. There are two types of glandular hairs: Stalked, which are found mainly on the seed hulls and usually not found intact, and the sessile (without a stalk) hair, which is found mainly on the lower surface of the leaves.

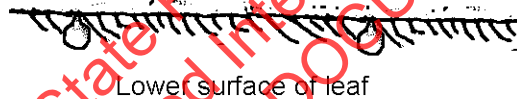


Cystolithic hairs - found on the tops of the leaves and most other parts of the plant.

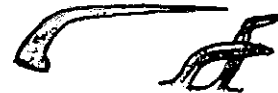
Upper surface of leaf



Lower surface of leaf



Glandular hairs - found on the lower surface of the leaves but mostly around the flowers.



Soft covering hairs found on the lower surface of the leaves

Figure 5 - Plant Hairs

3.4.0 STEMS:

The term "stalk" is a legal or vernacular term, which is defined by Webster as "the main stem of an herbaceous plant often with its independent parts". Botanists consider the correct term to be "stem" and define it as "the major supporting structure in plants, to which buds, leaves, and flowers are attached at regular intervals at points called nodes". In forensic work and in dealing with the legal system both terms will be used. It is important to know that botanists may take exception.

The “stalk” of the marijuana plant is angular with lengthwise fluting. The green outer covering contains tough fiber and is covered with cystolithic hairs that curve upwards with their tips pressing against the stem. Beneath the outer covering is a layer of woody material and within the woody material is the pith. The center is usually hollow. Rather inconspicuous nodes occur on the stalk at intervals of 4 to 20 inches and from these spring the leaves and branches - a branch immediately above each leaf.

The plant branches at the nodes. The branch attachments of marijuana are generally opposite near the bottom of the plant with each pair situated almost at right angles from those above and below them. Near the top of the plant the branch arrangement becomes alternate instead of opposite.

3.5.0 FLOWERS:

The flower of a plant is believed to be a grouping of highly modified leaves. In many plants, both the male and female sex organs are contained in the same flower. The typical flower is composed of four such groupings or whorls. The outermost whorl is composed of the sepals that is collectively called calyx. The sepals are usually green and are easily seen on flowers that aren't completely open such as the green objects surrounding partially opened rose petals. The next whorl proceeding toward the center are the petals, collectively known as the corolla. The next most inner whorl is composed of the stamens, which can be thought of as the male flower parts. Each stamen is composed of a long stalk called the filament, which is terminated by an anther. The anther is a pollen sack, which is made up of chambers that contain the pollen. The final innermost whorl is composed of a pistil, which can be thought of as the female flower parts. A pistil is composed of a swollen lower portion or ovary, which contains the ovules. Attached to the ovary is a stalk-like style, which expands at the tip. The expanded tip is called a stigma and is the receptive surface for the pollen.

Marijuana is “dioecious” i.e., the male and female flowers are borne on separate plants. Since marijuana is dioecious, not all flower parts are contained in the flowers of individual plants. Some parts are in the male flowers and some are in the female flowers.

The male inflorescence is loosely arranged, much branched and many-flowered, standing out from the leaves, with individual flowering branches. Each flower usually has five sepals and five stamens. The flowers, which are usually white, green and yellow, are covered with hairs. Some of these hairs are resin producing. The stamens hang freely from the flower. Each stamen consists of a short slender filament that leads to the anther. The anthers open lengthwise from the tip downwards to release the pollen that is carried by the wind to female flowers.

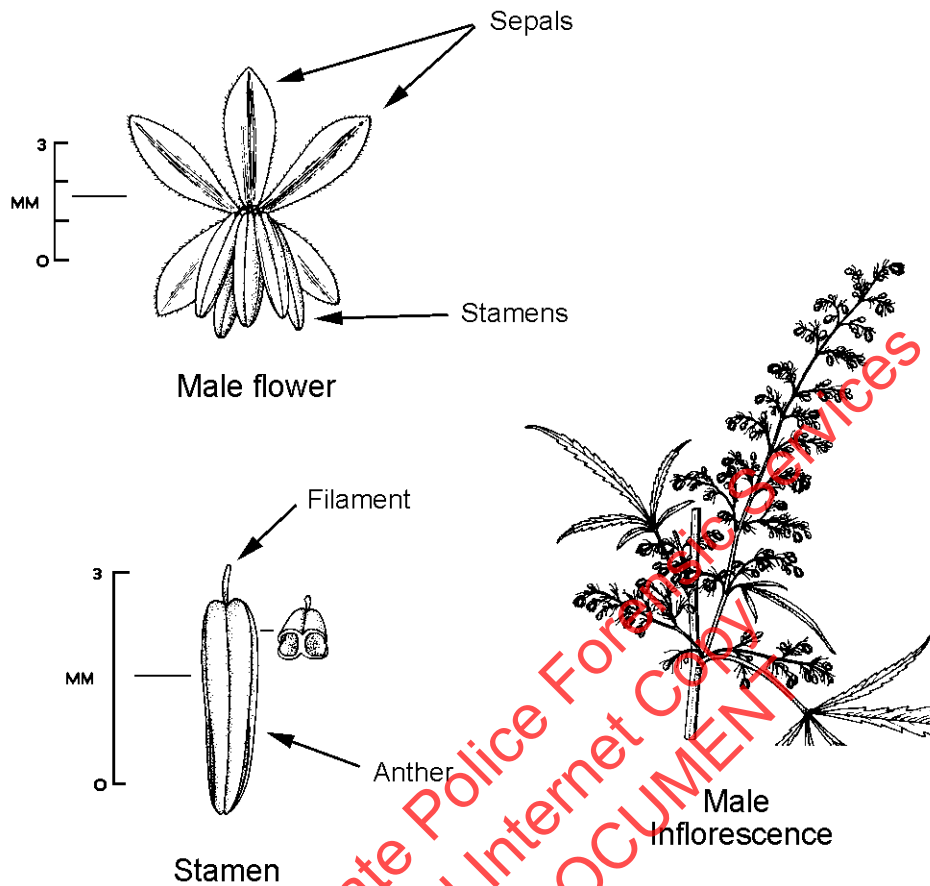


Figure 6 - Male Flower and Inflorescence

The female inflorescence does not project beyond the leaves. They are compact, short and few-flowered. The flowers occur in pairs that grow at the joints of the leaves. At the center of each flower is the pistil. The ovary contains one ovule. The flower also has a small green organ, sometimes called a bract, sometimes a calyx, which completely enwraps the ovary forming a tubular “sheath”. Out of this sheath project the stigmas. After pollination the stigmas quickly fall off. The sheath increases in size as the ovary matures into the fruit and the ovule becomes the seed.

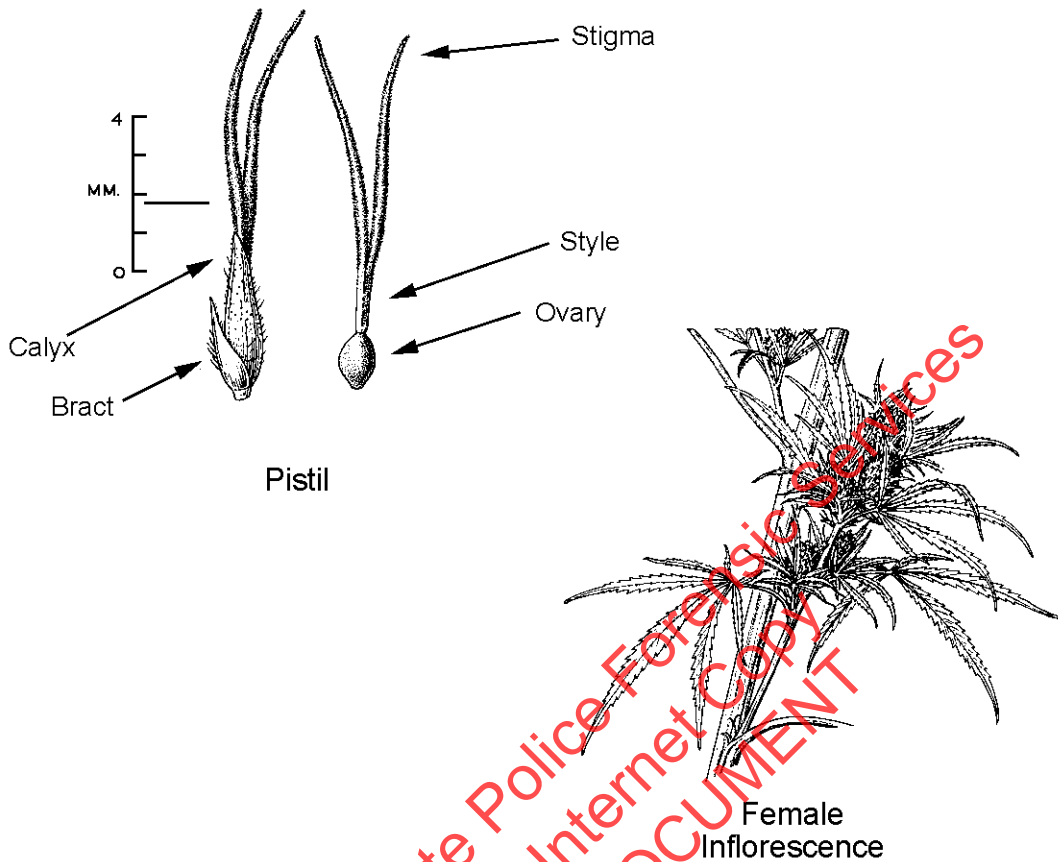


Figure 7 - Female Flower and Inflorescence

Marijuana plant sexes cannot be differentiated with certainty until flowers appear. Female plants tend to be shorter and have more limbs than the male. The female plants appear leafy to the top with many leaves surrounding the flowers while the male plants appear thinly leaved near the flowering limbs. The male flower develops about three to four weeks ahead of the female flower.

There are two theories regarding what determines the sex of a marijuana plant. The first is that sex is determined by physiological stimuli at some stage after fertilization. The second is that sex is determined by inheritance of the XY type. The best explanation is probably a combination of the two. Initially, sex is determined by inheritance. The final production of flowers on an individual plant is influenced by the environment that may override the inherited sex. It has been reported that the ratio of male to female plants can be influenced by exposure of seeds to ultraviolet light, by air temperature, by carbon monoxide concentration, by the age of pollen and the stigma, and by nitrogen concentration in the soil. Unfertilized female plants occasionally produce a few male flowers. This condition is described as “monoecious”. The offspring of these monoecious plants will be mainly female.

Resin is more abundant on the female plant. It is postulated that the copious resin protects the female flower and plant while the seed is developing. The male plant, on the other hand, dies after giving off pollen and does not need to be protected as long. Usually the development of glandular hairs stops on the male flowers when pollination occurs.

3.6.0 SEED:

The fruit in marijuana is technically an achene, i.e. it contains a single seed with a hard shell tightly covered by the thin wall of the ovary, the whole being regarded in practice as a "seed". The seed is 3-5 mm in diameter - about the size of a large kernel of wheat. It is ovoid in shape and has been described as resembling tiny melons. The surface is divided into two "halves" by a rather sharp ridge round the greatest circumference. The surface color may vary from a greenish-yellow to brown and it is frequently somewhat mottled. The surface is covered with characteristically peculiar lacy markings. The interior of the seed is white and oily and resembles coconut meat.

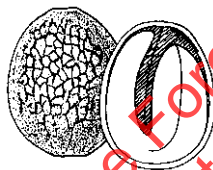


Figure 8 - Marijuana Fruit

Marijuana seeds germinate in 3-7 days. The first structure to emerge from the seed is the radicle or embryonic root. The radicle will eventually develop into a taproot that will have numerous lateral roots growing from it. The first leaves to emerge from the seed are termed cotyledons. There are usually two cotyledons but there can be three. They are slightly unequal in size, oblanceolate shaped and rounded at the base. The cotyledons have trichomes on the upper leaf surface and no trichomes on the lower leaf surface. About 2 cm above the cotyledons the first true leaves develop.

3.7.0 SINSEMILLA:

Occasionally, the term Sinsemilla is applied to marijuana. "Sinsemilla" is Spanish for "without seeds". Sinsemilla is a "high potency" marijuana that is produced by removing the male marijuana plants before they can pollinate the female plants. This causes the female plants to produce more THC rich resin in its flowering buds.

3.8.0 GLOSSARY OF BOTANICAL TERMS

Achene - A dry, one-seeded fruit with a firm close fitting wall that does not split open at maturity.

Alternate - Located singly at a node, as leaves on a stem; situated between other parts, as stamens between petals.

Annual - A plant that completes its development in one year or one season and then dies.

Anther - The pollen containing part of a stamen, usually consisting of two sacs.

Axel - The angle formed between two organs, as between a leaf and stem.

Maxillary (axile) - In the axis; designating flowers borne in the axils of leaves, and ovules or seeds produced in the angles formed by partitions in the ovary of a compound pistil.

Bract - A leaf on a flower, located just below the flower or flower cluster at the base

Bracteole (bractlet) - A secondary bract, often very small.

Calyx - The outermost series of flower parts; the sepals of a flower considered as a group

Cannabaceae - the hemp family

Cannabis sativa - Hemp plant or marijuana.

Capitate - Like a head; in a dense, more or less rounded cluster.

Compound - Composed of two or more parts; compound leaves have two or more leaflets.

Cotyledon - The first leaf or leaves developed in the seed. These leaves are present in the seed and they may or may not enlarge and become green when the seed germinates. Often food materials are stored in them.

Cystoliths - A calcified deposit within a hair.

Dioecious - The flowers are unisexual and the male flowers are on one plant and the female flowers are on a separate plant.

Embryo - The rudimentary plant within a seed.

Epidermis - The outer tissues of a plant.

Fertile - Capable of reproducing, as a stamen producing viable pollen or a carpel producing ovules.

Filament - The stalk of a stamen.

Fruit - A ripened ovary.

Flower - An aggregation of highly modified leaves that make up the reproductive structure of certain plants.

Gland - A secretory hair or other part that produces nectar or some other liquid.

Glandular hair - A hair that produces a resin.

Herb - A plant that dies at the end of a growing season and is not woody stemmed.

Hypocotyl - The part of a seedling below the cotyledons and above the radicle.

Inflorescence - A flower arrangement or cluster.

Lanceolate - Lance shaped.

Leaflet - One of the divisions of a compound leaf

Midrib - The main or central vein of a leaf.

Monoecious - Having separate staminate and pistillate flowers on the same plant.

Morphology - The study of external plant structures and their shapes and forms.

Node - The point of a stem where leaves or branches are attached.

Ob - A prefix meaning inverted, as in "oblongate," upside down lanceolate and broadest above the middle.

Opposite - (leaves) in pairs, one on either side of the node; (stamens) inserted in front of petals and thus opposite them.

Ovary -- The basal portion of a pistil containing one or more ovules.

Ovate - Egg-shaped, the broadest part below the middle.

Ovule - The structure that becomes a seed after fertilization.

Palmate - Compound leaves in which leaflets radiate from a common point, like the fingers of the hand.

Perennial - A plant that continues to live year after year.

Perianth - A collective term for the calyx.

Pericarp - The ovary wall in the fruiting stage.

Petiole - The stalk of a leaf.

Phenotype - The external, manifest, or visible characters of an organism, as contrasted with its genetic constitution (the genotype).

Pinnate - Resembling a feather in the leaflets are on each side of a stem.

Pistil - The organ of a flower which bears ovules and later seeds. It is composed of ovaries, stigma, and style.

Pistillate - A flower with only pistils; a female flower or plant.

Pollen - Minutes spores produced by the anther of a stamen.

Rapine - A seam that joins the two halves of a seed.

Radicle - The part of the seedling which becomes the root.

Root - The absorbing, usually underground part of a plant, without nodes.

Seed - A mature ovule consisting of an embryo and a surrounding protective coat.

Seminal root - The first or primary root produced by a seedling.

Sepal - The outer set of floral leaves (part of the calyx).

Sessile - Lacking a stalk, as some leaves and flowers.

Simple fruit - derived from a single flower and a single pistil

Simple leaf - having the blade in one piece

Simple pistil - consisting of a single carpel.

Sinsemilla - Spanish for "without seeds". When applied to marijuana - very mature female plants that have not been pollinated.

Stamen - The pollen producing part of a flowering plant, consisting of an anther and a filament or stalk.

Staminate - Bearing stamens and consequently male; usually used in reference to unisexual flowers or plants.

Stem - The major supporting structure in plants, to which buds, leaves, and flowers are attached at regular intervals at points called nodes.

Stigma - The part of a pistil on which pollen adheres and germinates, generally terminal in position, and often enlarged.

Style - The stalk-like part of some pistils, connecting the stigma and the ovary.

Taproot - A stout, tapering main root from which arise smaller, lateral branches.

Taxonomy - The study of plant classification.

Trichome - A plant hair.

Variety - A reproducing, natural population of genetically related individuals.

Vascular - Containing conductive tissues.

4.0.0 MICROSCOPIC ANALYSIS OF MARIJUANA

4.1.0 One of the tests performed on suspected marijuana samples submitted to the ISP laboratory is a microscopic examination. Marijuana displays a set of microscopic features that are unique. No other vegetation displays exactly the same appearance under the microscope. It is the view of many analysts that marijuana can be conclusively identified by microscopic examination alone. A positive microscopic examination is required for the identification of marijuana in the ISP laboratory. However, in this laboratory the microscopic examination must be confirmed by additional chemical tests. These tests will be discussed in a later section.

The microscopic examination is performed by viewing a representative sample of the questioned vegetation at 10 to 50 times magnification with a stereo microscope. For a positive identification to be made, leaf material must be present and that leaf material must have cystolithic hairs on one side and profuse covering hairs on the other side. Both types of hairs must in general be pointed towards the tips of the leaves (or if only leaf fragments are present, they must be pointed in the same direction). **Any other characteristics and plant parts that are also present (including vein structure, leaf texture and color, stem material, flower parts, and seeds) should also be examined and considered before arriving at any conclusions.** There are some variations in the appearance of different samples of marijuana due to growing conditions and other factors, but all marijuana samples exhibit common characteristics which can usually be recognized quite easily. To be proficient at identifying marijuana microscopically requires much practice and experience in examining a wide variety of marijuana samples as well as a large number of non-marijuana samples with a microscope.

The stereomicroscope is a low power magnifying instrument which produces a three-dimensional image of a specimen. This is achieved using two separate optical paths showing two slightly different views of the specimen. These two different views allow the user's brain to create a three dimensional image. The viewed image is "erect", that is it is not inverted, which makes the microscope much easier to use. The stereomicroscope basically allows the user to see an image that is similar to that seen by the unaided eye, only magnified. Depending on the lighting, the user can see color, texture, shape, glossiness, and other useful characteristics.

The total magnification of a microscope is the product of the ocular magnification and the objective magnification (total magnification = ocular magnification x objective magnification). For the stereo microscope, the objective is a group of lenses built into the body of the microscope. The oculars are the eyepieces of the microscope and they usually have a fixed magnification printed on them. The total magnification of the microscope can be easily changed by installing oculars with different levels of magnification, or by varying the objective magnification via adjusting the zoom

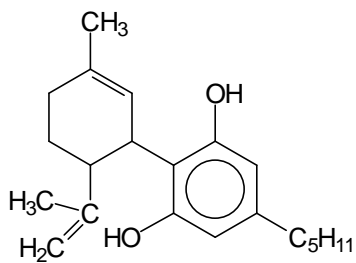
knob.

Most stereo microscopes in forensic labs are mounted on boom stands, allowing a large distance between the microscope head and the heavy base of the stand. This allows the examination of large or bulky objects. Stereomicroscopes also usually have a large working distance. The working distance is the clearance between the upper surface of the object being viewed and the lowest edge of the objective of the microscope. The practical working distance generally decreases with an increase in total magnification.

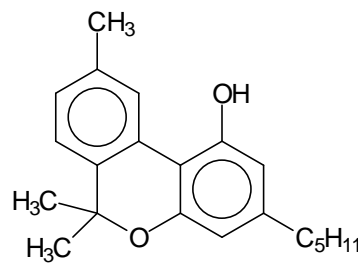
There are two types of lighting that are commonly used: point source and annular. Point source lighting is from a single lamp and allows shadows to form behind high points in the specimen which allows a greater depth awareness of the specimen. Annular or ring lighting is created by a “ring” of light that completely encircles the specimen. This tends to cancel out shadows and provide even illumination across the entire field of view. Bifurcated fiber optic lights can be used as one- or two-point light sources to illuminate the specimen in a variety of ways.

5.0.0 CHEMISTRY AND CHEMICAL TESTING OF MARIJUANA

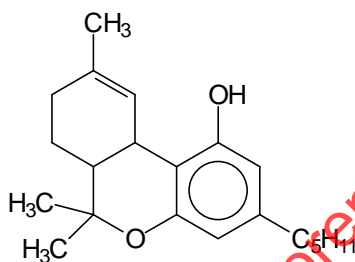
5.1.0 There are over 400 compounds in the marijuana plant, including cannabinoids, amino acids, proteins, sugars, hydrocarbons, steroids and terpenes. Forensic scientists are concerned chiefly with the cannabinoids. Cannabinoids are a group of structurally similar compounds which usually contain 21 carbon atoms. Of the 61 known cannabinoids three are most abundant. These three are; cannabitol (also known as CBN), cannabidiol (CBD) and tetrahydrocannabinol (THC). Tetrahydrocannabinol is considered to be the most pharmacologically active.



Cannabidiol (CBD)



Cannabinol (CBN)



Tetrahydrocannabinol (THC)

Figure 5 - 1 Common Cannabinoids

In attempting to assign chemical names to the cannabinoids, several numbering systems have been used. This has resulted in more than one name being assigned to each compound. The two most common numbering systems are the "Dibenzopyran" system and the "Monoterpene" system.

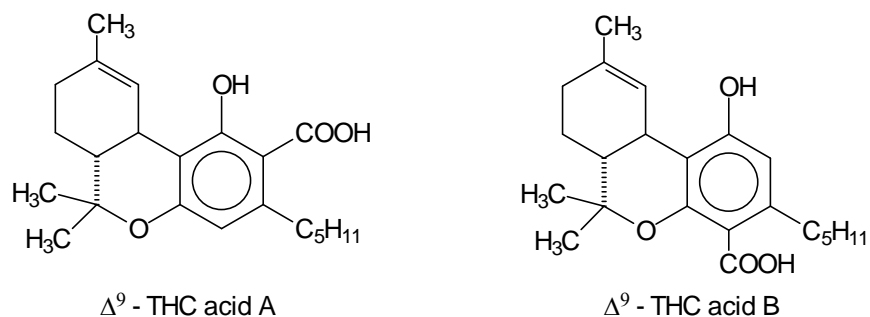


Figure 5 - 3 THC Acids

Both isomeric forms are converted on decarboxylation to Δ^9 - THC. Literature reports that most marijuana contains the A form. It would also seem to be the preferred molecular structure considering that steric hindrance from adjacent groups is minimal in comparison to the B form. However, the fact that the B form has been found in a few marijuana samples indicates the possibility that different variants of cannabis may have slightly different biosynthetic pathways.

Decarboxylation of the acidic cannabinoids is suspected to begin when marijuana is harvested and continues rather slowly on storage. The acid cannabinoids decarboxylate in solvent media especially when exposed to daylight. Under the influence of heat, decarboxylation occurs instantly and completely.

At room temperature, Δ^9 -THC is oxidized to cannabinol at the rate of about 3-5% per month. This conversion is hastened at elevated temperatures. Δ^9 -THC also slowly isomerizes to Δ^8 -THC which decomposes at a lower rate. The decomposition of cannabinoids stored in solvent media is also hastened by light. Various literature references indicate that CBD condenses into THC. Analysis of old samples shows relatively large amounts of CBN and CBD with very little THC so the conversion of CBD to THC must not be nearly as efficient as the conversion of THC to CBN.

5.2.0 CHEMICAL TESTS FOR MARIJUANA

Chemical tests resulting in color production are widely used to test for the presence of marijuana, more specifically, for the cannabinoids produced by marijuana.

5.2.1 Duquenois Test:

The most popular color test is the modified Duquenois-Levine test. The Duquenois test was first reported in 1938; modifications which increase the specificity of the test for cannabinoids have resulted in renaming the test the "modified Duquenois-Levine" test. Various literature articles^{15, 16, 17} have explored the possibility of obtaining false-positives using the modified version of the Duquenois-Levine test. The authors of these articles subjected various chemical compounds and plant materials to the test in an effort to obtain the same color formation as that obtained with marijuana. As a result of these studies, it appears that, when properly used, the modified Duquenois-Levine test can furnish presumptive evidence for the presence of marijuana or a marijuana product. The reagent will not only react with THC, but it will also react with CBD and/or CBN to

provide the same blue-purple color. Combining this chemical test with a careful examination of the morphology of the sample plant material can definitely serve as a reliable screen for marijuana

The procedure for performing the Modified Duquenois-Levine test is given below:

Duquenois Reagent -- 2 grams vanillin, 2 ½ ml acetaldehyde in 100 ml ethyl alcohol (This reagent may be kept for some time in glass-stoppered bottles. Place approximately 1/4 gram of dry crushed sample in a test tube and extract with about 1 ml. of petroleum ether.

1. Transfer the petroleum ether to another test tube evaporate to dryness.
2. Add ~5 drops of Duquenois reagent.
3. Add ~ 5 drops of concentrated hydrochloric acid and mix. The presence of marijuana is indicated by an indigo-violet shade.
4. Add ~ ½ ml. of chloroform and stir. The indigo violet color produced by the action of the reagents on marijuana will partition into the chloroform layer.

NOTE: No strict rule need be observed with respect to amounts of sample or reagents. Those given are convenient. The test succeeds with a much smaller amount of vegetation. With differences in sample size, it is a good idea to adjust the amounts of reagent accordingly.

Below is a proposed mechanism for the Duquenois reaction. There may be other possible mechanisms.

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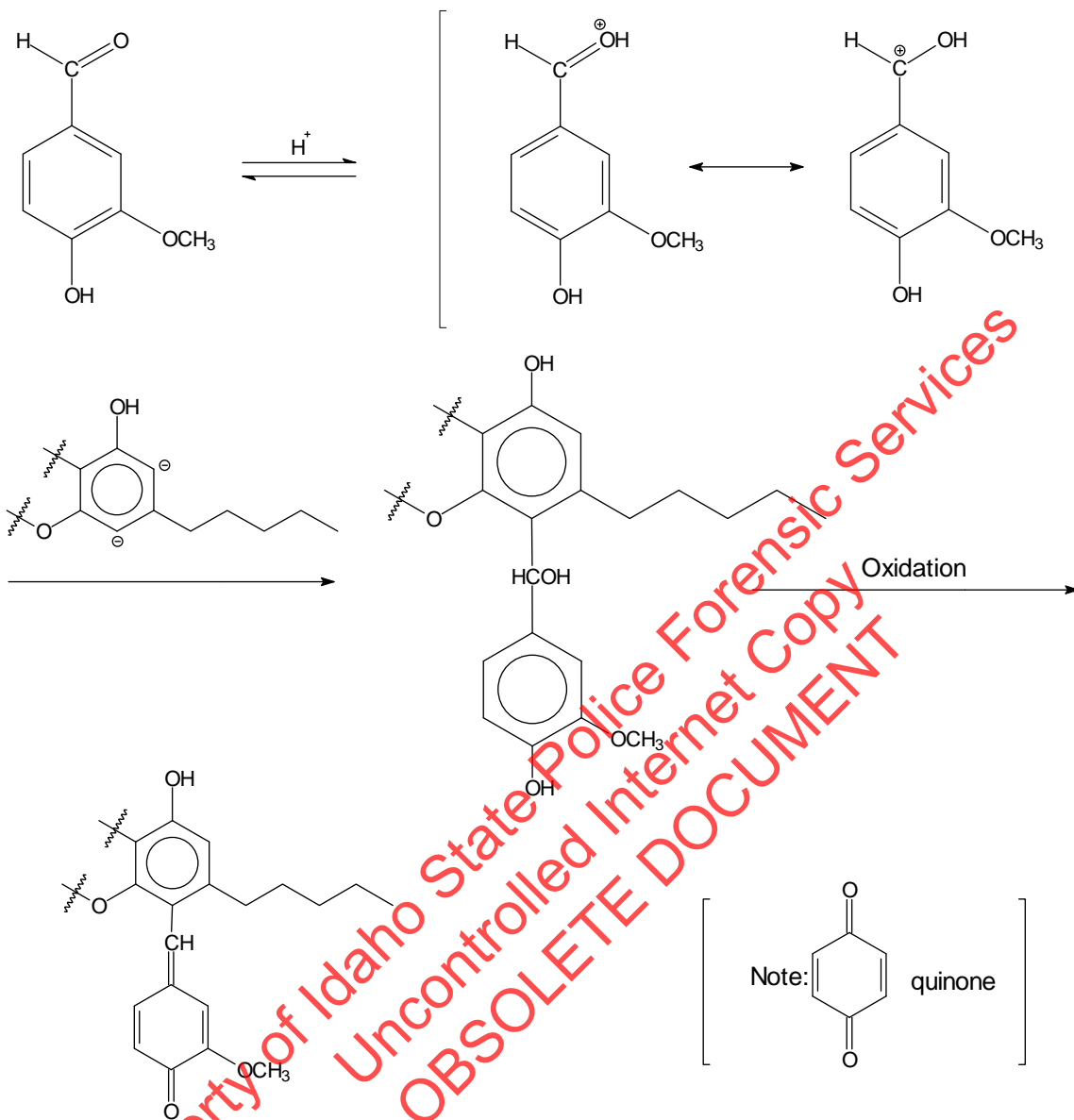


Figure 9 - Duquenois Reaction

The petroleum ether extract removes the cannabinoids from potentially interfering substances. A non-polar solvent is used because many of the compounds present on the plant material are insoluble in this type of solvent. The cannabinoids by virtue of the n-amyl side chain of the phenolic ring are soluble in non-polar solvents.

Vanillin is protonated under acidic conditions. This is why the Duquenois reagent is not mixed with acid until the test is conducted. The protonated aldehyde group of vanillin acts as an electrophile which attacks the phenolic ring of the cannabinoids. The para-hydroxy group of vanillin is not disturbed in the initial attack on the cannabinoid. The intermediate product resulting from the initial attack then undergoes further oxidation to

form a highly conjugated quinone-like compound. Conjugated compounds are usually colored.

The exact role of the acetaldehyde in the reaction is unknown. The reaction will proceed without it, albeit more slowly. Its role is probably as an oxidizing agent. Since it is an aldehyde like vanillin, it can also substitute at the ortho and para positions of the phenolic ring of the cannabinoids and then undergo further condensation.

The reaction is acid catalyzed and the final colors are pH dependent. This is the reason why a range of colors is considered positive for the cannabinoids and why it is important to always use a consistent amount of Hydrochloric Acid when performing the Duquenois test. The range of colors is also dependent on the relative proportions of the cannabinoids which are present in the sample.

The Duquenois test is designed to detect molecules containing structures similar to the cannabinoids. Like any other color test, it reacts with the chemical moiety and not just a specific compound. The Duquenois reagent does react with other phenolic and terpene derivatives but the product, if colored, may not be in the correct color range. There may, however, be materials which produce a color which cannot be distinguishable from the color produced by the cannabinoids. The fact that this possibility exists does not necessarily mean that there are other plant materials which produce a similar color or that the color will be soluble in chloroform.

5.2.2 There are several variations of the Duquenois Test.

Duquenois-Negm: The original Duquenois test where the Duquenois reagent and Hydrochloric acid were added to the evaporated plant extract to form the purple color.

Modified Duquenois-Levine: The Levine modification to the Duquenois-Negm test is the addition of the chloroform.

Physical tests used to identify THC in suspected marijuana samples vary from TLC to GC/MS to IR. TLC is widely used in forensic laboratories because of the availability of many solvent systems, supports, and sensitive visualizing reagents. There is little doubt that a mass spectrum, an infrared spectrum, or a nuclear magnetic resonance spectrum will unequivocally identify THC, as well as CBN and CBD.

5.3.0 THIN LAYER CHROMATOGRAPHY:

Thin Layer Chromatography (TLC) is a very common analytical technique that has many applications. It will be covered in greater depth in a later phase of the forensic scientist training program. For now, a brief introduction is all that is necessary to use this technique for marijuana analysis.

Thin Layer Chromatography is a technique that incorporates a solid stationary phase and a liquid mobile phase to effect the separation of the constituents of a mixture. A TLC plate is prepared by coating a glass plate with a thin layer of an adsorbent material. Silica

gel is a commonly used stationary phase. A small amount of the sample to be analyzed is placed near the lower edge of the TLC plate. The plate is then placed in a closed chamber called a development chamber that contains a selected liquid or solvent. The solvent slowly travels up the plate by capillary action. This rising solvent serves as the mobile phase. As the solvent moves past the sample spot, the components of the sample will become distributed between the solid stationary phase and the liquid mobile phase. Those components with the greatest affinity for the mobile phase will travel up the plate faster, and hence farther, than those that have a greater affinity for the stationary phase. When the solvent front, has moved a sufficient distance (usually almost the entire height of the plate) the development of the plate is complete. The plate is removed from the chamber and allowed to dry.

Often the various components of the sample cannot be seen with the naked eye. A number of methods are available to "visualize" them. Often a chemical reagent is sprayed on the plates. This reagent reacts with the various components of the sample forming colored spots.

The distance a compound moves up a TLC plate can be assigned a numerical value known as the R_f value. This value is defined as the distance traveled by the compound divided by the distance traveled by the moving liquid phase. The actual R_f value for a given compound can vary due to a number of different factors including: humidity, relative concentration of components in the developing solvent, thickness of the stationary phase, chamber saturation, etc. Because of the variability of the R_f values, questioned samples are developed on the same plate alongside an authentic standard. If both the sample and the standard travel the same distance up the plate, they can be tentatively identified as being the same. It must be cautioned that such an identification cannot be considered definitive, for the possibility exists that other substances can migrate the same distance up the plate when chromatographed under similar conditions. Thus, a single TLC cannot by itself provide an absolute identification. It must be utilized in conjunction with other testing procedures to prove absolute identity.

In years past, many forensic laboratories, used a combination of two TLC procedures using two different developing solvents to identify THC. The two solvent systems separate the various components in the samples differently. The combination of the two solvent systems greatly enhance the specificity of identification. An authentic Δ^9 -THC standard is run alongside the sample in these tests. The samples are visualized with either Fast Blue B ((3,3'-dimethoxybiphenyl - 4,4' - bisdiazonium chloride also known as ortho-dianizadine) or Fast Blue 2B (4 - benzoylamino - 2,5 - diethoxybenzenediazonium chloride). Both of these reagents react with the cannabinoids to give unique colors. Literature reports that very few compounds will react with these reagents to give the exact same color as THC. Those compounds that do give the same color will not migrate the same distance on the TLC plates as THC. Therefore, the two different developing solvents in combination with the specific visualizing reagents makes the dual TLC identification procedure highly specific for identifying Δ^9 -THC.

Marijuana identification at ISP is accomplished via microscopic examination backed up by a single TLC procedure and a Duquenois-Levine test. For samples that cannot be identified as marijuana via a microscopic examination, Δ^9 -THC is identified by a combination of two TLC systems and Duquenois-Levine test.

5.4.0 GAS CHROMATOGRAPHY/MASS SPECTROMETRY (GC/MS):

Gas Chromatography/Mass Spectrometry (GC/MS) is a very common instrumental technique that has many applications. It is considered state of the art for many forensic analyses. Both gas chromatography and mass spectrometry will be covered in depth in a later phase of the forensic scientist training program. For now, a brief introduction is necessary to use this technique for marijuana analysis.

A GC/MS is really two analytical instruments attached in sequence. The first instrument is the gas chromatograph. Like all forms of chromatography, gas chromatography employs a mobile phase and a stationary phase. A tube known as a "column" contains the stationary phase. In the instruments used in this laboratory for THC analysis, the stationary phase is a very viscous liquid (typically made of cross-linked polymerized methyl or phenyl-methyl siloxane compounds) bonded to the inside walls of the column. The mobile phase is an inert gas such as Helium flowing through the column. This mobile phase is often called the "carrier gas". The sample is dissolved in a solvent and injected on one end of the column. The column is contained within an oven. The temperature within the oven is raised to a point where the components within the sample mixture vaporize. The components are carried through the column by the carrier gas. As they move through the column, they continuously partition between the mobile phase and the stationary phase. The amount of time it takes a given component to travel through the column depends on how much time it spends in the stationary phase, and the gas flow rate. The amount of time a component spends in the stationary phase depends on a variety of factors including the component's vapor pressure, molecular weight, polarity, and atomic makeup. Different chemical compounds will travel through the column at different rates. The amount of time between the point where a compound is injected on one end of the column and when it elutes from the other end of the column is called the retention time. In most instances, compounds can be tentatively identified by comparing their retention times with the retention times of known standards.

In the typical GC/MS system, compounds pass from the end of the GC column into the mass spectrometer. The compounds initially enter an area of the mass spectrometer known as the ion source where they are bombarded by electrons. The neutral molecules are ionized to form a variety of products, including positive ions. Initially the ions consist of whole molecules with a single electron missing. This is known as the "molecular ion". Loss of an electron often destabilizes the molecule causing it to fragment into both neutral and charged species. While fragmentation can occur by breaking any bond in the molecule, the bond cleavage tends to occur at certain preferred locations, giving rise to a reproducible distribution of ions which is unique for most compounds.

The positive ions are electronically ejected from the ion source through a series of electronic “lenses” where they are focused into the mass filter.

The quadrupole filter consists of four parallel electrodes (rods) held in a square array. To each diagonally paired set of rods a combination of radio frequency (rf) and dc voltage is applied. One pair receives an rf voltage and positive dc voltage, and the other pair receives an rf voltage with an 180° phase shift and a negative dc voltage. These voltages create an electrostatic field that causes the ions to oscillate as they travel along the space between the rods. Only ions with the proper mass to charge ratio will have a stable trajectory through the quadrupole array at a given voltage and rf combination. The voltage and rf values are “ramped” through a range of appropriate values to sequentially pass ions with ascending or descending (depending on the model of instrument) mass to charge ratios.

Once ions are ejected from the mass filter, they are quantitatively detected by an electron multiplier which creates an amplified signal. This signal is fed to an appropriate data handling system.

The data handling system depicts the data in the form of a bar graph in which each bar represents a mass of ion detected, with the height of the bar being proportional to the ion’s abundance. This graph is known as a mass spectrum. The majority of chemical compounds give unique mass spectrums. In most instances, comparing the mass spectrum and GC retention time of an unknown compound with those of a known compound run on the same instrument under identical conditions is enough for absolute identification of a unknown compound. Some isomers and diastereoisomers have similar or identical ion distributions and retention times. These instances are not a problem in THC analysis and will be covered later in the training program.

5.5.0 Exercises:

E5-1. Extract a sample of marijuana with petroleum ether and place equal amounts of the extract into five test tubes and evaporate. Place an equal amount of Duquenois reagent into each of the test tubes. To the first test tube add 10 drops concentrated HCl. To the second add 1 drop concentrated HCl. To the third add one drop of 9 parts concentrated HCl diluted with 1 part water. To the fourth add one drop of 5 parts concentrated HCl diluted with 1 part water. To the fifth add one drop of 1 part concentrated HCl diluted with 1 part water. Add an equal amount of chloroform to each test tube. Does the Duquenois-Levine reaction appear to be very dependent on acid concentration? Explain.

E5-2. Take a marijuana sample and divide it equally among three test tubes. Extract one sample with petroleum ether. Extract one sample with methanol. Evaporate the extracts and perform the Duquenois-Levine on these extracts. At the same time perform the rapid Duquenois-Levine test on the third vegetation sample.

Are there any color differences between the three different tests? If so, explain why these color differences could have arisen.

- E5-3. Perform TLC and DL tests on a fresh samples of dry coffee, octanol, and patchulli oil.
- E5-4. Draw the 8 possible isomers of THC. Indicate, by name only, which one(s) are referenced in your readings as being naturally occurring.

6.0.0 MISCELLANEOUS

6.1.0 The purpose of this section is to cover the information what was not covered in the other sections. For this reason, it will appear as a sort of hodgepodge of information.

Cigarettes that appear to have gotten wet or smell like organic chemicals may contain PCP and should be subjected to further analysis.

Crack cocaine is sometimes mixed with the vegetation in cigarettes. If small white masses are noticed during the microscopic exam, the sample should be subjected to further analysis.

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8.0.0 HISTORY

Prior to revision 3 modules in the training manual did not have individual history pages.

Revision #	Issue or review date	History	Author or Reviewer
3	7/08/11	Added 8.0.0	David Sincerbeaux