The Objectives and Purposes of the California Association of Criminalists

1. Foster an exchange of ideas and information within the field of criminalistics.
2. Foster friendship and co-operation among the various laboratory personnel.
3. Stimulate research and the development of new techniques within the field.
4. Encourage financial support for worthy research projects.
5. Encourage the compilation of experience data of value in the field.
6. Promote wide recognition of the practice of criminalistics as an important phase of jurisprudence.
7. Promote a high level of professional competence among criminalists.
8. Encourage uniform qualifications and requirements for criminalists and related specialists.
9. Disseminate information to the law profession concerning minimum qualifications for physical evidence consultants.
10. Provide a board of review in cases involving differences of professional opinions when requested.
11. Encourage the use of improved testing procedures and methods of presentation of conclusions.
12. When appropriate, to review and act upon any pending legislation which appears to be related to the field of criminalistics.
13. Encourage the recognition of this Association and its purposes among other appropriate groups and societies.
14. Lend assistance, whenever possible, in the formulation of college curricula and law enforcement training programs.
15. Establish a code of ethics for criminalists.

J. Forens. Sci. Soc. (1972), **12**, 461

The Identification of Marijuana

J. I. THORNTON
School of Criminology, University of California, Berkeley, California, U.S.A., 94720
and
G. R. NAKAMURA*
Bureau of Narcotics and Dangerous Drugs, U.S. Department of Justice, San Francisco, California, U.S.A., 94102

The chemistry of the phenolic constituents of the resin from the leaves and flowering tops of the marijuana plant is reviewed, and discussed in terms of possible chemical precursors to synthetic tetrahydrocannabinol which may be encountered. The Duquenois test is studied and support presented for the hypothesis that the mechanism of the test is in part an electrophilic substitution type of reaction. Evidence is presented to suggest that the product of the Duquenois reaction is not a reticulated polycondensate, but exists as a resole. A study is made of the Levine modification of the Duquenois test involving a terminal extraction of the color with chloroform. The ability of Duquenois reactants to extract into chloroform is related to the structure of the phenolic constituents of the resin. Thin-layer chromatographic separation of the major constituents of marijuana resin is described, as well as the infrared absorption of tetrahydrocannabinol isomers. The occurrence of cystolith hairs are an important criterion in the identification of marijuana leaf fragments. Cystolith hairs, however, occur among several dicotyledonous families, notably the Moraceae, Boraginaceae, Loasaceae, Ulmaceae, and Cannabinaceae. Since these develop in different forms in these families, marijuana can be differentiated in many cases. However, in other cases where similarity in cystolith development occurs, other morphological features, such as the trichomes on the other side of the leaf, are helpful in differentiation. In any event, cystolith hairs cannot be used as a sole criterion for marijuana identification. The Duquenois-Levine test is found to be useful in the confirmation of marijuana, since none of the 82 species possessing hairs similar to those found on marijuana yield a positive test. Where marijuana cannot be morphologically delineated, thin-layer chromatographic methods are indicated.

PART I: CHEMISTRY

Introduction

The possession of marijuana is an offense proscribed by law in every one of the United States and by the Federal Government. Although the importation, sale, or possession is subject to rigid control or heavy penalty, the illicit use of marijuana has proliferated in all areas of the country.

In any case involving the possession of marijuana, a rigorous identification of the material is universally considered to be necessary before proceeding with the administration of criminal justice. When the leaves, seeds, or flowering tops of the plant are submitted for examination, an identification may be based upon the botanical appearance of these parts as observed microscopically. No other plant has ever been reported as having an identical microscopic appearance.

Although a rigorous identification of the marijuana plant may be effected through an examination of its botanical chracteristics, it is generally considered advisable to perform a chemical test in most instances, and necessary to perform it in others. The chemical test is a parameter of the identification which is unrelated to the microscopic appearance and independently confirms the botanical examination. In addition, instances may arise in which no recog-

* Present address: Los Angeles County, Dept. of Chief Medical Examiner-Coroner, Los Angeles, California, U.S.A. 90033.

nizable plant material is available, either as an extract of the plant as in the case of hashish, or in which only a residue of resin or a charred ash is encountered.

The Duquenois test, the most widely used chemical test, is a somewhat enigmatic reaction whose mechanism is poorly understood. The investigation of this chemical test for marijuana and its reactivity with isolated phenolic and terpene constitutents of the marijuana plant comprises the major portion of this work. In addition, the thin-layer chromatographic separation of the phenolic constituents provides a technique for the unequivocal identification of one or more isomers of tetrahydrocannabinol, the pharmacologically active principle of marijuana.

Chemical Constituents of Marijuana

The chemical investigation of the constitutents of marijuana began slightly over a century ago, although no substantial progress was made until the early 1930's. All of the constitutents of marijuana are purified with extreme difficulty and are exceedingly difficult to crystallize. In addition, many of their derivatives are also difficult to crystallize. This has no doubt been one of the more severe impediments to the elucidation of the chemistry of marijuana. The application of the separation techniques of column and gas chromatography, along with more sophisticated analytical instrumentation such as nuclear magnetic resonance, has recently partially compensated for the lack of sustained interest in the chemistry of marijuana.

Cannabinol

In 1847 two Scottish brothers, T. and H. Smith, demonstrated that the pharmacologically active principle of marijuana was contained in a high boiling portion of the resin from the plant (Smith and Smith, 1847). Vacuum distillation of the resin resulted in a "red oil" which was eliminated as being alkaloid in nature (ibid.).

The literature does not indicate further interest in the chemistry of marijuana until 1896, when the British chemists Wood, Spivey and Easterfield isolated an active oil to which they gave the name cannabinol (Wood, Spivey and Easterfield, 1896). Although originally considered to be a pure product, the same workers later reported a further purification as a crystalline acetate from the oil in about 25% yield (Wood, Spivey and Easterfield, 1899). The name cannabinol was then transferred to the material isolated as the acetate, and the original material referred to as "crude cannabinol". The isolation of a crystalline cannabinol acetate was reported in the previous year by Dunstan and Henry (1898), but no experimental details were given.

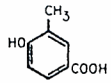
Attempts to confirm the work of Wood, Spivey and Easterfield were made by Fraenkel (1903), Czerkis (1907), Casparis and Baur (1927), and Bergel and Wagner (1930). None of these workers were successful in the isolation of cannabinol in a crystalline form, either as cannabinol or as the acetate.

The isolation of a crystalline cannabinol acetate was again reported in 1930 by the English chemist Cahn (1930), who subsequently elucidated the structural skeleton of the cannabinol molecule (1930, 1931, 1932, 1933). The results of his work may be briefly summarized. Natural cannabinol may be nitrated to a trinitrocannabinol. Nitric acid oxidation of this trinitrocannabinol yields a nitrocannabinolactone, along with caproic, valeric, and n-butyric acids. This nitrocannabinolactone may be reduced to the corresponding aminolactone, and replacement of the amino group with subsequent reduction yields a cannabinolactone. This cannabinolactone was subjected to exhaustive further examination.

Upon fusion with alkali, cannabinolactone yields m-toluic acid (I), and upon oxidation with permanganate followed by alkali fusion gives isophthalic acid (II).

(I)

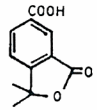
(II)



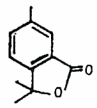
(III)

Diazotization of the aminolactone described above and replacement of the amino group with a hydroxyl group yields a hydroxycannabinolactone. Alkali fusion of this hydroxycannabinolactone produces 6-hydroxy-3-toluic acid (III) and acetone, while oxidation resulted in hydroxytrimellitic acid.

Oxidation of cannabinolactone with alkaline permanganate gave cannabinolactonic acid (IV). The identity of this acid was firmly established by comparison with a synthetic 4-carboxydimethylphalide. Cahn proposed that on the preceding basis, cannabinolactone be given the structure (V). The structure was unequivocally established by synthesis of this lactone by Bergel and Wagner (1930).

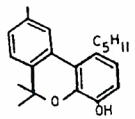


(IV)



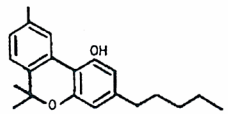
(V)

The structure of cannabinolactone accounts for eleven of the twenty-one carbons in cannabinol. Cahn noted that cannabinol contains a hydroxyl group as evidenced by the formation of an acetate, and that it reacts in the manner of a phenol. He further noted that this phenolic ring must bear an n-amyl substituent, as n-caproic acid is a product of oxidation of cannabinol. Cahn suggested the structure (VI) for cannabinol, in which the positions of the hydroxyl and amyl groups are not precisely elucidated.



(VI)

Cahn's work on cannabinol terminated in 1934. The precise determination of the structure of cannabinol was completed in 1940 by the independent work of Adams (Adams, Pease and Clark, 1940; Adams, Pease, Clark and Baker, 1940; Adams, Cain and Baker, 1940; Adams, Baker and Wearn, 1940; Adams and Baker, 1940c; Adams and Baker, 1940a) in the United States and Todd (Work, Bergel and Todd, 1939; Ghosh, Todd and Wilkinson, 1940; Jacob and Todd, 1940) in England. Both groups of investigators proved by synthesis that cannabinol has the structure 1-Hydroxy-3-n-amyl-6,6,9-trimethyl-6-dibenzopyran (VII).

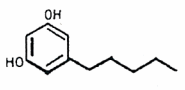


(VII)

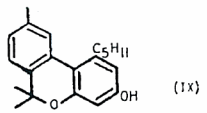
The work of Adams and Todd was quite similar in approach and in the conclusion reached. Only the work of Adams is outlined here to avoid duplication.

The position of the n-amyl group and the hydroxyl group in the structure as proposed by Cahn was given careful scrutiny. Assuming the basic dibenzopyran skeleton to be correct, twelve structural variants are possible. Of these, eight could be eliminated in consideration of the fact that cannabinol gives a strong indophenol test, indicating the presence of an unsubstituted position *para-* to the phenol. Two addition variants were considered unlikely on the basis of the facile introduction of two nitro groups into the phenolic ring.

Adams had meanwhile been successful in isolating another material from marijuana resin which was to be of assistance in the elucidation of the structure of cannabinol. This new material, $C_{21}H_{30}O_2$, was given the name cannabidiol. The degradative work and ultraviolet and infrared absorption suggested that it contained an olivetol (VIII) moiety.



(VIII)



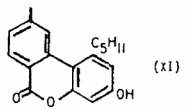
(IX)

Reasoning that structural relationships might prevail between cannabinol and cannabidiol, as they originate from the same plant and may share a metabolic pathway, Adams suggested two possible alternatives for cannabinol, 1-Hydroxy-3-n-amyl-6,6,9-trimethyl-6-dibenzopyran (VII) and 1-n-Amyl-3-hydroxy-6,6,9-trimethyl-6-dibenzopyran (IX).

It is apparent that both (VII) and (IX) contain an olivetol moiety, differing only in the relative orientation of the phenolic ring.

The two isomers were produced by synthetic methods by Adams and were compared with natural cannabinol. The (IX) isomer was prepared in the following manner: Olivetol was condensed with 4-methyl-2-bromobenzoic acid (X) in the presence of dilute alkali and copper sulfate to form the pyrone, 1-n-Amyl-3-hydroxy-9-methyl-6-dibenzopyrone (XI), which was converted to the pyran (IX) by methylmagnesium iodide.

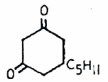
(X)



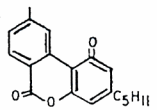
(XI)

Comparison of (IX) with natural cannabinol indicated that the synthetic isomer was dissimilar to natural cannabinol.

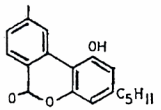
Condensation of dihydroolivetol (XII) with 4-methyl-2-bromobenzoic acid in the presence of dilute alkali and copper sulfate gave a tetrahydropyrone (XIII). This pyrone was dehydrogenated with sulfur to yield the dibenzopyrone (XIV). The dibenzopyrone was converted to the pyran (VII) with



(XII)



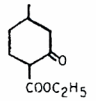
(XIII)



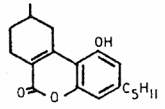
(XIV)

methylmagnesium iodide. Comparison of this material with natural cannabinol confirmed the structural identity of cannibinol.

Cannabinol was synthesized in a second manner by Adams, who subsequently utilized this method for the synthesis of a number of tetrahydrocannabinol analogs. Olivetol was condensed with ethyl 5-methylcyclohexanone-2-carboxylate (XV) to give the tetrahydropyrone (XVI).

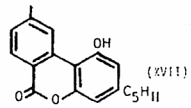


(XV)



(XVI)

This pyrone was dehydrogenated with sulfur to yield the dibenzopyrone (XVII). This was converted to the dibenzopyran cannabinol (VII) by



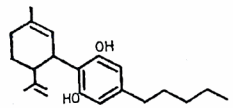
(XVII)

methylmagnesium iodide. Cannabinol produced by this method was shown by Adams to be indistinguishable from natural cannabinol.

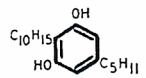
Cannabidiol

Before the structure of cannabinol had been firmly established, Adams and his group had isolated a crystalline material from marijuana resin as the bis-(3,5-Dinitrobenzoate) (Adams, Hunt and Clark, 1940b). Hydrolysis of this derivative by anhydrous ammonia in toluene produced a crystalline dihydric phenol which was given the name cannabidiol (XVIII).

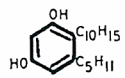
(XVIII)



Infrared and ultraviolet absorption spectra indicated cannabidiol to be a resorcinol derivative (Adams, Cain and Wolff, 1940). Oxidative degradation with alkaline permanganate produced caproic acid, just as it had with cannabinol. On this basis, Adams suggested two partial structures for cannabidiol, (XIX) and (XX).



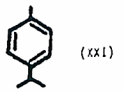
(XIX)

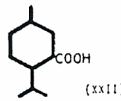


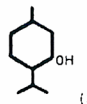
(XX)

Treatment of cannabidiol with hydrogen in the presence of a platinum catalyst resulted in the uptake of 2 moles of hydrogen, and as the olivetol moiety was not affected by this reduction, the absorption of the hydrogen indicated the presence of two double bonds in the remaining part of the molecule (Jacob and Todd, 1940; Adams, Hunt and Clark, 1940a). Treatment of cannabidiol with hot pyridine resulted in the cleavage of the molecule to p-cymene (XXI) and olivetol. The formation of p-cymene indicated that cannabidiol was likely to be a conjugation of olivetol and a menthyl moiety.

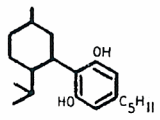
Oxidation of tetrahydrocannabinol produced by catalytic hydrogenation of cannabidiol produced a menthane-carboxylic acid (ibid.) (XXII) which was identical to that previously synthesized from l-menthol (XXIII).

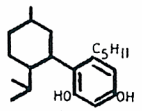

(XXI)


(XXII)

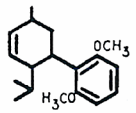

(XXIII)

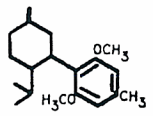
The olivetol moiety of cannabidiol was concluded by Adams to be attached to the remainder of the molecule at the position of the carbon adjacent to that bearing the isopropenyl group, as the acid (XXII) is identical to that produced by the treatment of menthylmagnesium chloride with carbon dioxide and structural similarities may prevail. From these data, Adams suggested two possible structures, (XXIV) and (XXV), for the tetrahydro- derivatives of cannabidiol.

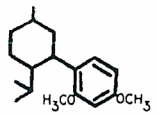

(XXIV)

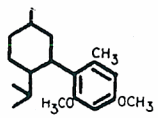

(XXV)

The position of the linkage between the menthyl and the olivetol moieties was further confirmed by ultraviolet absorption spectra. Adams and his group synthesized the following model compounds for purposes of comparison (Adams, Wolff, et al, 1940b:


(XXVI)


(XXVII)


(XXVIII)

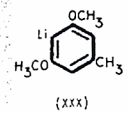

(XXIX)

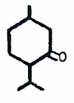
The ultraviolet absorption spectrum of the tetrahydro derivative of cannabidiol was quite similar to (XXVI) and (XXVII), but was significantly dissimilar to that of (XXVIII) and (XXIX). On the basis of the preceding, Adams suggested that the menthyl group in the tetrahydrocannabidiol dimethyl ether was joined to the olivetol moiety at the position of the carbon between the two ether groups (ibid.).

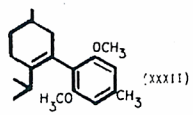
Adams concluded that the structure (XXIV) be assigned to the tetrahydro derivative of cannabidiol. The carbon skeleton of cannabidiol was now elucidated.

The determination of the position of the two double bonds in the menthyl portion of the cannabidiol molecule was attended by some difficulty. The approach utilized by Adams and his group was a combination of degradative procedures and synthesis of model compounds for comparison purposes.

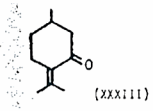
Condensation of 2-lithioorcinol dimethyl ether (XXX) with menthone

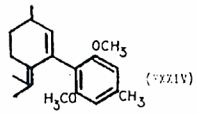

(XXX)

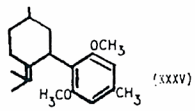

(XXXI)


(XXXII)

1940a). Condensation of 2-lithioorcinol dimethyl ether with pulegone (XXXIII) followed by dehydration produced (XXXIV) (ibid.).


(XXXIII)


(XXXIV)

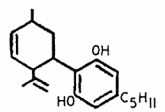

(XXXV)

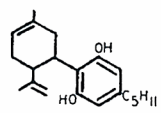
The ultraviolet spectrum of (XXXII) was found by Adams to be totally unlike that of natural cannabidiol dimethyl ether. The absorption spectrum of (XXXIV), likewise, did not agree with that of natural cannabidiol dimethyl ether, although it agreed closely with that of (XXXII), in which one double bond is conjugated to the aromatic ring. Reduction of (XXXIV) gave (XXXV), 2-(2′-Isopropylidene-5′-methylcyclohexyl)-orcinol dimethyl ether. The ultraviolet spectrum of this compound closely agreed with natural cannabidiol dimethyl ether.

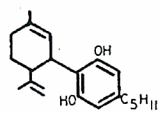
On the basis of the close agreement in ultraviolet absorption between cannabidiol and the reduced synthetic model, Adams concluded that the two double bonds in the menthyl moiety are not conjugated with the aromatic nucleus (ibid.). Todd and co-workers reached the same conclusion independently through a consideration of the ultraviolet spectrum of cannabidiol with particular emphasis being placed on the comparatively weak absorptivity of the material at its absorption maxima (Jacob and Todd, 1940).

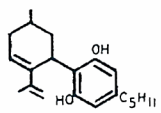
Ozonolysis of cannabidiol was observed by Adams to result in the formation of formaldehyde, enabling him to determine the position of one of the double bonds in the menthyl moiety (Adams, Wolff et al, 1940a). On this basis, two possible positions for the methylene group are possible, and one may be eliminated on the basis of the facile ring closure observed in cannabidiol.

Adams then concluded that the structure of cannabidiol is limited to one of the following compounds (Adams, Pease, Cain and Clark 1940), differing only in the position of the alicyclic double bond:


(XXXVI)


(XXXVII)


(XXXVIII)


(XXXIX)

Adams made an attempt to assess the relative likelihood of the existence of the various positions of the alicyclic double bond. Structure (XXXVI) was

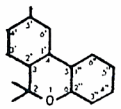
was successful in distinguishing (Adams, Cain et al., 1941). This point has been clarified by subsequent work and will not be considered here in further detail. Structure (XXXVI) was also favored from the standpoint of the lack of formation of a Diels-Alder adduct from cannabidiol and maleic anhydride (Adams, Loewe et al., 1940). Structure (XXXVII) was dismissed by Adams as acid-catalyzed cyclization would result in only one tetrahydrocannabinol, and a driving force for a double bond migration necessary for the isolation of two isomers is not apparent (Adams, Cain et al., 1941).

Depending upon the conditions of acid-catalyzed isomerization, Adams could instigate either double bond migration alone or double bond migration with ring closure. He further suggested that under the conditions for ring closure the alicyclic double bond would migrate into a more stable position conjugated with the phenyl nucleus. As the ultraviolet absorption spectrum of the tetrahydrocannabinol thus formed did not support a double bond conjugated with the phenyl nucleus, Adams dismissed structures (XXXVIII) and (XXXIX) (Adams, Loewe et al., 1940).

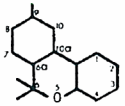
The exact position of the alicyclic double bond in cannabidiol was not determined until 1963, when Mechoulam and Shvo (1963) in Israel investigated this compound using nuclear magnetic resonance (NMR). The NMR spectrum of cannabidiol indicated the presence of three olefinic protons, two of which could be accounted for in the methylene portion of the isopropenyl moiety. The remaining proton must, therefore, be on the double bond.

It is convenient here to clarify the various ring numbering systems utilized to illustrate the structures of cannibis compounds.

The first numbering system to be applied was that of Todd, who numbered the rings as substituted pyrans. The base numbers denote positions on the pyran ring, the prime numbers denote positions on the terpene ring, and the double-prime numbers are denoted to the aromatic ring.

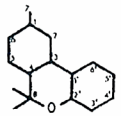


Another numbering system is that which employs the Chemical Abstracts convention. Under this system, the cannabis compounds are regarded as substituted dibenzopyrans.



These two numbering systems have one serious failing, in that those compounds with an open pyran ring, such as cannabidiol, requires that the numbers be totally changed.

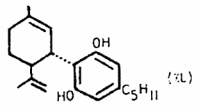
A more satisfactory numbering system is one which distinguishes the terpene and the aromatic moieties in the molecule. The aromatic carbons are denoted by prime numbers, while the terpene molecule is assigned base numbers.



It is this last system that will prevail in further discussion in this work.

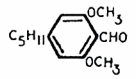
The structure (XXXVI) suggested by Adams may be eliminated on the basis of the NMR spectrum, as the Δ^6-bond position would demonstrate four olefinic protons, two for the terminal methylene group and two for the double bond. Further considerations based on the NMR spectrum led Mechoulam and Shvo to conclude that the double bond is in the Δ^1-position.

Turning their attention to the stereochemistry of cannabidiol, Mechoulam and Shvo concluded that the isopropenyl group and the phenyl ring are *trans*- to one another (ibid.), based on a consideration of the menthane-carboxylic acid (XXII) obtained from oxidation of tetrahydrocannabidiol with permanganate. Structure (XVIII), modified to indicate the stereochemical relationships, may be illustrated as (XL).

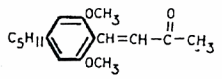


(XL)

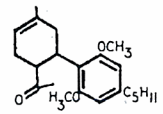
Hackel has reported the synthesis of the Δ^6-isomer of cannabidiol (Hackel, 1963). Alkaline acetone was condensed with 2,6-Dimethoxy-4-n-amylbenzaldehyde (XLI) to form a benzalacetone intermediate (XLII). A Diels-Alder reaction of this benzalacetone with isoprene gave (XLIII) which gave Δ^6-cannabidiol dimethyl ether (XLIV) when treated with methylene triphenylphosphine.



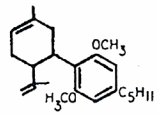
(XLI)



(XLII)

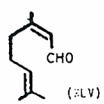


(XLIII)

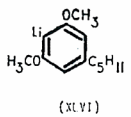


(XLIV)

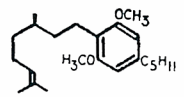
The total synthesis of Δ^1-cannabidiol was reported by Gaoni and Mechoulam (1965). Citral (XLV) was condensed with lithioolivetol dimethyl ether (XLVI) to yield a mixture of products, which presumably contained (XLVII) as one of its constitutents.



(XLV)

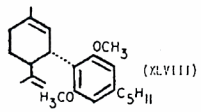


(XLVI)



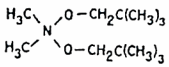
(XLVII)

Treatment of this mixture with p-toluenesulfonyl chloride in pyridine yielded Δ^1-*trans*-cannabidiol dimethyl ether (XLVIII) among other products. This was demethylated with methylmagnesium iodide to give (XL). Cannabidiol prepared in this manner was identical to natural cannabidiol as shown by infrared and NMR spectroscopy.

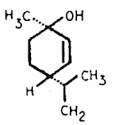


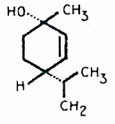
(XLVIII)

Early in 1967, an elegant synthesis was described by Petrzilka and co-workers which involved the one step synthesis of cannabidiol (Petrzilka et al., 1967). The use of optically active starting materials yielded a levorotary product, identical in all respects to the natural material. N,N-Dimethylformamide-dineopentylacetal (XLIX) in methylene chloride mediates the direct formation of *l*-cannabidiol (XL) from d-*trans*-p-menthadiene-(2,8)-ol (L) or d-*cis*-menthadiene-(2,8)-ol (LI) and olivetol. The resulting *l*-cannabidiol


(XLIX)

may undergo mild acid cyclization to *l*-tetrahydrocannabinols. This represents a very strong synthesis which could lead to production of significant quantities of optically active tetrahydrocannabinols. The substitution of 5-n-hexylresorcinol for olivetol would increase the psychopharmacological action of the resulting tetrahydrocannabinol by a factor of two (Adams, Harfenist and Loewe, 1949) while replacement of the aliphatic chain in the resorcinol derivative with a dimethylheptyl group should result in a product approximately 70 times more active than natural *l*-Δ^1-tetrahydrocannabinol (ibid.; Taylor, Lenard and Loev, 1967).


(L)


(LI)

Tetrahydrocannabinol

The psychopharmacologically active principle of the marijuana plant is tetrahydrocannabinol, now known to exist in at least two natural isomers.

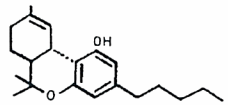
Jacob and Todd reported the isolation of a material from the marijuana plant in 1940 which they considered to be a tetrahydrocannabinol, but the small amount of material isolated did not permit an exhaustive identification (Jacob and Todd, 1940). Haagen-Smit and co-workers also isolated a crystalline compound from American marijuana in the same year, but as in the case of Jacob and Todd the limited amount of material did not permit the nature of the isolated material to be established (Haagen-Smit et al., 1940).

A natural tetrahydrocannabinol was isolated as a non-crystalline acetate by Wollner and co-workers in 1942 (Wollner et al., 1942). The material was isolated by column chromatography on alumina followed by vacuum molecular distillation. Hydrolysis of the acetate could be accomplished by either ethanolic acid or ethanolic base, although the hydrolysis product did not have as strong a pharmacological activity as did the acetate. Wollner and co-workers considered this to be an indication that isomerization had taken place during hydrolysis. Support for this contention came in the form of the observation that a change in specific rotation had also taken place during hydrolysis.

Korte and Sieper were successful in the isolation of a crystalline tetrahydrocannabinol in 1960 through the use of countercurrent extraction (Korte and Sieper, 1960a). The material which was obtained was quite similar to that isolated by Haagen-Smit in 1940 (Haagen-Smit et al., 1940).

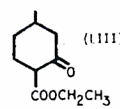
In the same year, De Ropp (1960) described a column chromatographic system for the separation of the constituents of marijuana resin. Using Celite saturated with N,N-Dimethylformamide as the stationary phase and cyclohexane saturated with N,N-Dimethylformamide as the mobile phase, he was successful in isolating tetrahydrocannabinol from Mexican marijuana. The ultraviolet absorption spectrum of this material agreed closely with that obtained from Indian marijuana by Wollner and co-workers (Wollner et al., 1942).

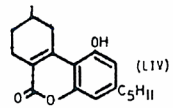
The isolation and structural determination of a tetrahydrocannabinol was reported by Gaoni and Mechoulam (1964a). Tetrahydrocannabinol from Israeli marijuana was isolated by column chromatography using Florisil and alumina. A further purification of the compound was effected by conversion of the material to its 3,5-dinitrourethane derivative. Hydrolysis of the urethane gave a tetrahydrocannabinol to which Gaoni and Mechoulam assigned the structure (LII).


(LII)

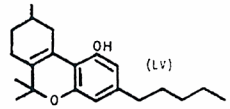
Dehydrogenation of this tetrahydrocannabinol with sulfur gave cannabinol. The structure, with particular reference to the Δ^1-bond, was determined primarily through the application of NMR spectroscopy. Gaoni and Mechoulam prepared tetrahydrocannabinol by refluxing cannabidiol in 0.05% ethanolic hydrochloric acid (ibid.). This prepared tetrahydrocannabinol was compared with the Δ^1-tetrahydrocannabinol isolated from marijuana and was found to be identical in all respects.

Tetrahydrocannabinol was first synthesized in 1940 by the independent work of Adams and co-workers (Adams and Baker, 1940b) and Todd and co-workers (Ghosh, Todd and Wilkinson, 1940). The methods are in essence identical. Methyl cyclohexanone carboxylic acid ethyl ester (LIII) was condensed with olivetol in the presence of phosphorous oxychloride to form the dibenzopyrone (LIV). The resulting pyrone was treated with methylmagnesium


(LIII)


(LIV)

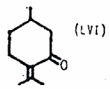
iodide to form the tetrahydrocannabinol, 1-Hydroxy-3-n-amyl-6,6,9-trimethyl-7,8,9,10-tetrahydro-6-dibenzopyran (LV).


(LV)

The psychopharmacological activity of this tetrahydrocannabinol is approximately one-seventh of that observed with natural tetrahydrocannabinol or that derived from the cyclization of cannabidiol (Adams and Baker, 1940b). It must be noted, however, that the synthetic material is racemic, while the natural tetrahydrocannabinols are strongly levorotary (Taylor, Lenard and Shvo, 1966).

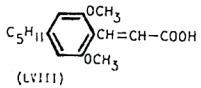
Korte and Sieper duplicated the synthesis of tetrahydrocannabinol described by Adams and co-workers (Korte and Sieper, 1960b). While Adams was not able to isolate a crystalline tetrahydrocannabinol, Korte and Sieper purified the reaction product by vacuum distillation and countercurrent distribution and obtained two crystalline compounds. The two isomers shared an identical ultraviolet absorption spectrum, but had different melting points. Korte and Sieper suggested that the isomers differed from one another in the position of the alicyclic double bond.

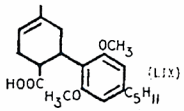
Another method for the synthesis of a tetrahydrocannabinol was reported by Adams (Adams, Loewe et al., 1942) and simultaneously by Todd (Ghosh, Todd and Wright, 1941; Leaf, Todd and Wilkinson, 1942). Pulegone (LVI) was condensed with olivetol to form the tetrahydrocannabinol (LV) above. Several other products were formed by this method, which imposes severe complications upon the purification of the tetrahydrocannabinol.


(LVI)


(LVII)

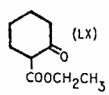
Adams attempted to synthesize tetrahydrocannabinol (Adams and Carlin, 1943) in which the alicyclic double bond is not conjugated to the phenyl nucleus. Isoprene (LVII) was condensed with 2,6-dimethoxy-4-n-amyl-cinnamic acid (LVIII) to form the material (LIX).

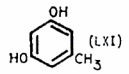

(LVIII)


(LIX)

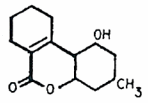
Demethylation of (LIX) should yield a lactone which could be converted to the dibenzopyran by methylmagnesium iodide. Although several methods of demethylation were employed, no successful method was found.

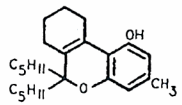
Bembry utilized the general technique of Adams to synthesize a number of tetrahydrocannabinols (Bembry and Powell, 1941), none of which were found to possess notable psychopharmacological activity. Bembry condensed ethyl cyclohexanone-2-carboxylate (LX) with orcinol (LXI) to form the dibenzo-


(LX)

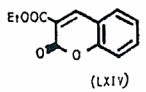

(LXI)

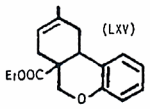
pyrone (LXII). Treatment with the appropriate Gringard produced a family of tetrahydrocannabinols with the 6-position substituted from methyl up through n-amyl (LXIII).


(LXII)

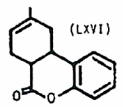

(LXIII)

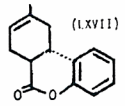
Strojny and Taylor reported the successful synthesis of materials related to tetrahydrocannabinol in that the alicyclic double bond is not conjugated to the phenyl nucleus, but which differ from natural tetrahydrocannabinol in not possessing the methyl and amyl groups on the phenyl ring (Taylor and Strojny, 1960). Isoprene was condensed with 3-carbethoxycoumarin (LXIV) to yield the 6a-carbethoxy-9-methyl-6a,7,10,10a-tetrahydro-6-dibenzopyrone (LXV).


(LXIV)

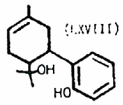

(LXV)

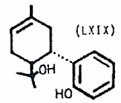
This pyrone was hydrolyzed in base and then decarboxylated to yield the *cis-* and *trans-* isomers of 9-methyl-6a,7,10,10a-tetrahydro-6-dibenzopyrone (LXVI) and (LXVII).


(LXVI)

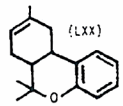

(LXVII)

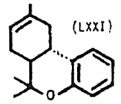
These isomers were separated by fractional crystallization and converted to the *cis-* and *trans-* diols (LXVIII) and (LXIX) by methylmagnesium iodide.


(LXVIII)


(LXIX)

Treatment of the diols (LXVIII) and (LXIX) with p-toluenesulfonic acid resulted in the dehydration and cyclization of the materials to give the *cis-* and *trans-* isomers of 6,6,9-trimethyl-6a,7,10,10a-tetrahydro-6-dibenzopyran (LXX) and (LXXI).

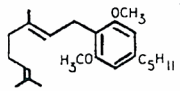

(LXX)


(LXXI)

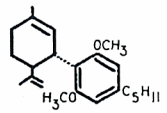
Strojny and Taylor suggested that Δ^6-*cis-* and Δ^6-*trans-*tetrahydrocannabinol could be prepared in the same manner by using an appropriately substituted coumarin.

Gaoni and Mechoulam reported the successful synthesis of Δ^1-*trans-*tetrahydrocannabinol in 1965 by a somewhat more simplified technique (Gaoni and Mechoulam, 1965). Citral (LXXII) was reacted with lithioolivetol (XLVI) to give a number of products, one of which was assumed to be

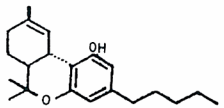

(LXXII)


(XLVII)

(XLVII). The resulting mixture was treated with p-toluenesulfonyl chloride in pyridine to yield cannabidiol dimethyl ether (XLVIII).

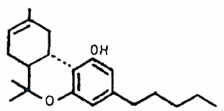

(XLVIII)

This was treated with methylmagnesium iodide to form cannabidiol (XL). Cyclization of the cannabidiol was accomplished with ethanolic hydrogen chloride. The resultant is Δ^1-*trans*-tetrahydrocannabinol (LII).



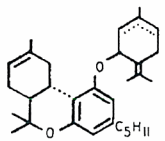
(LII)

Hively, Mosher and Hoffman (1966) reported the isolation of the Δ^6-*trans*-tetrahydrocannabinol isomer (LXXIII) from marijuana in 1966. Chromatography of a petroleum ether extract of marijuana followed by additional purification by column chromatography on silicic acid-silver nitrate gave separation of the Δ^1- and Δ^6-isomers. The Δ^6-isomer was found to represent 10% of the total tetrahydrocannabinol in a sample of Mexican marijuana.

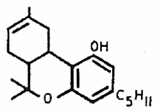


(LXXIII)

Taylor and co-workers described the synthesis of the Δ^6-isomer in the same year (Taylor, Lenard and Shvo, 1966). Citral (LXXII) was reacted with olivetol in cold benzene in the presence of boron trifluoride. The reaction product was chromatographed on a Florisil column to give the tetrahydrocannabinol derivative (LXXIV) followed by a mixture of isomers. The mixture of isomers was separated by preparative gas chromatography into Δ^6-*cis*-tetrahydrocannabinol (LXXV) and Δ^6-*trans*-tetrahydrocannabinol (LXXIII).

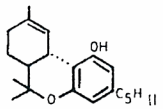


(LXXIV)

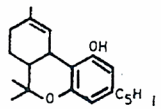


(LXXV)

When the condensation of the citral with olivetol was carried out under exceedingly mild conditions, the reaction mixture was separated by column chromatography into Δ^1-*trans*-tetrahydrocannabinol (LII) and Δ^1-*cis*-tetrahydrocannabinol (LXXVI).



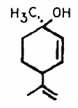
(LII)



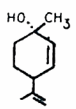
(LXXVI)

The structure, position of the alicyclic double bond, and stereochemistry of all four isomers was accomplished, primarily through the application of NMR spectroscopy.

In early 1967, Petrzilka and co-workers described a two step synthesis of levorotary Δ^1-tetrahydrocannabinol (Petrzilka et al., 1967). The synthesis is described in detail above in the cannabidiol section. Olivetol is reacted with d-*cis*-p-menthadiene-(2,8)-ol (L) or d-*trans*-p-menthadiene-(2,8)-ol (LI) to form *l*-cannabidiol. Mild acid catalyzation leads to *l*-Δ^1-tetrahydrocannabinol in an unspecified yield.



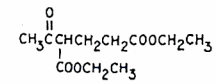
(L)



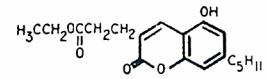
(LI)

The levorotary form of tetrahydrocannabinol is much more active in the pharmacological sense than is the dextrorotary form (Todd, 1946). The acid catalyzed cyclization of *l*-cannabidiol to *l*-tetrahydrocannabinol is attended to with some difficulty with regard to the purity of the product. Mechoulam and co-workers have apparently been engaged in this work, and although their work has not been published as of this writing, it has been described by Efron (1967). Cyclization of cannabidiol with dilute acid leads to a mixture of *d*- and *l*-tetrahydrocannabinols. Separation of the racemic forms is difficult with a resultant poor yield of *l*-tetrahydrocannabinol.

In the Fall of 1967, several tetrahydrocannabinol syntheses were described by Fahrenholtz, Lurie and Kierstead (1967). Olivetol was condensed with diethyl α-acetoglutarate (LXXVII) in the presence of phosphorous oxychloride to give the chromone (LXXVIII). Treatment of this chromone with sodium hydride in dimethyl sulfoxide gives the coumarin chromaphore (LXXIX).

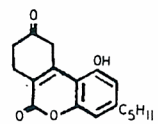


(LXXVII)

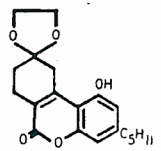


(LXXVIII)

Treatment with ethylene glycol yields the corresponding ketal (LXXX). Reaction of the ketal with methylmagnesium iodide followed by acid hydrolysis gives (LXXXI). Further reaction of this product with lithium in liquid

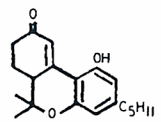


(LXXIX)

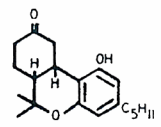


(LXXX)

ammonia at −70°C gives a mixture of two ketones, (LXXXII) and (LXXXIII).

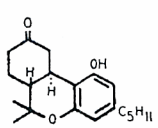


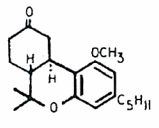
(LXXXI)



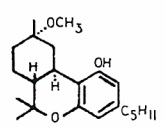
(LXXXII)

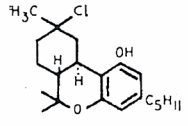
The major product of this reduction gives the *trans*-isomer (LXXXIII), the corresponding methyl ester of which (LXXXIV) is reacted with methylmagnesium iodide to give the carbinol (LXXXV).


(LXXXIII)

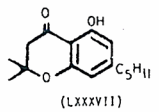

(LXXXIV)

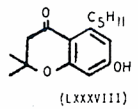
Treatment of the carbinol with a catalytic amount of p-toluenesulfonic acid in benzene gives Δ^6-*trans*-tetrahydrocannabinol (LXXIII). Reaction of the carbinol with Lucas reagent in acetic acid gives the chloride (LXXXVI). This product, on dehydrohalogenation with sodium hydride in tetrahydrofuran gives predominately Δ^1-*trans*-tetrahydrocannabinol (LII).

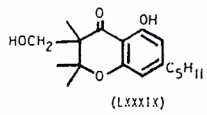

(LXXXV)


(LXXXVI)

A second scheme described by the same authors (ibid.) involved the reaction of olivetol with 3-methylcrotonic acid in the presence of boron trifluoride etherate to form the chromones (LXXXVII) and (LXXXVIII). The predominating chromone (LXXXVII) may be condensed with ethyl formate in the presence of sodium hydride to give (LXXXIX).

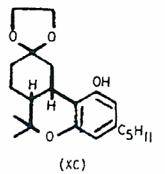

(LXXXVII)

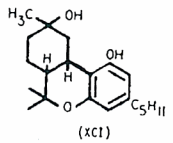

(LXXXVIII)


(LXXXIX)

Ring annealation of this product with methyl vinyl ketone gives the dibenzopyrone (LXXXI). The steps leading to the final product from this material have been outlined above.

A third scheme described by the same authors (ibid.) involves the ketal (LXXX) which undergoes catalytic hydrogenation with Raney nickel to give the *cis*-lactone (XC). Treatment of this lactone with methylmagnesium


(XC)

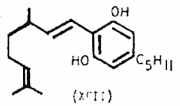

(XCI)

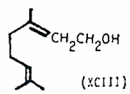
iodide followed by hydrolysis gives the *cis*-ketone (LXXXII) previously described. Reaction of (LXXXII) with methylmagnesium iodide gives the methylcarbinol (XCI). This product may be dehydrated with p-toluenesulfonic acid to give Δ^1-*cis*-tetrahydrocannabinol (THC) (LXXVI).

Stereospecifically labelled Δ^6-THC has been described by Burstein and Mechoulam (1968). Δ^1-THC was deuterated and refluxed in benzene for 2 hours, at which time Δ^6-THC was extracted from the mixture. NMR indicated that one atom of deuterium had been introduced at carbon atom 2. This labeled THC is reported as having possible value in demonstrating the metabolic fate of tetrahydrocannabinol in animals.

Cannabigerol

In 1964 Gaoni and Mechoulam (1964) isolated a material from a hexane extract of marijuana by column chromatography. The name cannabigerol was given to this fraction, which possesses an ultraviolet absorption spectrum indistinguishable from that of cannabidiol. Cannabigerol has no optical activity, eliminating a carbon-carbon bond between the two asymmetric centers such as that which exists in cannabidiol. The NMR spectrum established unequivocally that cannabigerol (XCII) has the following structure:


(XCII)

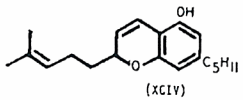

(XCIII)

Gaoni and Mechoulam prepared cannabigerol by refluxing geraniol (XCIII) with olivetol in decalin for 36 hours (ibid.). The synthetic material was identical to that isolated from the natural marijuana resin.

No psychopharmacological activity has been ascribed to cannabigerol.

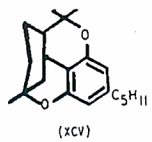
Cannabichromene

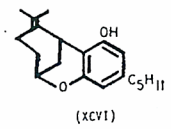
A new substance occurring in marijuana was reported simultaneously in 1966 by Gaoni and Mechoulam (1966) and by Claussen, Spulak and Korte (1966). This material was called cannabichromene (XCIV) and is observed not to possess the closed alicyclic ring of tetrahydrocannabinol.


(XCIV)

The structure was determined by NMR, ultraviolet absorption spectrum, and mass spectrometry.

The total synthesis of cannabichromene has been described (Mechoulam, Yagnitinsky and Gaoni, 1968). Cannabigerol (XCII) is dehydrogenated with chloranil in benzene to give cannabichromene and a tetracyclic diether (XCV). When treated with p-toluenesulfonic acid in boiling benzene, the diether is converted to the Δ^3-isotetrahydrocannabinol (XCVI).


(XCV)


(XCVI)

Although the material was originally considered to possess some psychopharmacological activity (ibid.), it has more recently been reported to have considerably less activity than Δ^1-tetrahydrocannabinol, or possibly no action at all (Isbell, 1967).

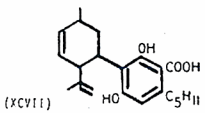
Cannabidiolic acid

Several constituents of the resin of the marijuana plant, related structurally to the phenolic materials previously described, possess a carboxylic acid functional group. The occurrence of acidic materials in extracts of the marijuana plant was first described by Fulton (1942). He extracted marijuana with petroleum ether, and shook out an acidic fraction with dilute sodium hydroxide. This fraction was separated into two components by extraction of the aqueous alkali with ether. The ether solution gave a material consistent with cannabinol. The basic solution was acidified and extracted with ether. This ether extract gave a material consistent with cannabidiol.

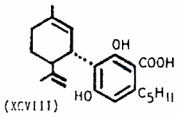
The same two components were separated by Todd and co-workers in the same year (Madinaveitia, Russel and Todd, 1942). Like Fulton, Todd was unable to isolate the acidic fractions but related the fractions to cannabidiol and cannabinol.

Krejci and Santavy (1955) in Czechoslovakia isolated an acidic constituent from the resin of the marijuana plant. This was established as being a β-resorcylic acid derivative by infrared and ultraviolet spectroscopy (Krejci, Horak and Santavy, 1958). This acidic fraction was identified as the phenolic constituent (Adams, Loewe et al., 1940) responsible for the microbiological activity of the marijuana plant (Kabelik, Krejci and Santavy, 1960). Krejci and Santavy gave the material the name cannabidiolic acid, considering it to be a carboxy derivative of cannabidiol, cannabinol, or tetrahydrocannabinol.

In the same year Schultz and Haffner (1958) in Germany also reported the isolation of this acid, which they found to decarboxylate to cannabidiol. At this time, the best information available was that the alicyclic double bond in cannabidiol was in the Δ^5-position as suggested by Adams. Schultz and Haffner reasoned that the mild conditions under which the decarboxylation occurred would not result in a double bond migration, and that cannabidiolic acid should be given the structure (XCVII).



(XCVII)

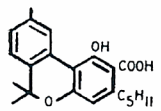


(XCVIII)

Mechoulam and Shvo (1963) repositioned the alicyclic double bond in the Δ^1 position, on the basis of its NMR spectrum. Cannabidiolic acid may be assigned the verified structure (XCVIII).

Cannabinolic acid

Mechoulam and Gaoni (1965) reported the isolation of an acidic constituent from the marijuana plant, to which they gave the name cannabinolic acid, relating it to the structure of cannabinol. The total acid fraction of the marijuana resin was esterified with diazomethane and separated into three different constitutents by column chromatography. The most polar material was identified as the methyl ester of cannabigerolic acid. The next in order of decreasing polarity was identified as the methyl ester of cannabidiolic acid. The least polar material of the three was identified as the methyl ester of cannabinol. The structure of cannabinolic acid (XCIX) was established by

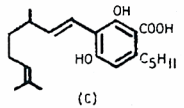


(XCIX)

comparison of its NMR spectrum with that of cannabinol (ibid.). Decarboxylation of this acid by potassium hydroxide in methanol gave cannabinol.

Cannabigerolic acid

Mechoulam and Gaoni (ibid.) reported the isolation of an acidic constituent from the marijuana plant in 1965, to which they gave the name cannabigerolic acid, relating it to the structure of cannabigerol. The total acid fraction of the marijuana resin was esterified with diazomethane and separated into three different constituents by column chromatography. The least polar was identified as the methyl ester of cannabinolic acid. The next in order of increasing polarity was identified as the methyl ester of cannabidiolic acid, and the most polar was identified as the methyl ester of cannabigerolic acid. The structure of cannabigerolic acid (C) was established primarily by comparison of its NMR spectrum with that of cannabigerol (ibid.). Decarboxylation of cannabigerolic acid yields cannabigerol.

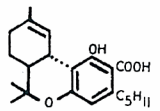


(C)

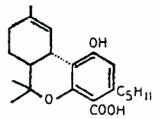
Tetrahydrocannabinolic acid

The corresponding acid of tetrahydrocannabinol (CI) was reported in 1967 (Yamauchi et al.). This was shown to be pharmacologically inactive, but may be converted into the active Δ^1-tetrahydrocannabinol by smoking (Gaoni and Mechoulam, 1964b).

A second tetrahydrocannabinolic acid (CII) was reported in 1969 (Mechoulam et al.). This second isomer was given the name Δ^1-THC acid



(CI)



(CII)

"B", with the name Δ^1-THC acid "A" being suggested for the first acid isolated. The THC acid "B" is reported to be converted into Δ^1-Tetrahydrocannabinol by heating, and presumably by smoking.

The Duquenois Test

The preceding overview of the chemistry of marijuana relates to the Duquenois test in a number of apparent instances. If the Duquenois test is in fact a test for resorcinol-type compounds as will be discussed later, then all of the phenolic constituents discussed above would be expected to give a positive Duquenois test of some type. The occurrence and relative proportions of the various constituents appear to vary somewhat depending upon the geographical location at which the plant is grown (Farmilo, 1961; Hively and Hoffmann, 1966; Farmilo et al., 1962).

Under the extant conditions of the Duquenois test, cannabidiol cyclizes to tetrahydrocannabinol.

The n-amyl chain on the phenolic ring is common to all of the materials discussed, and may have a bearing upon the solubility of these materials in non-polar solvents.

The discussion above also provides some information regarding possible chemical precursors which may be encountered in instances involving the illicit production of synthetic tetrahydrocannabinols.

The chemical test for marijuana which has gained wide acceptance, to the virtual exclusion of all others, is the so called "Duquenois" test. Duquenois and Negm (1938a) reported the development of a sensitive test which, under

specified conditions, they regarded as being specific for the resin from the plant *Cannabis sativa*, otherwise known as marijuana. The test involves treating a petroleum ether extract of the plant or resin material with the reagent. The test actually consists of two parts: the first step involves the addition of an ethanolic solution of vanillin and acetaldehyde to the evaporated petroleum ether extract; the second step involves the addition of concentrated hydrochloric acid to the mixture. In the presence of marijuana or marijuana resin, an intense color formation is observed. Although some controversy exists over the progression of transient colors formed, there appears to be universal agreement that the final color is deep blue to deep violet. The writer is of the impression that a portion of this controversy, particularly with reference to a transient green, may have been clarified by certain of the work described in this article.

Duquenois made no attempt to give an explanation of the chemical basis of the reaction, and the mechanism of the reaction is not yet entirely elucidated.

The specificity of the Duquenois reaction has been established, empirically at least, over the past three decades. No plant material other than marijuana has been found to give an identical reaction (Farmilo, et al., 1962; Nakamura, 1969). The reagent is observed, however, to react with a number of phenolic materials and a few terpenes to give a color formation. In the case of the terpenes, the intensity of the color formed is quite low and the reaction appears to be of a decidedly different type. In the case of some aromatic nuclei with a phenolic moiety, the color formed is vivid but of a shade or sequence clearly distinguishable from that observed with marijuana resin.

Cheronis (1960) offered criticism of the specificity of the Duquenois reaction, stating that "It should be noted that Adams and co-workers found the Duquenois color reaction inconclusive". Reference to the original article of Adams and co-workers cited by Cheronis reveals that the inconclusiveness referred to is an interpretation of Cheronis, and the criticism offered by Cheronis appears to be without foundation. Adams and co-workers (1940b) performed the Duquenois test on the crude "red oil" and on isolated pure cannabidiol and found them to be virtually indistinguishable. In the context of a specific test for marijuana this cannot be construed as an inconclusive test. Adams and co-workers did not comment upon the significance of their observations in this regard.

Fulton (1942) has reported that Minnesota-grown marijuana does not give the same color sequence observed by Duquenois on marijuana of Egyptian origin, while Manchurian-grown material does give the sequence noted by Duquenois. Blackie (1941), working on marijuana of Indian origin, also reports a somewhat different sequence of colors than that observed by Duquenois. The difference in the sequence of the transient intermediate colors is attributed to differences in the relative proportions of the various constituents of the resin arising from agronomic variations.

Blackie (ibid.) observed that several aromatic aldehydes other than vanillin would give vivid color tests for marijuana, and that the presence of acetaldehyde in the reagent is not critical. Blackie states that after color formation with the reagent prepared without acetaldehyde, subsequent addition of the aldehyde does not result in the modification of the color. This is contrary to the observations of Duquenois (1938) and those of Kingston (1961).

The original Duquenois reaction was adopted as a preferential test by the League of Nations Sub-Committee of Cannabis (Duquenois, 1950). A modification of the test has been proposed by the United Nations Committee on Narcotics (1960a) as a universal and specific test for marijuana. The modification referred to is the addition of chloroform to the final colored complex, a technique suggested by the U.S. Treasury Department Bureau of Narcotics (Butler, 1962). The modification is generally referred to as the "Duquenois-Levine" test. This modification of the test would seem to insure the specificity of the reaction, as the reactive phenolic materials other than the constituents of marijuana resin do not give colors soluble in chloroform. This has lead the UN Committee on Narcotics to conclude that there is nothing other than marijuana which will give exactly the same Duquenois reaction (Farmilo et al., 1962). This will be considered in further detail later, but it should be noted that the Duquenois reaction as conceived by the UN Committee on Narcotics is characterized only by the final color, and not by the sequence of transient intermediate colors.

The history of the chloroform extraction of the colored complex of the Duquenois reaction appears to be somewhat apocryphal. The U.S. Treasury Department (1962) originally published the modification in a booklet of field tests. The United Nations Laboratory adopted the modification in 1960 based upon personal communications with the Bureau of Narcotics (United Nations Secretariat, 1960b). The Treasury Department again published the test (Butler, 1962), asserting that it had been in effect in their laboratories since 1941. In the same communication, it was reported that tetrahydrocannabinol was responsible for the color extracted into the chloroform, with a personal communication from Adams cited as the authority.

Kingston investigated the Duquenois reaction in a study directed toward the elucidation of the mechanism involved (1961). On the basis of similarities in ultraviolet absorption spectra of the condensation products of a number of simple aromatic aldehydes and simple phenols, he suggested that the mechanism of the test is at least partly a phenol-aldehyde condensation. His investigation was directed toward the nature of the final product of the reaction, and the actual mechanism by which the condensation takes place was given only peripheral consideration.

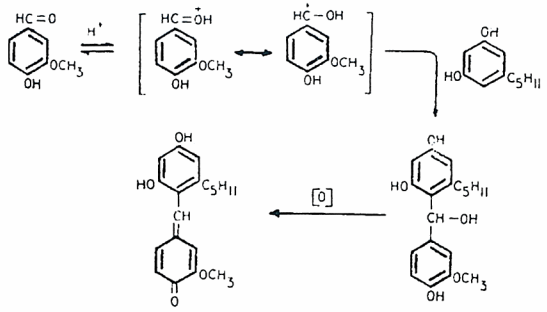
In addition, Kingston was able to gas chromatographically separate twelve fractions from a petroleum ether extract of marijuana of Mexican origin. One of these fractions was tentatively identified as cannabinol, and was observed to give a blue color with the Duquenois reagent. The remaining fractions were separated into five cuts, all of which gave a violet color of greater or lesser intensity with the reagent. Kingston concluded that the color observed when an extract of the plant is tested with the reagent is the result of the interaction of the various constituents, giving blue to violet depending upon the relative proportions of the various components. It should be noted that Adams obtained a blue color, not with cannabinol, but with cannabidiol, when tested with the Duquenois reagent (Adams, Hunt, and Clark, 1940b). The UN Committee on Narcotics (1960a) reports a violet color with cannabidiol when tested with the Duquenois reagent.

Although the interpretation of the colors formed is to a certain extent subjective, it is apparent that no significant agreement with respect to the colors obtained with the phenolic constituents of marijuana is to be found in the literature. There is disagreement as to the colors obtained with cannabinol and cannabidiol, and the remaining constituents do not appear in the literature as having been tested in the pure form.

The reactivity of the Duquenois reaction has been subjected to rather extensive empirical tests. While a number of phenolic materials have been described as giving some color with the Duquenois reagent, there has been very little attempt to relate the reactivity to the structure of the aromatic phenol, and no attempt has apparently been made to explain the solubility in chloroform of the color formed.

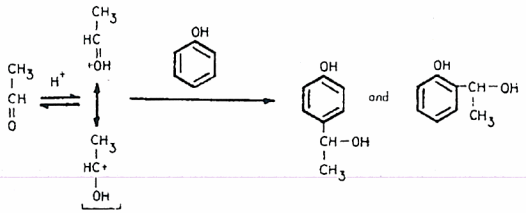
An entirely adequate explanation of the chemical basis of the Duquenois reaction does not lie in its similarity with a phenol-aldehyde condensation such as Bakelite, as has been suggested by Kingston (1961). Under alkaline conditions the phenoxide ion is sufficiently activated to react with the weakly electrophilic free aldehyde. These, however, are not the conditions of the Duquenois reaction, which is acid catalysed.

A possible explanation of the reaction mechanism is that in acid solution, protonation of the aldehyde makes it a stronger electrophile that can bring about substitution of the undissociated phenol:



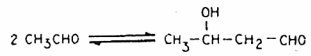
Substitution at the *ortho-* and *para-* positions would be expected, with the product possibly undergoing further condensation to yield a resinous material of considerable complexity. Oxidation of this product could lead to quinone structures with a resultant intensely colored solution.

There is little question but that the reaction is considerably more complex than outlined above. The contribution of the acetaldehyde is also uncertain, but may also substitute at available *ortho* and *para* positions:



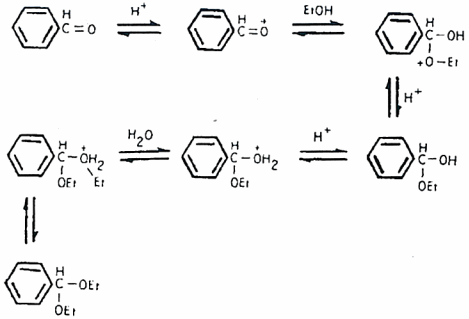
It should be noted that vanillin is itself a phenol as well as an aldehyde, and may be substituted by either vanillin or acetaldehyde. The acetaldehyde may, however, be dispensed with, as intense reactions are obtained when the aldehyde is deleted from the reagent.

Several other mechanisms may be occurring concommitantly. The acetaldehyde, having free α-hydrogen, may be participating in a self-addition reaction:



The acetaldehyde and vanillin may both be reaching hydration equilibria, leading to species of the type:

The vanillin may participate in a reaction with the alcohol to form an acetal or hemiacetal:



The acetaldehyde may also enter a similar reaction to form an acetal. The elimination of water in an acetal formation may further the attainment of a hydration equilibrium by the aldehydes.

These reactions are of necessity somewhat speculative, as it is difficult to establish the effect of ancillary reactions which may be taking place. If a resin is formed in a linear or cross-linked chain with condensation taking place in the ortho and para positions, it would be of interest to establish at what point condensation terminates. It would also be of interest to establish whether or not certain tangential reactions have a determinative effect upon the Duquenois reaction in general.

Kingston has established that the reaction product of the Duquenois test is similar to the product of an aromatic phenolaldehyde condensation. Several simple experiments were attempted by this writer to further establish the nature of the product of this type of reaction. Hexylresorcinol and p-hydroxybenzaldehyde were selected as representative reactants for purposes of simplicity. The intensely colored complex produced by the reaction of these two materials in acid was extracted into chloroform. The chloroform was then evaporated to dryness under mild heat. The isolated material was an intensely colored gummy resin. A molecular weight determination was attempted on the product using the camphor melting point depression technique. The depression of the melting point was exceedingly severe, and the writer is unable to draw a meaningful conclusion from the results observed.

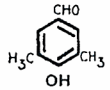
An experiment was then attempted to ascertain the presence or absence of cross-linkage in the reaction product. A resole or "A-stage" resin, not being cross-linked, is fusible and soluble in organic solvents, while cross-linked resins are characteristically insoluble in organic solvents (D'Alelio, 1955). A quantity of the product of the reaction of p-hydroxybenzaldehyde and hexylresorcinol was heated at 80°C for 8 hours. At the end of this period the product was not perceptively fused and was quite soluble in organic solvents. This cannot be interpreted as having unequivocally established the nature of the resin, but is a clear indication that the product of the reaction is not cross-linked but is a resole of limited size.

A quantity of the product of the reaction of hexylresorcinol and p-hydroxybenzaldehyde was subjected to infrared analysis. The infrared spectrum of the resin was poorly defined with broad absorption bands. Structure correlation with the infrared absorption of this material does not appear feasible.

The contribution of the methoxy- group on the vanillin molecule to the Duquenois reaction does not appear to have been previously investigated. To test this, marijuana was tested with p-hydroxybenzaldehyde substituted for vanillin. No significant difference in reactivity or in the colors formed was noted. Marijuana was also tested with 3,5-dimethyl-4-hydroxybenzaldehyde. Again, no significant difference in reactivity or in the colors formed was noted. In the first instance, the positions *ortho-* to the phenol are unoccupied; in the second, the positions are masked by methyl groups.

Vanillin

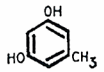
p-Hydroxybenzaldehyde



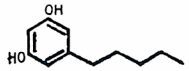
3,5-Dimethyl-4-hydroxybenzaldehyde

Resorcinol

p-Hydroxybenzaldehyde and acetaldehyde in ethanolic HCl was reacted with phenol. Phenol was observed to be weakly reactive, giving a pink-orange coloration. If *ortho-* and *para-* electrophilic substitution is taking place, resorcinol would be expected to be more strongly reactive with the Duquenois reagent or with p-hydroxybenzaldehyde. This was confirmed; resorcinol is strongly reactive, giving a pinkish violet which, like phenol, does not extract into chloroform. Orcinol would be still more strongly reactive, as the presence of the methyl group would tend to orient the substitution. This was also confirmed; orcinol gives an intense orange-violet with the Duquenois reagent and with p-hydroxybenzaldehyde. The color does not extract into chloroform, except with very great concentrations of orcinol. Hexylresorcinol would be expected to be strongly reactive, and this was confirmed. But in hexylresorcinol a long aliphatic chain is attached to the resorcinol molecule, and it would appear reasonable to expect that the solubility of the colored complex in non-polar solvents might be modified. This is confirmed when testing hexylresorcinol with either the Duquenois reagent or with p-hydroxybenzaldehyde. The color formed with hexylresorcinol is easily extracted into chloroform, bromoform, or methylene chloride.



Orcinol



Olivetol

The phenolic moiety of the principal marijuana constituents is n-amylresorcinol, or olivetol. Curiously, olivetol does not appear in the literature as having ever been tested with the Duquenois reagent. Olivetol is observed to give an intense reaction with the vanillin-acetaldehyde reagent. The color formed, when adjusted for concentration, is virtually indistinguishable from that of pure Δ^1- and Δ^6-tetrahydrocannabinol.

Thymol and eugenol would be expected to be less strongly reactive than resorcinol or phloroglucinol, and this is confirmed. p-Phenylphenol would be expected to react on the order of phenol, or rather weakly, and this was confirmed.

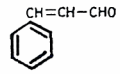
Styphnic acid (2,4,6-trinitroresorcinol), having all *ortho* and *para* positions to the phenols masked, was observed to be unreactive. Hydroquinone was observed to be reactive.

Quebrachitol (inositol mono methyl ether), having been reported as occurring naturally in marijuana (Adams, Pease and Clark, 1940), is unreactive.

While the methoxy- group on the vanillin molecule does not appear to materially affect the Duquenois test as generally performed, several observations are worthy of note when an extract of marijuana is tested with p-hydroxybenzaldehyde. Upon extraction of the color into chloroform, there is an inversion of the colors from that observed with the Duquenois reagent. The aqueous layer is violet, and the color extracted into the organic layer is blue. Another observation of interest is that while the color formed with the Duquenois reagent fades in a matter of hours, the color formed with p-hydroxybenzaldehyde appears to be unaffected by time and persists for days.

When orcinol, resorcinol, and an extract of marijuana resin are tested with ethylvanillin substituted in the reagent for vanillin, no significant differences are noted in the colors formed. Isovanillin is observed to be strongly reactive with the above phenolic materials, although the colors formed are decidedly different from those observed with the vanillin reagent. Benzaldehyde and acetaldehyde in the presence of ethanolic HCl is not as reactive as the Duquenois reagent, the color taking much longer to form, being less intense and more transient in nature. The presence of acetaldehyde in the Duquenois reagent is not indispensable, as in its absence a strong blue color is observed with an extract of marijuana resin. The color extracts into chloroform to give a light blue aqueous layer and a green organic phase. Nor is the presence of acetaldehyde indispensable to the test when the vanillin is substituted by p-hydroxybenzaldehyde, benzaldehyde, or cinnamaldehyde. Deletion of the acetaldehyde results in no color being formed when the vanillin is substituted by *ortho-* and *para*-anisaldehyde, and o-hydroxybenzaldehyde (salicylaldehyde).

Isovanillin



Cinnamaldehyde

o-Anisaldehyde

p-Anisaldehyde

Inclusion of acetaldehyde with these three aromatic aldehydes gives intense colors with extracts of marijuana and with resorcinol analogs.

Salicylaldehyde

Resorcylaldehyde

Terephthaldehyde

m-Hydroxybenzaldehyde

Resorcylaldehyde is strongly reactive both with and without the presence of acetaldehyde, giving colors virtually indistinguishable from those observed with p-hydroxybenzaldehyde. Terephthaldehyde is unreactive.

It is noted that the more reactive aldehydes are those with a p-phenol. This would appear consistent with the postulated resin formation and quinone structure. The question of the oxidation of the resin to a quinone was investigated briefly by testing an extract of marijuana with the Duquenois reagent, purging the solution with nitrogen to remove oxygen. The HCl was then added under a nitrogen atmosphere. Instead of the characteristic blue-violet color being formed, a faint light green was observed which failed to extract into chloroform

This green color was of the same relative intensity as certain other materials tested which were interpreted by the writer and by Farmilo (Davis, Farmilo and Osadchuk, 1963) as an essentially negative reaction. Shaking of the green solution with air resulted in the gradual development of a blue-violet color which extracted violet into the chloroform layer. It is noted that the intensity of the blue-violet color developed in this manner does not compare with that obtained by performing the test in the normal manner.

An attempt was made to reproduce the work of Blackie (1941) to ascertain the effect of the solvent upon the Duquenois reaction, as it is possible that any difference in the color formed could have some basis in the reaction of the aldehydes with the different alcohols to form acetals or hemiacetals. The vanillin-acetaldehyde reagent was made up in methanol, ethanol, n-propanol, 2-propanol, n-butanol, n-amyl alcohol, iso-amyl alcohol, tert-amyl alcohol, and benzyl alcohol. With the exception of methanol, no significant differences were observed in the color formed with a petroleum ether extract of marijuana, suggesting that acetal formation does not have a determinative effect in the total reaction. With methanol, the color formed was quite cloudy and faded much more rapidly than with other alcohols. The writer is unable to determine the factors predicating the cloudiness. It may be due to a lack of solubility of the reaction product in the methanol, or it may be due to the greater amount of acid it was necessary to add to develop the color in this solvent.

The results of these experiments tend to suggest then an aldehyde-phenol reaction leading to a resin formation by *ortho-* and *para-* electrophilic substitution is the likely mechanism involved in the Duquenois reaction, other reactions being of less consequence. Although this mechanism is reasonable, and fits the observations made, it cannot be considered as proved.

The modification of the Duquenois test which incorporates the extraction of the color formed into chloroform does not appear to have been previously investigated in other than an empirical manner. A petroleum ether extract of marijuana was tested with the Duquenois reagent in the regular manner and the color extracted into chloroform to give a blue aqueous layer and a violet chloroform layer. The organic layer was drawn off and fresh chloroform added. This process was repeated several times, after which all of the blue color of the aqueous layer had been extracted into the organic layer as a violet color. The chloroform fractions were combined and evaporated to dryness under mild heat. Upon drying, the violet color changed back to the original blue color of the aqueous layer. These results suggest that the color of the organic layer is predicated by solubility and pH effects.

To test this further a Duquenois test was carried out in the normal manner, the color extracted with chloroform, and conc. NaOH added to the tube. The color was destroyed in both layers upon shaking. A Duquenois test was carried out in the normal manner, the color extracted with chloroform, and the chloroform layer separated and concentrated by evaporation. When the chloroform layer was dried to a fraction of its original volume, fresh Duquenois reagent was added and the mixture shaken. A blue color developed in the aqueous phase. Experiments with resorcinol analogs, hexylresorcinol, orcinol, and olivetol suggest that the solubility of the color in the chloroform may be a function of the length of the aliphatic chain attached to the phenol.

Testing of the Duquenois reagent with Pyrahexyl (Δ^3-hexyl analog of tetrahydrocannabinol, Abbot Laboratories) supports this contention. The material, possessing a six carbon aliphatic chain, produces a colored complex which is virtually indistinguishable from that of marijuana resin, and extracts easily into chloroform.

Pure Δ^1- and Δ^6-tetrahydrocannabinol were tested with the Duquenois reagent and the color developed extracted into chloroform. The final colors developed with the two isomers were indistinguishable from one another, both in the organic and the aqueous layers. The colors were also exceedingly similar to those obtained with olivetol and with a petroleum ether extract of marijuana.

Work was then attempted to ascertain with precision the nature of the colors formed with the Duquenois reagent and the various constitutents of natural marijuana resin. Samples of pure synthetic Δ^1- and Δ^6-tetrahydrocannabinol and Δ^1-tetrahydrocannabinol acetate, prepared for the Psychopharmacology Research Branch, National Institute of Mental Health, were made available to the writer (Kenneth Parker, Hinc Laboratory). The pure materials were subjected to Duquenois tests performed in tubes and, as will be discussed below, to thin-layer chromatography. As performed in the tubes, the Δ^1-tetrahydrocannabinol isomer gave a blue-green color which changed instantaneously into a blue-violet. After addition of chloroform, the aqueous layer was blue, the organic layer violet. With the Δ^6-tetrahydrocannabinol isomer, the color formed was a blue-violet. No indication of transient green as in the Δ^1-isomer was observed. With the addition of chloroform, the aqueous layer was blue, the organic layer violet. At this point the colors in the aqueous and organic layers were indistinguishable between the two isomers.

With the Δ^1-tetrahydrocannabinol acetate, no color was immediately observed. Gradually a blue color developed over a period of several minutes. With the addition of chloroform, the aqueous layer was blue and the organic layer violet. This confirms the observation made by Kingston (1961) that the phenolic moiety of the resorcinol derivative must be intact for the reaction to take place. Under the conditions of the Duquenois test, the acetate is hydrolyzed and frees the tetrahydrocannabinol to react in its characteristic manner.

Infrared absorption spectra of these pure compounds were prepared for future comparison with materials eluted from columns or thin-layer chromatographic plates. The ultraviolet absorption spectra of the two tetrahydrocannabinols were also determined and found to be virtually indistinguishable from one another. It was found that the absorption maxima reported for tetrahydrocannabinol in the literature (Korte and Sieper, 1960a) was in fact two close but separate peaks with absorptivities which are nearly identical.

The unequivocal identification of the remaining constituents of the marijuana resin was attended to with somewhat more difficulty, due to the lack of availability of known materials. The approach was one of separation by means of thin-layer chromatography, and identification by means of chemical tests and ultraviolet spectrophotometry.

Cannabidiol is reported to give a pink color with 2,6-dibromoquinonechloroimide, cannabinol a bluish-green, and tetrahydrocannabinol (presumably a mixture of the isomers) a blue (Miras, Simos and Kiburis, 1964). The Beam's test, methanolic KOH, is reported to react only with cannabidiol, giving a red-violet to purple color (Grlic, 1962). Fast Blue B is reported to give an orange with cannabidiol, a violet with cannabinol, and a scarlet to brick-red with tetrahydrocannabinol (Grlic, 1964). Ultraviolet absorption data is available for cannabinol (Adams, Cain and Baker, 1940), cannabidiol (Adams, Cain and Wolff, 1940), cannabidiolic acid (Schultz and Haffner, 1958), and for a synthetic tetrahydrocannabinol with the alicyclic double bond in the Δ^3-position (Hively and Hoffmann, 1966). No great reliance was placed on the colors reported with the Duquenois reagent with the various constituents, as no accord is found in the literature on this subject. The colors obtained with the various constituents by Kingston must be questioned, as on the basis of the emergence of pure tetrahydrocannabinol isomers and cannabinol from the gas chromatograph we must now conclude that the identification of Kingston's gas chromatographic cuts d, e, and j is subject to considerable doubt (Kingston' 1961).

As Rf values for cannabinol, cannabidiol, and tetrahydrocannabinol are reported by Korte and Sieper (1960a), an attempt was made to duplicate the thin-layer chromatographic system described by these authors. This system involves the development of Kieselguhr G plates which have been impregnated with carbon tetrachloride saturated with N,N-dimethylformamide. The developing solvent is cyclohexane. This system was found to be susceptible to an erratic

solvent front and to a lack of reproducible Rf's. As this system was considered to be unsatisfactory, the writer applied several other solvent systems to the thin-layer chromatographic separation of the phenolic constitutents of marijuana.

Polar solvents were found to be entirely unsuited for the separation of these constituents. The materials tended to travel with the solvent front and run off the plate. Several non-polar solvent systems were tried with substantially greater success, although in several non-polar systems no separation was achieved of the Δ^1- and Δ^6-tetrahydrocannabinol isomers. Certain solvents were observed to be better suited for the separation of specific constituents. Straight benzene was observed to be the solvent of choice for the separation of cannabinol from the tetrahydrocannabinols, while being inferior for the separation of the two tetrahydrocannabinol isomers. Hexane:diethyl ether was found to provide the best separation of the two tetrahydrocannabinols. This solvent system is perhaps the best all-round system, as the Rf's of the tetrahydrocannabinols are fairly high, allowing a better separation of the less mobile cannabinol, cannabidiol, and cannabidiolic acids.

In spraying the developed TLC plate with Beam's reagent (methanolic KOH), two discrete spots were developed, one reacting intensely, the other reacting with considerably less intensity. The higher, more strongly reactive spot was considered to be cannabidiol, while the lower spot, possessing a carbonyl and therefore expected to be less mobile in this solvent system, was considered likely to represent cannabidiolic acid. Although the ultra violet absorption spectrum of cannabidiolic acid has been reported by Schultz and Haffner (1958), the amount of acid present was insufficient to elute from the thin-layer plate for confirmatory ultraviolet or infrared spectrophotometric analysis. In addition, it is doubtful that the ultraviolet absorption spectrum would be of value in the identification of the acid due to its similarity with that of cannabidiol.

The spot tentatively identified as cannabidiol by reason of its reactivity with Beam's reagent was investigated further. The spots at the Rf of this component were removed from an unsprayed portion of a thin-layer plate and eluted with ethanol. Although 5× scale expansion was necessary to provide a meaningful spectrum, the ultraviolet absorption of this constituent agrees with that of cannabidiol reported by Adams, Cain and Wolff (1940).

Returning to the lower spot which reacts with Beam's reagent, the plate was sprayed with methanolic ferric chloride. The spot at this Rf was observed to be moderately reactive with the reagent. No other reactive spots were observed.

Both spots gave an orange color with Fast Blue B and Fast Blue 2B, and both spots gave a weak pink with 2,6-dibromoquinone-4-chloroimide.

On the basis of the preceding, it was concluded that the lower spot is cannabidiolic acid and the higher spot cannabidiol.

A spot was observed which stained green with 2,6-dibromoquinone-4-chloroimide and violet with Fast Blue B. The material at this Rf was removed from an unsprayed portion of a thin-layer plate and eluted with ethanol. The ultraviolet absorption of this constituent agrees with that of cannabinol as reported by Korte and Sieper (1960a). On the basis of the preceding, it was concluded that this spot represents cannabinol.

The identity of the tetrahydrocannabinol isomers was established by comparison with authentic samples of these materials run on the same thin-layer plate.

A prominent spot at a high Rf was observed, particularly with the Fast Blue 2B spray reagent. The presence of Δ^1-tetrahydrocannabinol acetate in marijuana resin has been suggested (Wright and Parker, 1967). The writer is unable, however, to find any detectable quantity of the acetate in samples of marijuana grown in Mexico or in the San Francisco Bay area. The spot at high Rf may be eliminated as being the Δ^1-tetrahydrocannabinol acetate on the basis of the disparity of the Rf's when authentic pure acetate is run on the same thin-layer plate. The material at this Rf was removed from an unsprayed portion of a thin-layer plate and eluted with ethanol. The ultraviolet absorption spectrum of this material was determined and observed to agree closely with that of a tetrahydrocannabinol reported by Korte and Sieper (1960a), although maximum scale expansion was required to obtain a meaningful spectrum. The quantity of this material in the resin of Mexican marijuana is roughly equivalent to the concentration of the Δ^6-tetrahydrocannabinol isomer and may represent a *cis*-isomer of tetrahydrocannabinol. The writer is unable to establish the identity of this component due to the lack of availability of authentic samples of the *cis*-isomers of tetrahydrocannabinol. Although the ultraviolet absorption spectra of the *cis*- isomers has been reported (Hively and Hoffmann, 1966), the spectra are not discriminating and would be of little value in the identification of this component. The identification of this component at a high Rf as a tetrahydrocannabinol isomer must be considered to remain tentative and guarded.

With the identities of the various components thus established, plates were developed and sprayed with the Duquenois reagent to ascertain the reactivity of the phenolic constituents with this reagent. The Δ^1-tetrahydrocannabinol isomer gives a transient blue-green immediately changing to a violet. The Δ^6-isomer gives a violet. Cannabinol gives a blue color. Cannabidiolic acid gives a blue-green. Cannabidiol gives a green which changes to a violet over a period of several minutes.

The change of cannabidiol from a green to a violet appears to be a very important consideration, inasmuch as the cyclization of cannabidiol to tetrahydrocannabinol has been reported to take place in alcoholic HCl, the conditions of the Duquenois test (Adams, Pease, Cain and Clark, 1940). These observations suggest that the transient green observed when performing the Duquenois test in a tube is due to the reaction of the Duquenois reagent with cannabidiol, which under the conditions of the test is converted to tetrahydrocannabinol, giving then the violet color characteristic of tetrahydrocannabinol.

This cyclization of cannabidiol to tetrahydrocannabinol was confirmed by further experimentation. Alternating spots of an extract of marijuana resin at the origin of a thin-layer plate were overspotted with 0·2N HCl. Upon development of the plate, no cannabidiol was observed and the quantity of Δ^1-tetrahydrocannabinol was perceptively increased.

It was observed that when the plates were sprayed with the Duquenois reagent and the spots removed, the colors developed would not extract into chloroform. This was investigated briefly to ascertain if the extraction of the color into chloroform was possibly dependent in part upon interaction of the phenolic constituents when the test is performed in a tube. This was considered unlikely as pure tetrahydrocannabinol isomers, single chemical entities, react and extract the color into chloroform. Spots were removed from an unsprayed portion of a plate and eluted with petroleum ether. The petroleum ether was evaporated and the eluate tested with the Duquenois reagent in a tube. The appropriate color was observed, indicating that the lack of solubility of the color in chloroform when the spot is removed from the plate is an effect of the absorption of the Silica Gel layer and not the result of an interaction of the phenolic constitutents in the tube.

Samples of marijuana with verified geographical origins of Mexico (Volk, 1967), Kansas (Vejar, 1967), and Northern California (Thornton) were extracted and subjected to thin-layer chromatography. In each instance, the phenolic constituent present in the greatest quantity was observed to be Δ^1-tetrahydrocannabinol. The extract of marijuana utilized for the majority of the thin-layer chromatographic analyses was an ethanolic solution of an extract of Mexican marijuana containing 7·2μg/μl of Δ^1-tetrahydrocannabinol and 0·18μg/μl of Δ^6-tetrahydrocannabinol as determined gas chromatographically.

When the quantity of cannabidiol which is available for conversion to tetrahydrocannabinol by the ethanolic HCl is considered together with the quantity of tetrahydrocannabinol naturally present, it must be concluded

that the Duquenois test will be, in the majority of cases, essentially a test for tetrahydrocannabinol.

The United Nations Laboratory (1960a) reported that a color was obtained with the Duquenois reagent and a number of plants other than marijuana, although in each instance the color developed could be easily distinguished from that obtained with marijuana. It was suggested that the reactions were in fact due to the presence of certain terpenes, which although reacting much less intensely than the phenolic constituents of marijuana, nevertheless give colors with the Duquenois reagent.

The terpene fraction of marijuana was investigated by the writer to ascertain the effect of these constituents upon the total Duquenois reaction. Simonsen and Todd (1942) reported that the essential oil from marijuana consisted mainly of p-cymene, caryophyllene, and 1-methyl-4-isopropenylbenzene. Wood, Spivey and Easterfield (1896) reported myrcene in the oil from marijuana of Indian origin. Farmilo and co-workers (1962) investigated the terpene fraction marijuana grown in Canada, and identified myrcene, limonene, α- and β-caryophyllene. Obata and Ishikawa (1960) isolated eugenol and guaiacol from marijuana grown in Japan.

As much of the illicit marijuana in California is of Mexican origin, a sample of verified Mexican marijuana was subjected to steam distillation. The quantity of oil obtained by distillation represented 0·2% by weight of the dry marijuana, including seeds and stem fragments. This oil was analyzed by gas-chromatographic means, the peaks being identified by comparison with authentic samples of the various terpenes. Myrcene, p-cymene, limonene, α-caryophyllene, and β-caryophyllene were identified. Several components were recognized which remain unidentified. Guiacol and eugenol were eliminated as being present on the basis of the disparity of retention times. The total distillate was tested with the Duquenois reagent and found to give a cloudy violet color which failed to extract into chloroform. By means of a disc integrator the relative amounts of the various terpene constituents were determined. As is apparent from the gas chromatographic separation and relative proportions involved, this test must be almost entirely due to the two caryophyllene isomers. On an equivalent weight basis, the amount of caryophyllene present in the quantity of marijuana generally tested (10 to 100mg) by the Duquenois test would be of the order of 20 to 200μg. β-Caryophyllene was diluted with ethanol to give a concentration of 100μg/ml and tested with the Duquenois reagent. A very weak cloudy violet color formed which could not credibly be mistaken for that obtained with the phenolic fractions of marijuana. The remaining terpene fractions identified gave negative tests when diluted to the concentration which would be encountered in a petroleum ether extract of 10 to 100mg of dry marijuana. On the basis of the preceding, it is concluded that the terpene fraction of marijuana does not have a determinative effect upon the total Duquenois reaction.

It is recognized that marijuana is not the richest natural source of caryophyllene. Hops contain a high concentration of this material (Nickerson and Likens, 1966). A brief experiment was conducted to ascertain whether or not the high concentration of caryophyllene in hops would give a false positive test with the Duquenois reagent. A few hundred milligrams of hops was extracted with petroleum ether and the Duquenois test carried out in the normal manner. A very weak cloudy violet color formed which did not extract into chloroform. Approximately 25g of hops were extracted with petroleum ether and the petroleum ether evaporated to dryness. The residue was taken up in a milliliter of ethanol and spotted on a thin-layer chromatographic plate. Development of the plate failed to reveal any constituents reacting with Fast Blue 2B or the Duquenois reagent utilized as an overspray. On the basis of the preceding, it is concluded that hops, botanically speaking a close relative to marijuana, has no phenolic constituents which could react with the Duquenois reagent, and that any caryophyllene concentration in the hops could not react with the Duquenois reagent in such a manner as to promote an erroneous interpretation of the test.

Experimental

The Duquenois Test

The reagent is prepared as follows:

Ethanol	20ml
Acetaldehyde	4 drops
Vanillin	0·4g

The other reagents were prepared similarly, substituting the particular aromatic aldehyde for the vanillin in the reagent. The concentration of o-vanillin was reduced to 0.1g as the solution would have otherwise been much too dense to observe normal color development.

Although the substitution of paraldehyde for the acetaldehyde has been proposed due to its greater stability, the writer has not perceived any instability in the prepared Duquenois reagent even after several months of refrigerated storage. The preparation of the reagent with paraldehyde gives colors with aromatic phenols which are indistinguishable from that given with the reagent prepared with acetaldehyde.

The Duquenois reagent is applied to the plant material or preferably an extract of the resin from the plant material, and an equal amount of concentrated HCl is added. An intense color formation, characteristic of marijuana, is observed.

A number of materials reactive to the Duquenois reagent are listed in Table 1.

TABLE 1

DUQUENOIS TEST REACTIONS

Material	*Color Developed with Duquenois Reagent*		*Color Extracted into Chloroform*
Cannabidiolic acid	Green	Blue-violet	Violet
Cannabidiol	Green	Blue-violet	Violet
Cannabinol	Blue		Violet
Δ^1-THC	Aqua	Blue-violet	Violet
Δ^6-THC	Blue-violet		Violet
Δ^1-THC Acetate	Slowly, blue-violet		Violet
Pyrahexyl	Blue-violet		Violet
Olivetol	Red	Blue-violet	Violet
Catechol	Pink		—
Carvacrol	Pink	Blue-violet	—
Caryophyllene	Weak violet		—
Cineole	Weak violet		—
Citronellal	Weak violet		—
Citronellol	Green	Blue	Green
Citral	Green	Red	—
Cymene	Weak green		—
Eugenol	Yellow	Green	—
Geraniol	Green	Blue-violet	—
Hexylresorcinol	Red	Violet	Orange
Hydroquinone	Pink		—
Limonene	Pink	Violet	—
Linalool	Blue		—
Menthol	Weak light green		—
Myrcene	Blue		—
Naphthoresorcinol	Red	Blue	Weak violet
Nerol	Green	Blue	—
Orcinol	Red	Blue-violet	Blue
α-Phellandrene	Pink	Violet	—
Phenol	Weak pink		—
p-Phenylphenol	Weak pink		—
Phloroglucinol	Pink	Blue-violet	—
Pinene	Pink	Brown	—
Pulegone	Pink	Blue-violet	Violet
Styphnic acid	No reaction		—
Thujone	Weak red		—
Thymol	Weak green	Violet	—

It should be noted that in terms of specificity the test is more specific than is immediately apparent upon review of this table. The test, as generally performed, is preceded by an extraction with petroleum ether. The petroleum ether extract is evaporated to dryness and the residue tested with the Duquenois reagent. While all of the terpenes listed in the table are soluble in petroleum ether. many of the aromatic phenols are not. As a result, certain of the phenols which would give a color with the Duquenois reagent do not survive the preliminary extraction with petroleum ether and could not interfere with the interpretation of the test. Table 1 also illustrates the terminal extraction of the color formed into chloroform. The enhancement of the specificity of the test is apparent upon reference to this portion of the table.

It is noted that the terpenes tend to react slowly and with intensities ranging from weak to moderate, even at high concentrations of the terpene.

Table 2 illustrates the colors formed when a petroleum ether extract of marijuana is tested with the Duquenois reagent and a number of other aromatic aldehydes.

Marijuana sources

The marijuana used in the study originated from four verified sources:

(1) Cannabin, or "red oil" (K. & K. Laboratories), a steam distillate of an extract of marijuana of unknown source. The age of this material was unknown, but gas chromatographic analysis showed less than 1% total tetrahydrocannabinol, approximately 30% cannabinol, and approximately 30% cannabidiol, suggesting that the material may be some years old. An alcohol insoluble component was observed which may be a paraffin previously reported, n-nonacosane.

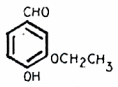
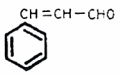
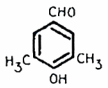
(2) Marijuana grown on the Military Reservation at Fort Leavenworth, Kansas, in the early Summer of 1967, and mailed to the Sheriff's Department, Contra Costa County, California.

(3) Marijuana grown by the writer in the Spring and Summer of 1967 in Martinez, California.

(4) Marijuana grown in the State of Nayarit, Mexico (Mazatlan-Tepic area), in the Summer of 1967. A quantity of the plant material was extracted with spectro-quality petroleum ether (30-60°C), filtered, and evaporated to an oil. Absolute ethanol was added and the solvent evaporated under nitrogen. Ethanol was again added and again evaporated under nitrogen, the process being repeated several times. Upon final addition of ethanol the material was shaken and centrifuged. Three distinct fractions were observed. Thin-layer chromatographic analysis showed the ethanolic top fraction to be richest in tetrahydrocannabinol. The middle fraction was an oil with a substantially

TABLE 2

REACTION OF MARIJUANA WITH AROMATIC ALDEHYDES

Aldehyde	*Color with Marijuana Resin*
 Vanillin	Blue-violet
 Isovanillin	Blue-violet
 Ethylvanillin	Blue-violet
 o-Vanillin	Weak amber
 Benzaldehyde	Brick red
 p-Hydroxybenzaldehyde	Blue-violet
 m-Hydroxybenzaldehyde	Weak violet
 Salicyaldehyde	Cloudy violet
 o-Anisaldehyde	Violet
 m-Anisaldehyde	Weak orange
 p-Anisaldehyde	Blue-violet
 Terephthaldehyde	No reaction
 Resorcylaldehyde	Violet
 Cinnamaldehyde	Burgundy
 3,5-Dimethyl-4-hydroxybenzaldehyde	Blue-violet

lower tetrahydrocannabinol content. The lower fraction was a solid, which was comparatively weak in tetrahydrocannabinol as shown by thin-layer chromatography. This solid is probably the paraffin n-nonacosane and traces of quebrachitol (inositiol mono-methyl ether), but was not further investigated.

The ethanolic fraction, richest in tetrahydrocannabinol, was gas chromatographically determined to contain 7·2μg/μl of Δ^1-tetrahydrocannabinol and 0·18μg/μl of the Δ^6-tetrahydrocannabinol isomer. This ethanolic solution was that which was utilized in the majority of the thin-layer chromatographic analyses conducted in this investigation. Assuming that the extraction was 100% efficient and that all of the tetrahydrocannabinol was in the top ethanolic fraction, then the total tetrahydrocannabinol would represent 0·105% of the dry weight of the plant, including the stems and seeds which are known to contain, respectively, little and no tetrahydrocannabinol.

The pure Δ^1- and Δ^6-tetrahydrocannabinol and the Δ^1-tetrahydrocannabinol acetate were provided by the Psychopharmacology Research Branch of the National Institutes of Mental Health through Mr. Kenneth D. Parker of the Hinc Laboratories, San Francisco, California.

Thin-layer Chromatography of Marijuana Resin

A number of thin-layer chromatographic systems were attempted with greater or lesser success. Silica Gel G or Silica Gel H gave better separations than did absorbant layers made up with Alumina G or Kieselguhr G. Silica Gel G-$AgNO_3$ (5:1) gave good results but not substantially different from that of Silica Gel G alone, and was not utilized in further thin-layer studies.

TABLE 3
THIN-LAYER CHROMATOGRAPHIC MOBILITY OF MARIJUANA CONSTITUENTS

	Rf in Solvent Systems				
Constituent	*S1*	*S2*	*S3*	*S4*	*S5*
Cannabidiolic acid	0·04	0·18	0·11	0·08	0·05
Cannabidiol	0·12	0·44	0·21	0·20	0·12
Cannabinol	0·38	0·66	0·29	0·54	0·26
Δ^1-THC	0·48	0·70	0·36	0·60	0·30
Δ^6-THC	0·50	0·72	0·40	0·64	0·34
Δ^1-THC acetate	0·71	0·84	0·80	0·85	0·60

Plates 250 layer Silica Gel HF, activated at 100°C. for 1 hour.
S1: Benzene
S2: Petroleum ether (60–80°C. b.p.): Ethanol (9:1)
S3: n-Hexane: p-Dioxane (9:1)
S4: n-Hexane: Diethyl ether (4:1).
S5: Petroleum ether (60–80°C. b.p.); Diethyl ether (9:1)

Table 3 illustrates the Rf's (distance traveled by material/distance traveled by solvent front) of the identified phenolic constituents in marijuana when chromatographed in a number of solvent systems, all run on a 250 micron thick plate of Silica Gel G or Silica Gel H which had been activated at 110°C for 1 hour. Polar solvents were observed to be unsatisfactory. Good separations were obtained with straight benzene, n-Hexane:Diethyl ether (4:1), Petroleum ether (30-60°C b.p.):Ethanol (9:1), and n-Hexane:p-Dioxane (9:1).

Color Development

Several reagents, in addition to the Duquenois reagent which was of most interest in this investigation, were utilized to visualize the components separated by the thin-layer chromatography. Fast Blue B, Fast Blue 2B, Scarlet Blue B, and Scarlet R were tried as diazo dye spray reagents. The Fast Blue B and Fast Blue 2B were observed to be more sensitive and were selected for this reason and also because the colors of marijuana phenolics with the Fast Blue B reagent had been reported in the literature. The Fast Blue B was found to be somewhat photosensitive, the developed and sprayed plate turning deep yellow and then light brown upon exposure to sunlight. Fast Blue 2B (Allied Chemical Co.) was observed to be much less sensitive to light while maintaining the same sensitivity and was used exclusively through the remainder of the thin-layer studies. The colors developed with Fast Blue B and Fast Blue 2B are identical.

The diazo dyes were made up as a 1% aqueous solution and sprayed on to the thin-layer plates, which were then heated with a forced air blower. The colors develop immediately, and are observed to persist for several months.

Beam's Reagent for cannabidiol and cannabidiolic acid was made up as 5% KOH in absolute ethanol.

A saturated solution of $FeCl_3$ in methanol was used as an indicator spray for cannabidiolic acid.

The Duquenois reagent was observed to be much less sensitive to the phenolic marijuana constitutents than the Fast Blue 2B salt. The limit of detectability of the Duquenois reagent on an undeveloped thin-layer plate is approximately 2 micrograms of either isomer of tetrahydrocannabinol, while the Fast Blue 2B can extend the sensitivity down to approximately 0·2μg. On the other hand, the Fast Blue 2B is observed to react with many compounds other than the phenolic constitutents of mariiuana and can hold no claim to specificity.

By using the Duquenois reagent as an overspray following development of a color with the Fast Blue 2B, the sensitivity of the reaction may be greatly enhanced. By the combination of the two spray reagents, the limit of detectability of tetrahydrocannabinol is of the order of 0·05 to 0·1μg.

As the materials of interest are phenolic in nature, the Folin-Ciocalteau phenol reagent was tried. This reagent was observed to possess poor sensitivity and gave the same colors (gray-green) with all of the separated constituents.

Infrared absorption spectra were prepared of the Δ^1- and Δ^6- isomers of tetrahydrocannabinol. Figure 1 illustrates the spectra of the isomers as determined by attenuated total reflectance infrared spectroscopy.

PART II: BOTANY

Introduction

The identification of marijuana by morphological examination can be readily made if the intact plant, or even the intact leaf and other parts such as flowers, seeds and stalks, are available (U.S. Treasury Dept., 1948). Many of the specimens submitted to forensic laboratories consist of crushed fragments, and no longer retain gross botanical characteristics. The smaller the fragments, the greater the expertise the examiner must exercise in the final identification of the species.

The forensic chemist also depends on the color reaction obtained in the Duquenois Negm test (Duquenois and Negm, 1938b), hereafter referred to in this paper as the "Duquenois N" test, and the subsequent transfer of this color to chloroform, as performed in the Duquenois-Levine test described by Butler (1962), and hereafter referred to as the "Duquenois L" test.

The identification of marijuana by microscopic methods depends largely on the presence of non-glandular hairs on the leaf surfaces, principally those which contain and are heavily encrusted with calcium carbonate deposits called cystoliths (Greek derivation of *kustis* and *lithos*, meaning "bag of stones"). Since the cystolith hairs have been used as an important diagnostic criterion for the identification of marijuana, this portion of the paper is devoted largely to a discussion of these formations. A literature search was conducted for information concerning the prevalence of cystolithic hairs in plants, and more precisely, concerning the taxonomic groups to which these hairs belong.

The voluminous works on plant anatomy by Solereder (1908), Metcalf and Chalk (1960), and a review, "Contribution à l'étude morphologique, histologique et physiologique des cystoliths" by Pireyre (1961) are rich sources of information on this topic. Hayward (1938) devotes a whole chapter to the detailed morphological treatment of *Cannabis*. Esau's discussion (1965) on trichomes, or epidermal appendages, served as an excellent introduction to the subject.

Leaf specimen collection was attempted for all the species described in the literature cited as having cystolith growths, with particular emphasis on trichomes incrusted with calcium carbonate. Where fresh specimens were not obtainable, those from herbaria were used for the study. Much of the screening, however, was done in the herbaria among families in which these species were known to bear cystolith hairs. Some 600 species belonging to the dicotyledons were examined, 82 of which were chemically tested by the Duquenois L method to determine if there were other plants having similar leaf hair characteristics and yielding the same color test as does *Cannabis*.

The United Nations Secretariat (1960a) reported the study of some 120 plant species belonging to 28 different families, using three different color-producing reagents, i.e., Ghamravy, Beam, and Duquenois N. Their study was largely concerned with seeking plants containing volatile oil and terpenes, while the present study was directed toward examining certain genera in which cystolith hairs are prevalent. Therefore, possibly only the two Moraceae species indicated in the UN document were duplicated by this study. Also, the present study was extended to those culinary herbs to determine if those in the UN study which yielded a positive blue to violet colors would also respond to the Duquenois L test. Most of these species are included in the family Labiteae which appears to be devoid of cystolith hairs.

Experimental

One hundred milligrams of leaf specimen was macerated in 25 ml of petroleum ether, filtered into a beaker, and evaporated to dryness without heating. The color reaction test was then conducted according to that described by Butler (1962) for the Duquenois L test.

For the morphological examination, leaf specimens were studied under stereoscopic binoculars, 10 to 50×, and a simple compound microscope, 50 to 100×. The subject was illuminated with narrowly directed reflected light from a "Fexilight" unit (Iota-Cam Corp., Wakefield, Mass.), capable of producing 3,000 to 11,000 candle power.

Photomacrography was conducted through a 16mm Zeiss Luminar lens mounted on a 35mm Leica by aid of Visoflex reflex and bellows attachments. Kodak Panatomic film was used. Unless otherwise indicated, all prints were enlarged to a final 60× magnification for all specimens to afford a size comparison of the hairs.

Table 4 indicates the taxonomic areas in which individual species were screened for hairs resembling those of *Cannabis*, including those reported in the literature (Solereder, 1908; Metcalf and Chalk, 1960; Pireyre, 1961; Esau, 1965). For confirmational purposes, the presence of calcium carbonate on the hairs was demonstrated by adding dilute HCl to the slide and observing for effervescence (U.S. Treasury Dept., 1948) under a microscope.

Discussion

One of the most widely accepted classification systems is that of Engler and Prantl (1924–1953), which includes the Cannabinceae within the Moraceae. However, most authors, including Solereder (1908), Porter (1967), Robbins, Bellue and Ball (1951), Metcalf and Chalk (1960), Pireyre (1961) and Core (1955), choose to isolate it as a distinct family. Marijuana is generally classified as follows:

DIVISION:	Spermatophyta (seed plants).
CLASS:	Angiospermae (flowering plants).
SUBCLASS:	Dicotyledons (dicots) 31,874 species.
ORDER:	Urticales (elms, mulberries, nettles, and hemps) 1,753 species.
FAMILY:	Cannabinaceae (hops and marijuana) 3 species.
GENUS:	*Cannabis*.
SPECIES:	*sativa*

TABLE 4

GENERA SCREENED BY MICROSCOPIC EXAMINATION

URTICACEAE *Boehmeria, Elatostema, Myriocarpa, Parietaria, Pellionia, Pilea, Urtica.*
MORACEAE *Antiaris, Artocarpus, Broussonetia, Cardiogyne, Cecropia, Conocephalus, Coussapoa, Cudrania Dorstenia, Fatoua, Ficus, Morus, Parartocarpus, Sorocea, Trophis.*
CANNABINACEAE *Cannabis, Humulus.*
ULMACEAE *Ampelocera, Aphananthe, Celtis, Gironiera, Lozanella, Holoptelea, Parasponis, Phyllostylon, Trema, Ulmus, Zelkova.*
ACANTHACEAE *Acanthus, Adhatata, Aphelandra, Asystasia, Barleria, Beloperone, Crossandra, Dicliptera, Diperacanthus, Fittonia, Hemigraphis, Hypoestes, Jacobinia, Peristrophe, Petalidium, Pseuderanthemum, Ruellia, Stenostephanus, Strobilanthes, Tetramerium, Thunbergia.*
BORAGINACEAE *Anchusa, Asperugo, Borago, Cerinthe, Cordia, Cynoglossum, Lycopsis, Lithospermum, Myosotis, Symphytum, Tournefortia.*
COMBRETACEAE *Anogeissus, Combretum, Quisqualis, Terminalia.*
CUCURBITACEAE *Benincasa, Bryonia, Cucurbita, Cucumis, Cyclanthera, Ecballium, Hamburia, Langenaria, Luffa, Melothria, Momordica, Thladiantha.*
LOASACEAE *Blumenbachia, Cajophora, Eucnide, Cronovia, Loasa, Mentzelia, Petalonyx.*
VERBENACEAE *Callicarpa, Clerodendron, Duranta, Lantana, Lippia, Tectona, Verbena, Vitex.*
SCROPHULARIACEAE *Antirrhinum, Calceolaria, Chelone, Craterostigma, Digitalis, Diplacus, Euphrasia, Gratiola, Linaria, Melampyrum, Mimulus, Odontites, Phygelius, Paraebe, Paulownia, Veronica.*
GESNERIACEAE *Aeschinanthus, Columnea, Gloxinia.*
CAMPANULACEAE *Campanula, Lobelia, Phyteuma, Trachelium.*
CISTACEAE *Cistus, Helianthemum.*
BEGONIACEAE *Begonia.*
HERNANDIACEAE *Hernandia.*
SAMYDACEAE *Homalium.*
PAPILIONACEAE *Cyamopsis, Indigofera.*
OLEACEAE *Nyctanthes.*
HYDROPHYLLACEAE *Eriodictyon, Hydrophyllum, Phacelis, Wigandia.*
COMPOSITAE *Artemisia, Inula, Tanacetum.*
LABIATAE *Lavendula, Mentha, Origanum, Rosemarinus, Salvia, Satureja, Thymus.*
MYRTACEAE *Eucalyptus.*
POLEMONIACEAE *Phlox.*
EUPHORBIACEAE *Euphorbia, Ricinus.*

This study was mainly concerned with cystoliths as they occur in the leaf epidermis and particularly in the trichomes; they are also found in the pith and cortex of certain plant stems (Solereder, 1908). Cystoliths of various types are found in the leaves of a number of dicots without taxonomic preference to any particular family. They are not considered to be common in occurrence nor particularly important for classification purposes. Hence, they are not diagnostic for a family, much less a genus. In families such as the Acanthaceae, Boraginaceae and Urticaceae, however, various forms or development of cystoliths have afforded criteria for genus and species identification (Solereder, 1908; Metcalf and Chalk, 1960).

While plants such as *Cannabis* and *Humulus* bear cystoliths as warty protuberances (Fig. 2) or as deposits in the basal position of hairs, others manifest cystolith growth in different forms, e.g. punctiforms, fusiforms, and stellate forms on the surface and in the mesophyll of the leaf. A typical fusiform cystolith of *Pilea* is shown in Fig. 3. This type lies elongated and parallel on the epidermis and has been found to be covered with a siliceous shell (Solereder, 1908). The stellate type of trichome is represented in *Cordia gerascanthus* in Fig. 4. Cystolith hairs of *Eucinide lobata* have anchor-shaped apices (Fig. 5). Fig. 6 shows the hairs of *Dorstenia contrajerva*, greatly magnified to delineate the ring of swollen subsidiary cells at the base of the hairs, a condition common to many hairs (Foster, 1949). Protuberances or "warts" as they occur on the epidermis of *Cannabis* and other cystolith-bearing plants, are protrusion of cystolith from within the cells, most of which occur independently of the hairs.

Solereder (1908) notes that an antagonistic relation exists between cystoliths and the development of hairs in *Cannabis* and in other species; the larger the cystolith at the base, the shorter the hairs. Therefore, the longer nonglandular hairs on the lower side of the leaf contain small cystoliths and do not assume the "rhinoceros horn" or "bear claw" shape characteristic of hairs on the upper surface. These unicellular hairs, although lacking distinctive characteristics for

diagnostic purposes, are of systematic value in *Cannabis* identification when correlated with cystolith hairs on the top side of the leaf specimens. None of the specimens examined in this study exhibited this correlation in an identical manner. These non-glandular hairs on the bottom side of the leaf should be noted for their size, shape, and proliferation. Dilute acid treatment of these hairs may indicate the presence of $CaCO_3$ on the base or the apex of these appendages. It may be well to note that non-glandular hairs by themselves vary greatly in plants and that a vast host of plants do not bear any hairs on the leaves. Different categories of trichomes are discussed in standard texts on plant anatomy, such as the one by Esau (1965).

When present, the glandular hairs in the *Cannabis* are of significance in identification due to the secreting heads at their apices. In the mature *Cannabis*, these hairs are decapitated at maturity and intact trichomes are usually observed only in the young leaf, according to Hayward (1938). The glistening heads of glandular hairs are abundant on the fruit hulls of marijuana.

Within the same genus, various types of hair can be found. In *Cordia*, for example, some species possess cystoliths which are entirely silicified, those which are calcified, or both types may occur side by side (Solererder, 1908). Figs. 7 and 8, *Lithospermum* and *Tournefortia* respectively, are examples of hairs whose walls are heavily encrusted with cystolith growth.

Of interest in this study of *Cannabis*, is *Humulus*, the only other genus in the family Cannabinaceae and a plant of considerable economic value as the source of "hops." Both glandular and nonglandular hairs of both genera are well-delineated by drawings in Esau (1965) and Hayward (1938). The cystolith hairs, also bear-claw shaped, are somewhat smaller in the mature leaves of *Humulus lupulus* while those of *Humulus japonica* are longer than those of *Cannabis* (compared in Figs. 2, 9 and 10). *Humulus* is differentiated from *Cannabis* by the presence of distinctive two-armed unicellular hairs seated on the epidermis directly or on a multicellular pedestal (Pireyre, 1961). The leaves of the *Humulus lupulus* do not give a positive Duquenois N test; however, the flower buds produce a moderate purple color with the Duquenois reagent. This color is not soluble in chloroform.

While difference in cystolith hair structure was observed between *Humulus lupulus* and *H. japonica*, the latter having its origin in China and Japan, the cystolith hairs of *Cannabis* showed no significant structural differences with respect to geographic origin or whether it is called *C. indica*, *C. americanus*, or *C. sativa*. Specimens from Europe and Asia were compared with those collected in America.

Urticaceae (nettles), Moraceae (mulberry), and Ulmaceae (elm) are taxonomically closely related to the Cannabinaceae inasmuch as these are grouped as Urticales (Core, 1955). For this reason, these families were screened more thoroughly than the others for trichomes having similar characteristics to those of *Cannabis*.

By and large, the cystoliths of Urticaceae and Moraceae are independent and are not associated with the hairs. However, certain *Urtica* species, e.g., *U. dioica* (Fig. 11) and *U. urens*, possess the so-called stinging hairs which are similar in appearance to the cystolith hairs of *Cannabis*. On closer examination, the apex of these stinging hairs bear a small knob which if broken, allows the liquid content of the hair to exude. *Boehmeria nivea* and *Parietaria officinalis*, listed by Pireyre (1961) as having cystoliths, were examined and their hairs are exhibited in Figs. 12 and 13 respectively. While the head and walls of the hairs of Urtica are silicified, the basal area is said to be calcified (Solererder, 1908). The base is, however, not enlarged as in *Cannabis*.

Of the genera examined in the family Moraceae, the *Broussonetia* has cystolith hairs (Fig. 14) resembling those of *Cannabis* while those of *Ficus* (Fig. 15) do not. Since *Ficus* is of common occurrence, many of its species were examined. In general, the cystoliths of the *Ficus* occur in the cells of the multiple epidermis (Esau, 1965; Foster, 1949) as independent ellipsoids; Solererder (1908) has referred to this type of formation as the "true cystolith". Although hairs were evident in *F. carica*, certain species such as *F. ribes*, *F. capaiefolia* and *F. sycomorus* showed only warty surfaces due to the protuberances of cystolith hairs from within the cells.

In the Ulmaceae, cystoliths existing as "true" forms in solitary or clustered crystals in the mesophyll of the leaves or as calcified "warts" in the epidermis have been recorded in a few genera (Solererder, 1908). Calcified hairs of *Aphananthe*, *Celtis* (Fig. 17), and *Trema* were observed to have structural appearances resembling those of *Cannabis*. They may, however, be differentiated by a close comparative examination.

Since the leaves of *Ulmus campestris*, *U. alata*, *U. divaricata*, and *U. foliacea* gave a positive Duquenois N test, but were negative to the Duquenois L test, leaves of five other different elms, i.e., *Ulmus rubra*, *U. scabra*, *U. pedunculata*, and *U. crassifolia*, and also those of other genera were tested. All of these failed to respond to the Duquenois N test. *Celtis occidentalis* yielded a light blue color with the Duquenois reagent, but it did not extract into chloroform.

The family Boraginaceae consists essentially of herbs in the north temperate zone and is characterized by bristle type hairs whose walls are wholly or partially encrusted with calcification. The cystolith hairs occur in such genera as *Cordia* (Fig. 4), *Tournefortia* (Fig. 8), *Asperugo*, *Myosotis*, *Anchusa* (Fig. 18) and *Borago*, and in a variety of sizes and shapes different even within certain genera. Elongated types of cystoliths, like in *Pilea* (Fig. 3), are found in *Cordia cuyabenis* and *C. alliodora* on the epidermis of the leaf while *C. gerascanthus* (Fig. 4) possesses hairs as well as independent crystalline structures. *C. macrocephala*, for example, has another type of stellate type growth. Solererder (1908) found these hairs of diagnostic importance among the *Cordia*. Notable was *Tournefortia scabra* (Fig. 8) which has cystolith hairs similar to those of *Cannabis* with fuzzy clothing hairs on the underside of the leaf. Although this same configuration is found with *Cannabis*, the cystolith hairs on *T. scabra* are conical and may be distinguished from those of *Cannabis*.

The cystolith hairs of *Anchusa* (Fig. 18), *Lithospermum* (Fig. 7), and *Symphytum* (Fig. 19) are large and pronounced with a multicellular base containing cystoliths. While many of the species of these genera possess cystolith hairs, none of those examined were positive to the Duquenois N test.

The bristle type hairs of the family Loasaceae are exemplified by those on *Loasa chelidonifolia* (Fig. 20), the verrucose walls of the trichomes being heavily encrusted with siliceous and calcified materials with barbs or spikes and by those with anchor-shaped apices found in *Mentzelia albescen* (Fig. 21) and *Eucinide lobata* (Fig. 5). As noted in Fig. 20, cystolith growth is manifested not only along the wall of the hairs, but is also exhibited in the subsidiary cells as a scale ring around the base hairs. Although these pronounced hair characteristics distinguish the species of *Loasaceae* from *Cannabis*, *Cajophora* (Fig. 22) had been classified, if incorrectly, in the family Cannabinaceae by Kohl as noted in Solererder (1908); the hairs on *Cajophora laterita* can be distinguished by barbs on the wall. *Petalonyx thurberi* (Fig. 23) has short, conical-shaped cystolith hairs superficially resembling those of *Cannabis*, but they grow profusely on a small leaf measuring approximately a centimeter long. Under high power, minute barbs can be observed on the hair surface. Loasaceae, for systematic interest, has been shown to be related to the Cucurbitaceae because of similar cystolith development (Solererder, 1908).

In the Cucurbitaceae, better known as the "gourd family", the cystoliths of this group are more characterized by their development in the epidermal and mesophyl cells associated with the hairs. Examination of such species as those of *Bryonia* and *Coccinia* indicate that the main difference between the cystolith hairs of these groups and those of *Cannabis* lies in the encrustation of the cell walls adjoining the hairs, forming a white round scale-like base. Hooked hairs of *Cucumis sativa* are relatively larger than those of *Cannabis* and are multicellular. On the other hand, the hairs of *Melothria malabarica* (Fig. 24) are comparable

in size to those of *Cannabis* but with marked thickening of basal structure.

Representative species of family Acanthaceae, which includes water willows, ruellia, etc. (Core, 1955), were examined for cystolith growth. A variety of independent types were observed. So varied are the cystoliths that they have been used as a basis for the identification of genera and species; however, neither Solererder (1908) nor Metcalf and Chalk (1960) describe cystolith growth in leaf hairs of Acanthaceae. Multicellular hairs of *Acanthus mollis* (Fig. 25) are abundant in this group since they also appear in the species of *Thunbergia*, *Strobilantes*, and *Ruellia*. No calcification was noted on these hairs. Most species of this family examined were characterized by having fusiform cystoliths growing on the epidermal cells of the leaves.

Calcification has been observed as a "delicate skeleton" on the hairs of *Campanula medium* (Solererder, 1908), of the family Campulaceae. The multicellular, curved hairs of *Campanula americana* are exhibited in Fig. 26. Of the Verbenaceae, *Lantana camara* (Fig. 27), and *Verbena officinalis* have cystolith hair resembling those of *Cannabis* in size and shape and would require a close scrutiny before they could be distinguished. *Lippia citriodora* possesses small cystolith hairs with large basal ring structure. In *Lantana* and *Lippia*, the cystoliths are concentrated in the cells at the base of the hairs (Metcalf and Chalk, 1960). *Tectona* species have large leaves with protuberances on the surface. The Duquenois N test was negative for these species.

Hernandiaceae includes species with cystolith growth occurring essentially as independent forms in the epidermis of the leaves and not associated with the hairs. Trichomes of the family Gesneriaceae were notable for their multicellular structure, e.g. *Gloxinia* and *Columnea*, while the family Cistaceae are characterized by a stellate type of hair such as those in *Helianthemum* and *Cistus*. The representative species of this group examined in the herbaria presented no hair structures of importance to this study.

In the family Labiteae, independent crystals or clusters of crystals of calcium oxalate (Solererder, 1908) occur in the leaves of some species but no cystolith hair is reported. *Origanum* (oregano) possesses unicellular, nonglandular hairs with a curved appearance not unlike those of *Cannabis*. These hairs are seated on a ring of swollen epidermal cells. Cross sectional drawings of oregano and other culinary herbs, showing hair and cell structures, are found in Parry's book on "Spices" (Parry, 1962).

Solererder (1908) listed the Hydrophyllaceae and Scrophulariaceae as having cystolith hairs and calcified protuberances in subsidiary cells of the hairs. Of the species of these families examined, the long, slender hairs of *Hydrophylum capitatum* and the short, flat type of *Melampyrum americanum* and *Calceolaria racemos* were found to be representative of the respective families but lacking in morphological similarities to the cystolith hairs of *Cannabis* and thus readily eliminated in forensic examination. Begonias (family Begoniaceae) bore largely smooth surfaced leaves and no trichomes of interest to this study. The paired cystolith deposits in the cells of certain other genera of Begoniaceae are considered, however, to be of systematic importance (Solererder, 1908). Pireyre (1961) listed Combretaceae as having cystoliths but an examination of the genera listed by him showed that the growths were not associated with the hairs.

Core (1955) noted that *Datisca cannabina* in Southeastern Asia and *D. glomerata* from Mexico and California and belonging to the family Datiscaceae, resemble the hemp plant, i.e. *Cannabis*; however, the leaves of these species were observed to have virtually no trichomes on either side, or sparse and isolated trichomes confined to the central vein on the underside of the leaf.

Representative species bearing cystolith hairs or hairs accompanied by independent calcified growth in the leaf, most of which are similar in structure to those of *Cannabis*, are listed in Table 5. No attempt was made to prepare a comprehensive listing because of the sheer enormity of the task to examine some 31,874 [illegible] dicotyledons; however, all species described by Solererder

TABLE 5

REPRESENTATIVE SPECIES BEARING CYSTOLITH HAIRS OR HAIRS RESEMBLING THOSE OF CANNABIS

URTICACEAE *Boehmeria nivea, Myriocarpa brachystachys, Parietaria officinalis, Urtica dioica, U. urens.*
MORACEAE *Broussonetia kaempferi, B. papyrifera, Dorstenia contrajerva, Fatoua japonica, Ficus elastica*, F. repens*.*
CANNABINACEAE *Humulus lupulus*.*
ULMACEAE *Aphananthe aspera, Celtis occidentalis, Parasponia andersonii, Trema cannabina, T. micrantha, Ulumus alata*, U. campestris*, U. divaricata*, U. foliaceae*.*
ACANTHACEAE *Acanthus mollis, Crossandra undulaefolia, Ruellia occidentalis, Strobilanthes isopricus, S. lactatus*, Tetramerium nervosum, Thunbergia alata.*
BORAGINACEAE *Anchusa officinalis, Asperugo procumbens, Borago officinale, Cordia gerascanthus, C. pubescen, Lithospermum officinale, L. purpureo-coeruleum, Myosotis sylvatica, Symphytum officinale, Tournefortia paniculata, T. peruviniana, T. scabra.*
CUCURBITACEAE *Bryonia dioica, Cucumis sativa, Ecballium elaterium, Melothria qualalupensis.*
LOASACEAE *Blumenbachia insignis, B. urens, Cajophora laterita, Gronovia scandens, Loasa bipinnata, L. chelidonifolia, L. picta, Mentzelia albescens*, Petalonyx thurberi.*
VERBENACEAE *Lantana camara, Lippia citriodora, Verbena officianalis.*
SCROPHILARIACEAE *Calceolaria racemos, Euphrasia officinalis*, Melampyrum americanum, Odontites verna.*
GESNERIACEAE *Gloxinia antirrhina.*
CAMPUNULACEAE *Campanula americana, Lobelia inflata.*
HYDROPHYLLACEAE *Hydrophylum capitatum.*

* Positive reaction obtained by Duquenois-Negm test; blue to purple colors produced were not $CHCl_3$ extractable.

(1908) and Metcalf (1960) as having cystolith hairs are included in this list. Such genera as *Loasa* and *Lithospermum* have a large number of species bearing cystolith hairs and only a few species were used to represent them in this Table. *Loasa* itself consists of some 80 species generally known to have stinging hairs (Core, 1955). Those species which reacted with the Duquenois N test to produce blue to violet colors are indicated. None of these colors was soluble in chloroform.

The United Nations document (UN Secretariat, 1960a) listed eight plants; namely, *Salvia officinalis, Thymus vulgaris, Satureja hortensis, Eucalyptus globus, Arthemisia dracunculus, Ficus carica, Pelargonium capitatum*, and *Rhamnus frangula*, which responded to the Duquenois N test. Since the Levine modification was not employed in their test, leaf specimens of these eight species were collected and tested to find whether the colors produced with the Duquenois N reagent could be extracted into $CHCl_3$; the results were negative on specimens from all eight species.

Earlier, Butler (1962) conducted an AOAC collaborative study to determine if the colors produced by some plant materials, including four of the species named above, consisting of Eucalyptus leaves, Turkish tobacco, Buckthorn flowers (*Rhamnus*), Dalmatian sage (*Salvia officinalis*), catnip (*Nepeta cataria*) and Thyme (*Thymus vulgaris*) would confuse analysts examining for the presence of marijuana and to assess the Duquenois L modification as a more useful test for marijuana identification. His seven collaborating chemists reported negative Duquenois L tests on all of them.

In addition, the present investigation tested the following other culinary herbs belonging to the Labitaeae family by the Duquenois L test: *Thymus herba-baron, T. serphyllum*, "lemon thyme," "Silver thyme," *Lavendula officinalis, L. dentata, L. tatifolia, L. pubescens, L. aurigerana, Salvia leucantha, S. divinorum, S. rutilaus, Satureja montara, Mentha citrata, M. crispa, M. gentilis, M. piperita, M. pulegium, M. spicata, M. requienii, M. rotundifolia, Marjoram hortensis, Origanum vulgare*, and *Rosemarinus officinalis*. None of these yielded a positive Duquenois L test, although a chloroform-insoluble violet color was produced in reaction with *M. pulegium* and *M. requientii*.

As indicated by the photomacrographs presented in this paper, the hairs of many dicotyledonous species have profiles which resemble the cystolith hairs of marijuana. Only after a studied examination, under high magnification, can the cystolith hairs of marijuana be tentatively identified. Microscopic identification of marijuana, therefore, depends not only on the presence of cystolith hairs but on its assocation with the longer clothing, or non-glandular

hairs, on the other side of the leaf, and if present, the fruits and their hulls, the glandular hairs and the flowering tops. This is set forth and described fully in the U.S. Treasury Department Manual (1948). The Duquenois L test should be used in final confirmation.

Loose cystolith hairs frequently found in hashish (marijuana resin) samples are of limited diagnostic value since they cannot be related to the plant structure from which they have originated. It is suggested that the thin-layer chromatography method described in Part I of this paper as well as the microscopic examination and the Duquenois L test should be employed for the positive identification of hashish.

The United Nations Laboratory (UN Secretariat, 1961) conducted Beam, Duquenois N and Ghamrawy tests on constituents of volatile oil of aromatic plants, in addition to the nine reported in the previous study (UN Secretariat, 1960a). It was reported that none of the different hues of violet color produced by the Duquenois reagent for some ten compounds were soluble in chloroform. The present study shows that a number of plants other than the aromatic plants will yield a positive Duquenois N test, although the color does not extract into chloroform.

De Faubert Maunder (1969a) listed some 25 species which exhibited violet to purple colors in the Duquenois N test and found to be extractable in chloroform.

The present authors collected and examined 23 of these specimens, i.e. *Calamus draco* (calamus), *Coffea arabica** (coffee), *Leptandra virginica* (culver root), *Asarum canadense* (ginger), *Dorema ammoniacum* (gum ammoniac), *Protium icicariba* (gum animi), *Hymenaea coubaril* (gum copal), *Caplafer conjugata** (gum copal), *Agathis australis** (gum Kawri), *Myrrhis odorata* (gum myrrh), *Lawsonia inermis* (henna), *Lactuca sativa* (lettuce opium), *Glyccyrrhiza glabra* (liquorice), *Myristia fragrans* (nutmeg), *Iris florentina* (orris), *Iris versicolor* (poison flag), *Adenanthera povinia* (sandal wood), *Thuja occidentalis** (thuja), *Myroxolon toluiferum* (tolu), *Pedicularis canadensis* (wood betony) and *Teucrium scorodonia** (wood sage) and *Callitris quabrivalvis** (sandarac). None of these specimens exhibited hair structures resembling those of the cystolith hairs of Cannabis nor produced a Duquenois violet color which was extractable in chloroform. Those leaf specimens yielding a purple to violet color with the Duquenois test are indicated (supra) by an asterisk.

The results indicate that in the above 23 species, the positive Duquenois L test was evidently obtained from parts of the plant other than the leaves. It is further noted that many of the samples consist of gums or resins which would be expected to be rich in reactive terpenes.

For specimens not conforming to the morphological description for marijuana (U.S. Treasury Dept., 1948), but yielding purple to blue colors with the Duquenois reagent, the TLC method using Fast Blue B as the detection reagent described in Part I of this paper should be employed to preclude the presence of hashish or any of its cannabinoid components. DeFaubert Maunder (1969b) tested the use of Fast Blue B on some 226 different samples of roots, rhizomes and barks having possible occurrence in compounded foods or drugs or "native medicines". Mace and nutmeg, which could not be credibly confused with marijuana on the basis of microscopic appearance, were the only two substances which yielded colors similar to that obtained with marijuana.

A reagent consisting of Fast Blue B salt in $CHCl_3$ is used by the government narcotic agency in Japan as a field test for marijuana and is called "KN" (for Kanto-Shinetsu Narcotic Control Office) reagent. Color development occurs in alkaline aqueous solution and is shaken into the chloroform phase. Koles (1969) in testing this kit found that it offered no advantage over the use of Duquenois-Levine test either in specificity or sensitivity. Several phenolic compounds yielded intensely colored reaction products, one of which was of an orange-colored hue produced by both phloroglucinol and marijuana.

Acknowledgements

The authors are indebted to Dr. Adriance S. Foster, Professor of Plant Anatomy, University of California, Berkeley, for his consultations; to the botanical gardens of the University of California at Berkeley, Davis and Los Angeles; to the Strybing Arboretum at San Francisco; to the State and County Botanical Gardens and Arboreta at Los Angeles and Santa Barbara; and to the Royal Botanic Garden, Kew, England, for kindly furnishing plant specimens; to Dr. John L. Strothers, University of California Herbarium, for his kind assistance; to Dr. Elizabeth McClintock of the Herbarium of the California Academy of Science, San Francisco, for her help and guidance; to the *Journal of the Association of Official Analytical Chemists* for permission to print a portion of the manuscript by Nakamura (1969) which appeared in a modified form in that journal; to Mr. Kenneth D. Parker of the Hine Laboratory, San Francisco, for his gift of tetrahydrocannabinol isomers; to Dr. Paul L. Kirk of the University of California School of Criminology and Dr. Donald Noyce of the Department of Chemistry, University of California, Berkeley, and to William P. Butler, Bureau of Narcotics and Dangerous Drugs, Washington, D.C., for having reviewed the manuscript.

References

Adams, R., and Baker, B. R., 1940a, *J.A.C.S.*, **62,** 2401.
Adams, R., and Baker, B. R., 1940b, *J.A.C.S.*, **62,** 2405–2408.
Adams, R., and Baker, B. R., 1940c, *J.A.C.S.*, **62,** 2208.
Adams, R., Baker, B. R., and Wearn, R. B., 1940, *J.A.C.S.*, **62,** 2204.
Adams, R., and Carlin, R. J., 1943, *J.A.C.S.*, **65,** 365.
Adams, R., Cain, C. K., and Baker, B. R., 1940, *J.A.C.S.*, **62,** 2201.
Adams, R., Cain, C. K., McPhee, W. D., and Wearn, R. B., 1941, *J.A.C.S.*, **63,** 2209.
Adams, R., Cain, C. K., and Wolff, H., 1940, *J.A.C.S.*, **62,** 732.
Adams, R., Harfenist, M., and Loewe, S., 1949, *J.A.C.S.*, **71,** 1624.
Adams, R., Hunt, M., and Clark, J. H., 1940a, *J.A.C.S.*, **62,** 735.
Adams, R., Hunt, M., and Clark, J. H., 1940b, *J.A.C.S.*, **62;** 196.
Adams, R., Loewe, S., Smith, C. M., and McPhee, W. D., 1942, *J.A.C.S.*, **64,** 694.
Adams, R., Loewe, S., Pease, D. C., Cain, C. K., Wearn, R. B., Baker, R. B., and Wolff, H., 1940, *J.A.C.S.*, **62,** 2466.
Adams, R., Pease, D. C., Cain, C. K., and Clark, J. H., 1940, *J.A.C.S.*, **62,** 2402.
Adams, R., Pease, D. C., and Clark, J. H., 1940, *J.A.C.S.*, **62,** 2194.
Adams, R., Pease, D. C., Clark, J. H., and Baker, B. R., 1940, *J.C.A.S.*, **62,** 2197.
Adams, R., Wolff, H., Cain, C. K., and Clark, J. H., 1940a, *J.A.C.S.*, **62,** 2215.
Adams, R., Wolff, H., Cain, C. K., and Clark, J. H., 1940b, *J.A.C.S.*, **62,** 1770.
Bembry, T. H., and Powell, G., 1941, *J.A.C.S.*, **63,** 2766.
Bergel, T., and Wagner, R., 1930, *Justus Liebigs Annalen der Chemie,* **482,** 55.
Blackie, W. J., 1941, *Ind. and Eng. Chem.*, **13,** 96.
Burnstein, S., and Mechoulam, R., 1968, *J.A.C.S.*, **90,** 2420.
Butler, W. P., 1962, *J.A.O.A.C.*, **45,** 597.
Cahn, R. S., 1930, *J. Chem. Soc.*, **1930,** 986.
Cahn, R. S., 1931, *J. Chem. Soc.*, **1931,** 630.
Cahn, R. S., 1932, *J. Chem. Soc.*, **1932,** 1342.
Cahn, R. S., 1933, *J. Chem. Soc.*, **1933,** 1400.
Casparis, P., and Baur, E., 1927, *Pharm. Acta Helvetiae,* **2,** 108.
Cheronis, N. D., 1960, *Microchem. J.*, **4,** 555.
Claussen, U., v. Spulak, F., and Korte, F., 1966, *Tetrahedron,* **22,** 1477.
Core, E. L., 1955, *Plant Taxonomy,* Prentice-Hall, Englewood Cliffs, N.J.

Czerkis, M., 1907, *Justus Liebigs Annalen der Chemie*, **351**, 467.
D'Alelio, G. F., 1955, *Experimental Plastics and Synthetic Resins*, Wiley, New York.
Davis, T. W. M., Farmilio, C. G., and Osadchuk, M., 1963, *Anal. Chem.* **35**, 1751.
De Faubert Maunder, M. J., 1969a, Laboratory of the Government Chemist, Stamford St., London, S.E.1, personal communication.
De Faubert Maunder, M. J., 1969b, *J. Assoc. Pub. Analysts*, **7**, 24.
De Ropp, R. S., 1960, *J. Amer. Pharm. Assn., Sc. Ed.*, **49**, 756.
Dunstan, W. R., and Henry, T. A., 1898, *Proc. Chem. Soc.*, **14**, 44.
Duquenois, P., 1950, *United Nations Bulletin on Narcotics*, **2**, 30.
Duquenois, P., and Negm, H., 1938a, *Bull. Sci. Pharm.*, **45**, 203.
Duquenois, P., and Negm, H., 1938b, *Ann. Med. Legale*, **18**, 485.
Efron, D. H., 1967, Reported to the Committee on Problems of Drug Dependence, National Academy of Sciences, 29th Meeting, 13 Feb. 1967, Lexington, Ky.
Engler, A. C., and Prantl, K., 1924–1953, *Die Naturlichen Pflanzenfamilies*, Engelmann (ed.), Leipzig, 2nd edn.
Esau, K., 1965, *Plant Anatomy*, John Wiley & Sons, New York.
Fahrenholtz, K. E., Lurie, M., and Kierstead, R. W., 1967, *J.A.C.S.*, **89**, 5934.
Farmilo, C. G., 1961, *United Nations Secretariat Bulletin* ST/SOA/SER.S/4.
Farmilo, C. G., McConnell Davis, T. W., Vandenheuvel, F. A., and Lane, R., 1962, *United Nations Secretariat Bulletin* ST/SOA/SER.S/7.
Foster, A. S., 1949, *Practical Plant Anatomy*, 2nd edn., Van Nostrand, New York.
Fraenkel, S., 1903, *Arch. f. exp. Path. u. Pharmakol.*, **49**, 266.
Fulton, C. C., 1942, *Ind. and Eng. Chem.*, **14**, 407.
Gaoni, Y., and Mechoulam, R., 1964a, *Proc. Chem. Soc.*, March, 2.
Gaoni, Y., and Mechoulam, R., 1964b, *J.A.C.S.*, **86**, 1646.
Gaoni, Y., and Mechoulam, R., 1965, *J.A.C.S.*, **87**, 3273.
Gaoni, Y., and Mechoulam, R. 1966, *Chem. Commun.*, **1**, 20.
Ghosh, R., Todd, A. R., and Wilkinson, S., 1940, *J. Chem. Soc.*, **1940**, 649
Ghosh, R., Todd, A. R., and Wright, D. C., 1941, *J. Chem. Soc.*, **1941**, 137.
Grlic, L., 1962, *United Nations Bulletin on Narcotics*, **14**, 37.
Grlic, L., 1964, *United Nations Bulletin on Narcotics*, **16**, 29.
Haagen-Smit, A. J., Wawra, C. Z., Koepeli, J. B., Alles, G. A., Feigen, G. A., and Pratner, A. N., 1940, *Science*, **91**, 602.
Hackel, E., 1963, Doctoral Dissertation, Rheinische Friedrick Wilhelms Universität, Bonn, Germany.
Hayward, H. E., 1938, *The Structure of Economic Plants*, The Macmillan Co., New York.
Hively, R. L., and Hoffman, F. W., 1966, *Edgewood Arsenal Technical Report*, EATR 4002, July.
Hively, R. L., Mosher, W., and Hoffmann, F. W., 1966, *J.A.C.S.*, **88**, 1832.
Isbell, H., 1967, Reported to the Committee on Problems of Drug Dependence, National Academy of Sciences, 29th Meeting, 13 February 1967, Lexington, Kentucky.
Jacob, A., and Todd, A. R., 1940, *J. Chem. Soc.*, **1940**, 649.
Kabelik, J., Krejci, Z., and Santavy, F., *United Nations Bulletin on Narcotics*, **12**, 5.
Kingston, C. R., 1961, Thesis, University of California School of Criminology.
Koles, J. E., 1969, BNDD, Washington, D.C., personal communication, Nov. 4, 1969.
Korte, F., and Sieper, H., 1960a, *Justus Liebigs Annalen der Chemie*, **630**, 71.
Korte, F., and Sieper, H., 1965, *Hashish: Its Chemistry and Pharmacology* G. E. W. Wolstenholme (Ed.), Little, Brown, and Company, Boston.
Korte, F., and Sieper, H. 1960b, *Angewandte Chemie*, **72**, 210.
Krejci, A., and Santavy, F., 1955, *Acta Universitatis Palackianae Olumucensis*, **6**, 59.
Krejci, Z., Horak, M., and Santavy, F., 1958, *Acta Universitatis Palackianae Oluwucensis*, **16**, 9.
Leaf, G., Todd, A. R., and Wilkinson, S., 1942, *J. Chem. Soc.*, **1942**, 185.
Madinaveitia, A., Russel, P. B., and Todd, A. R., 1942, *J. Chem. Soc.*, **1942**, 628.
Mechoulam, R., and Gaoni, Y., 1965, *Tetrahedron*, **21**, 1223.
Mechoulam, R., Ben-Zvi, E., Yagnitinski, B., and Shani, Al, 1969, *Tetrahedron Letters*, **28**, 2339.
Mechoulam, R., and Shvo, Y., 1963, *Tetrahedron*, **19**, 2073.
Mechoulam, R., Yagnitinsky, B., and Gaoni, Y., 1968, *J.A.C.S.*, **90**, 2418.
Metcalf, C. R., and Chalk, L., 1960, *Anatomy of the Dicotyledons*, Vol. 2, Oxford at the Clarendon Press.
Miras, C., Simos, S., and Kiburis, J., 1964, *United Nations Bulletin on Narcotics*, **16**, 13.
Nakamura, G. R., 1969, *J.A.O.A.C.*, **52**, 5.
Nickerson, G. B., and Likens, S. T., 1966, *J. Chromatography*, **21**, 1.
Obata, Y., and Ishikawa, Y., 1960, *Bull. Agr. Chem. Soc. Japan*, **24**, 667.
Parry, J. W., 1962, *Spices*, Chemical Publishing Co., New York.
Petrzilka, T., Haefliger, W., Sikemeier, C., Ohloff, G., and Eschenmoser, A., 1967, *Helv. Chim. Acta*, **50**, 719.
Pireyre, N., 1961, *Rev. Cytol. Biol. Veg.*, **23**, 93.
Porter, C. L., 1967, *Taxonomy of the Flowering Plants*, W. H. Freeman, San Francisco.
Robbins, W. W., Bellue, M. K., and Ball, W. S., 1951, *Weeds of California*, California Department of Agriculture, Sacramento, California.
Schultz, O. E., and Haffner, G., 1958, *Archiv. det Pharmazie*, **291**, 391.
Simonsen, J., and Todd, A. R., 1942, *J. Chem. Soc.*, **1942**, 188.
Smith, T., and Smith, H., 1847, *Pharm. J. and Transactions*, **6**, 127 and 171.
Solererder, H., 1908, *Systematic Anatomy of The Dicotyledons*, Oxford at the Clarendon Press.
Taylor, E. C., Lenard, D., and Loev, B., 1967, *Tetrahedron*, **23**, 77.
Taylor, E. C., Lenard, K., and Shvo, Y., 1966, *J.A.C.S.*, **88**, 367.
Taylor, E. C., and Strojny, E. J., 1960, *J.A.C.S.*, **82**, 5198.
Todd, A. R., 1946, *Experientia*, **2**, 55.
United Nations Secretariat, 1960a, *UN Bulletin* ST/SOA/SER.S/1.
United Nations Secretariat, 1960b, *UN Bulletin*, ST/SOA/SER.S/3.
United Nations Secretariat, 1961, *UN Bulletin*, ST/SOA/SER./S/5.
U.S. Treasury Department, 1948, *Marijuana, Its Identification*, U.S. Government Printing Office, Washington, D.C.
U.S. Treasury Department, 1962, U.S. Treasury Department Bureau of Narcotics Document No. 5104 (7–62), Washington, D.C.
Vejar, M., 1967, personal communication, Civil Division, Contra Costa County Sherriff's Department, September, 1967.
Volk, M. J., 1967, personal communication, Special Services Division, Contra Costa County Sheriff's Department, October, 1967.
Wollner, H. J., Matchett, J. R., Levine, J., and Loewe, S., 1942, *J.A.C.S.*, **64**, 26.
Wood, T. B., Spivey, W. T. N., and Easterfield, T. H., 1896, *J. Chem. Soc.*, **69**, 539.
Wood, T. B., Spivey, W. T. N., and Easterfield, T. H., 1899, *J. Chem. Soc.*, **75**, 20.
Work, T. S., Bergel, F., and Todd, A. R., 1939, *Biochem, J.*, **33**, 123.
Wright, J., and Parker, K. D., 1967, personal communication, Hine Laboratories, San Francisco, California, 14 July 1967.
Yamauchi, T., Shoyama, Y., Aramaki, H., Azuma, T., and Nishioka, I., 1967, *Chemical and Pharmaceutical Bulletin*, **15**, 1075.

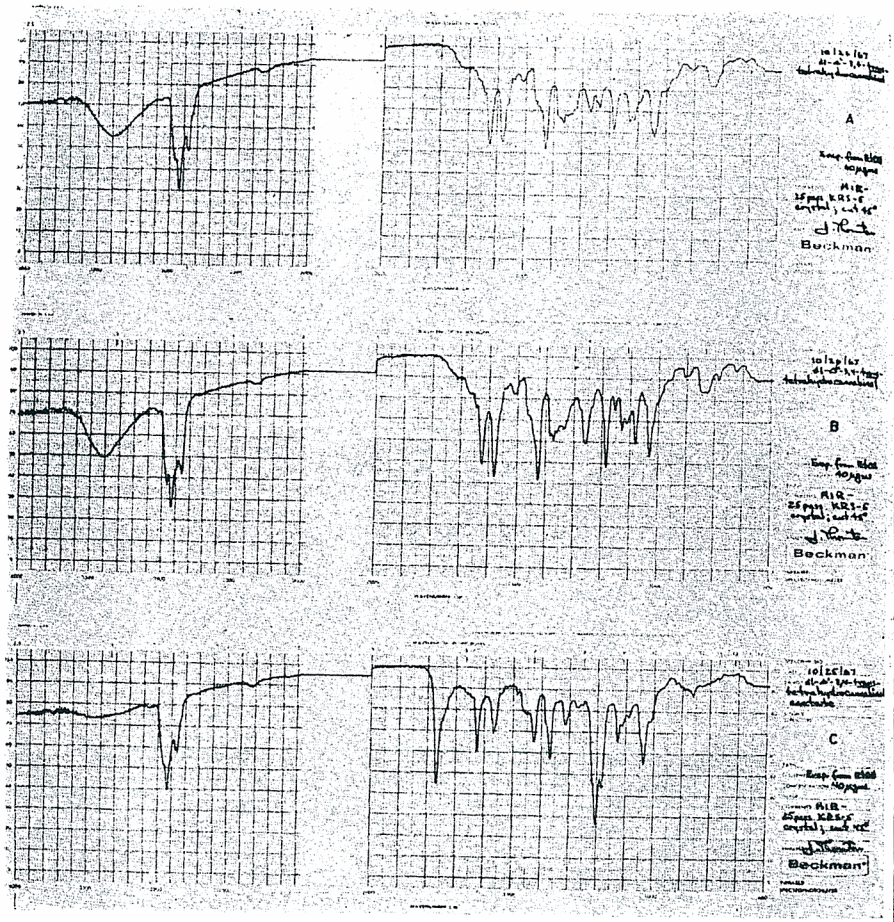


Fig. 1. Infrared absorption spectrum of (top) dl-Δ^1-3,4-*trans*-tetrahydrocannabinol, (centre) dl-Δ^6-3,4-*trans*-tetrahydrocannabinol, and (bottom) dl-Δ^1-3,4-*trans*-tetrahydrocannabinol acetate. The spectra were prepared by evaporating an ethanolic solution of the isomers on a 25 pass KRS-5 crystal, 45° cut, and run by multiple internal reflectance.

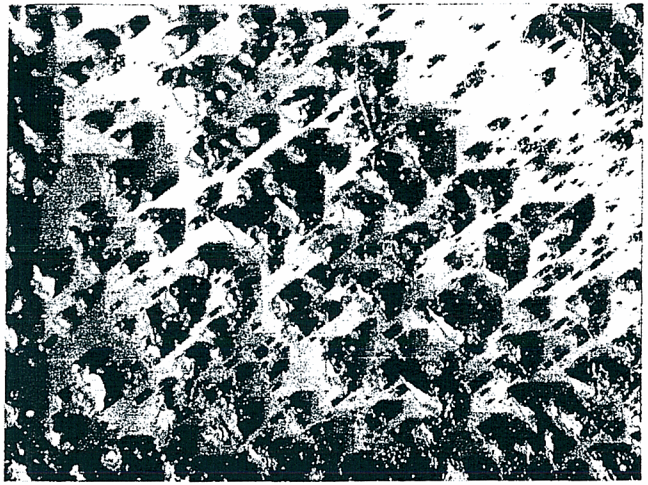


Fig. 2. Cystolith protuberances of *Humulus lupulus*, 60×.

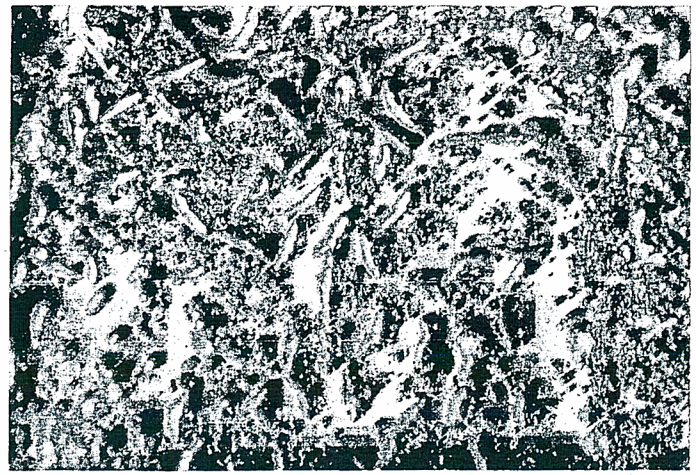


Fig. 3. Fusiform type of cystoliths on *Pilea pubescens*, 60×.

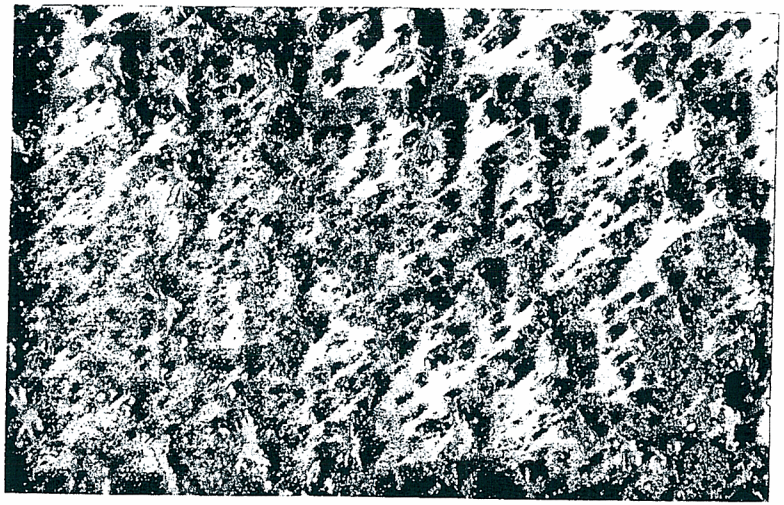


Fig. 4. Stellate type of cystoliths on *Cordia gerascanthus*, 60×.

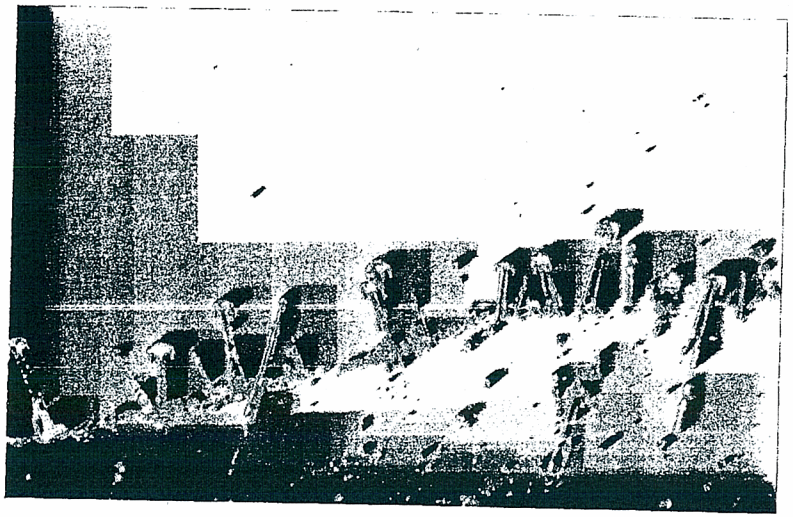


Fig. 5. Anchor-shaped heads on leaf hairs of *Eucinide lobata*, 60×.

Fig. 6. Close-up of leaf hairs of *Dorstenia contrajerva* showing basal subsidiary cells, 90×.

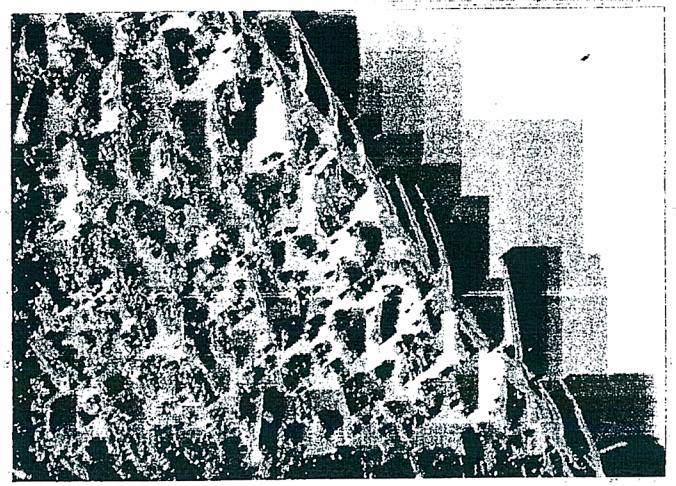


Fig. 7. Long, hypha-type hairs of *Lithospermum officinalis*, 60×.

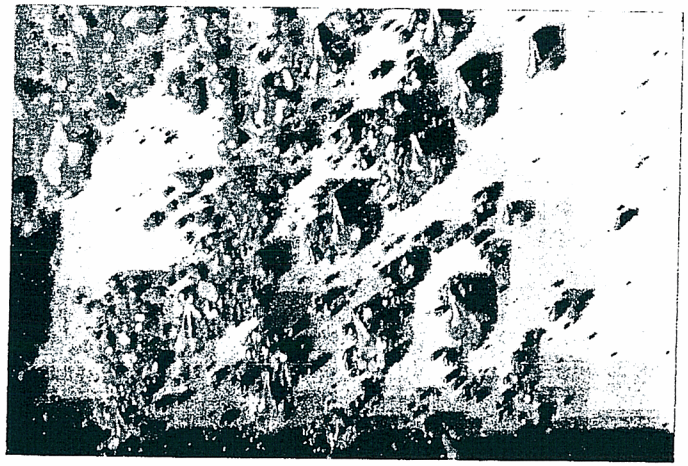


Fig. 8. Conical-shaped leaf hairs of *Tournefortia scabra*, 60×.

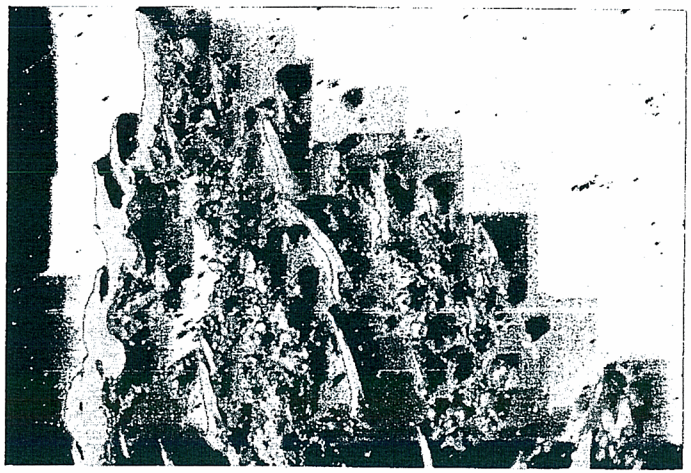


Fig. 9. Cystolith hairs of *Humulus japonica*, 60×; note similarity with hairs of Cannabis, Fig. 10.

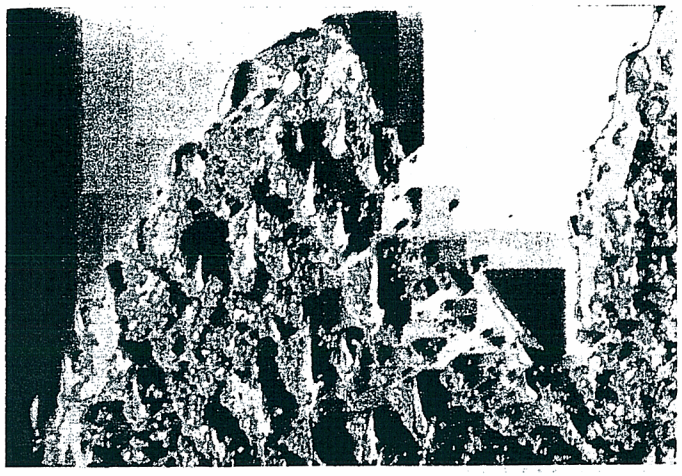


Fig. 10. Cystolith leaf hairs of *Cannabis sativa*, 60×.

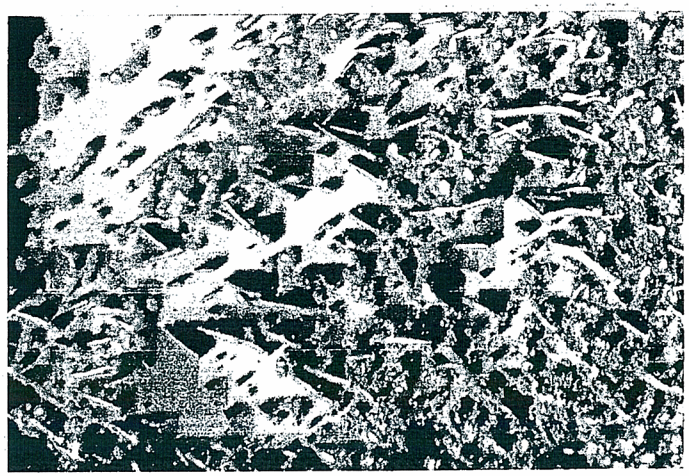


Fig. 11. "Stinging hairs" of *Urtica divica*, glandular-type, 60×.

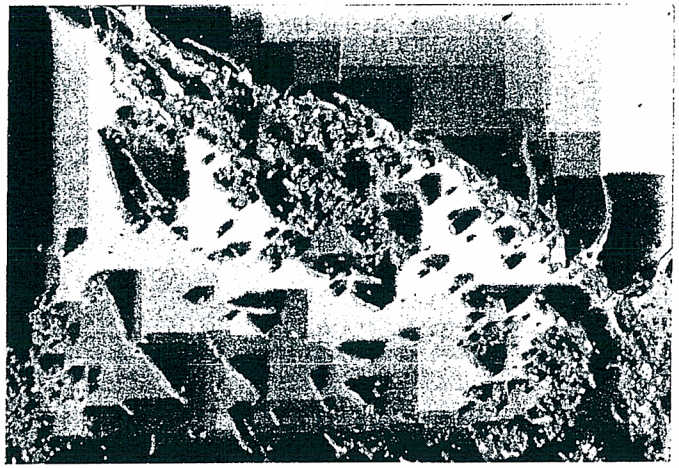


Fig. 12. Hook-shaped leaf hairs of *Boehmeria nivea*, 60×.

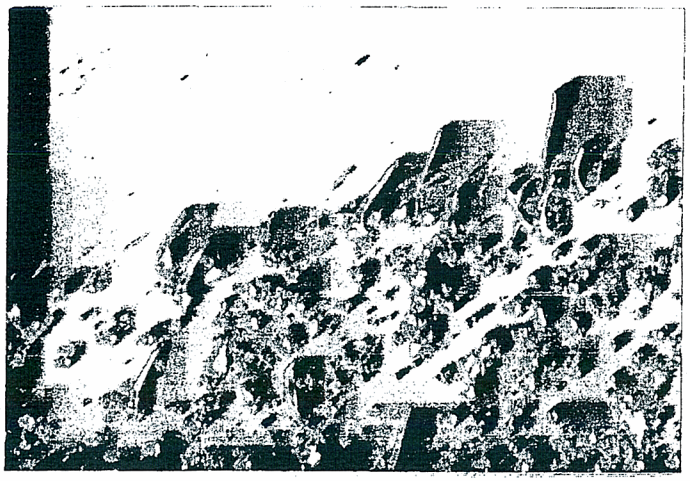


Fig. 13. Hook-shaped leaf hairs of *Parietaria officinalis*, 60×.

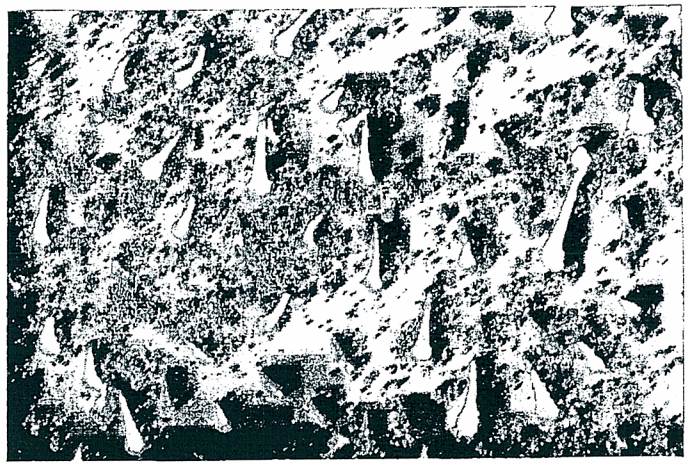


Fig. 14. Heavily calcified leaf hairs of *Broussonetia papyrifera*, 60×.

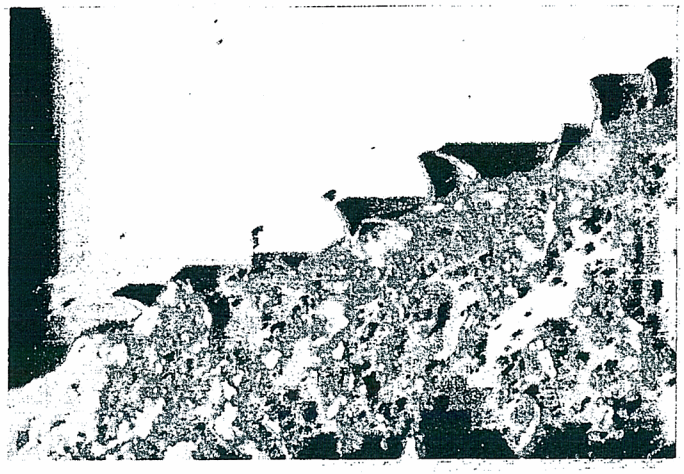


Fig. 15. Calcified upper portions of leaf hairs of *Ficus carica*, 60×.

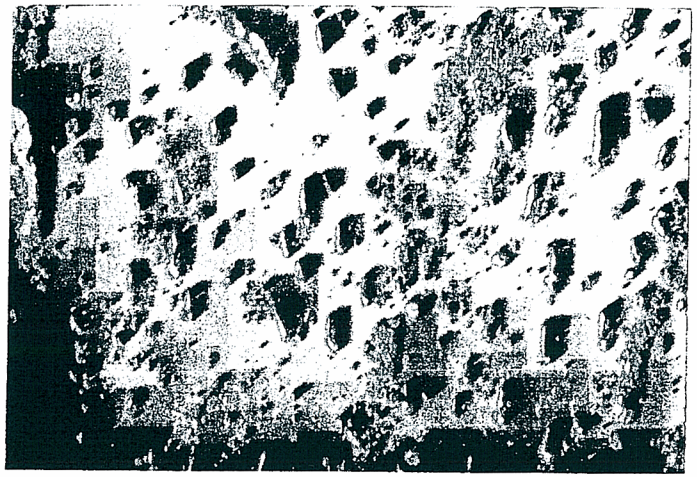


Fig. 16. Leaf hairs of *Ulmus campestris*, 60×.

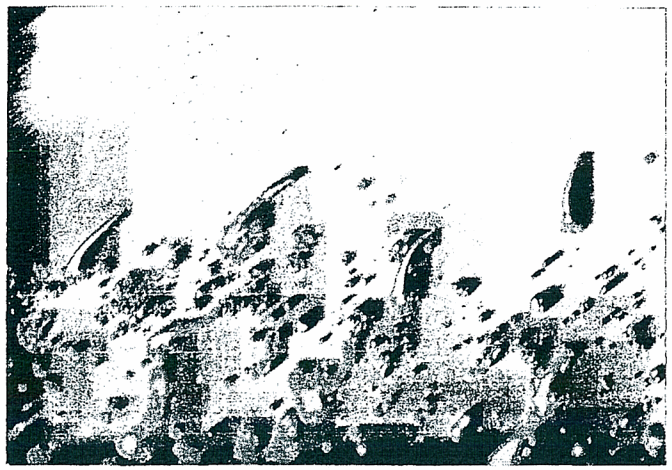


Fig. 17. Leaf hairs of *Celtis occidentalis*, 60×.

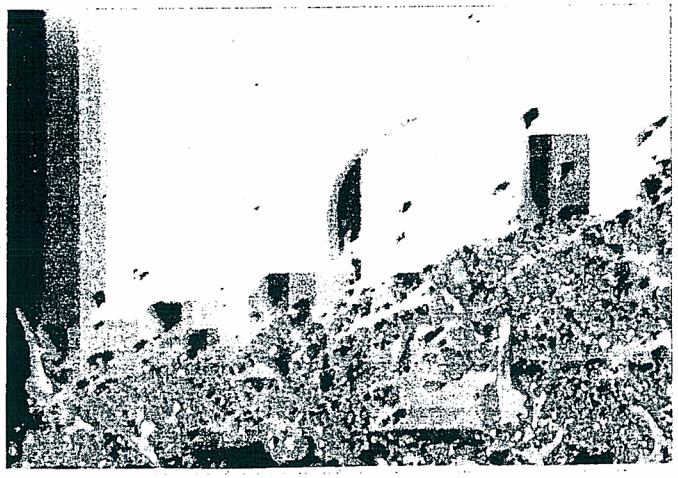


Fig. 18. Sickle-shaped leaf hairs of *Anchusa officinalis*; note enlargements of subsidiary cell at the base due to presence of cystoliths, 80×.

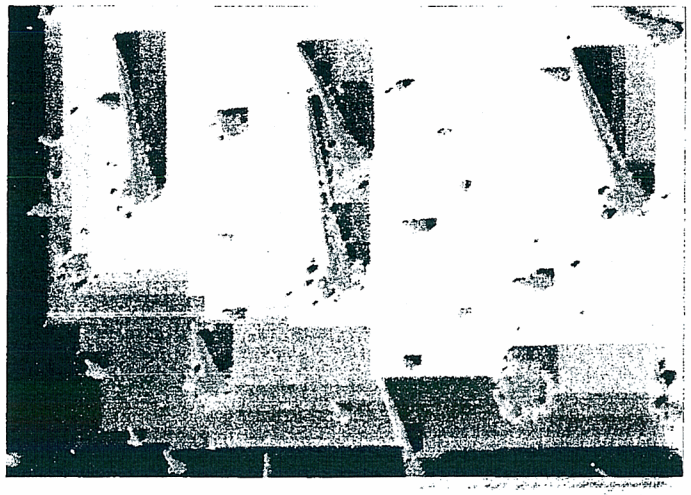


Fig. 19. Bristle hairs of *Symphytum officinalis*; note smooth, calcified wall and swollen base, 60×.

Fig. 20. Verrucose conical hairs of *Loasa chelidonifolia* that are oblique to surface; thickening of base is due to cystolith attached to wall, 60×.

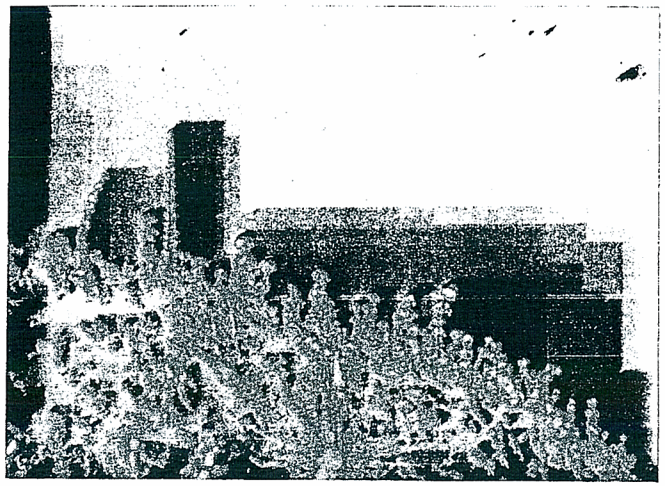


Fig. 21. Many-fluked anchors on leaf hairs of *Mentzelia albescens*, 60×.

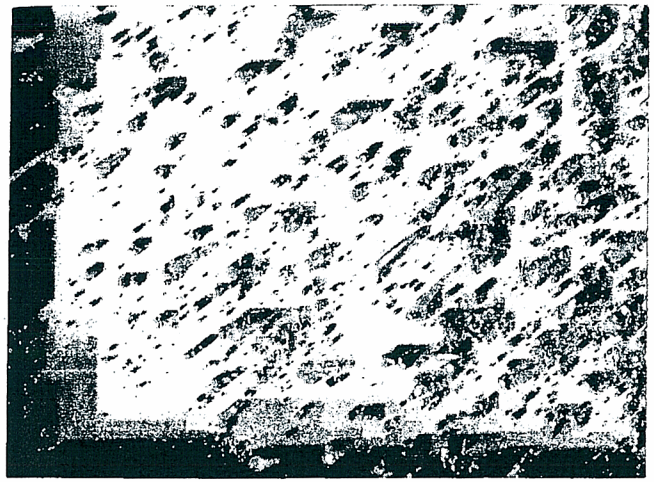


Fig. 22. Leaf hairs of *Cajophora laterita* with spines along the wall as in most species of Loasaceae.

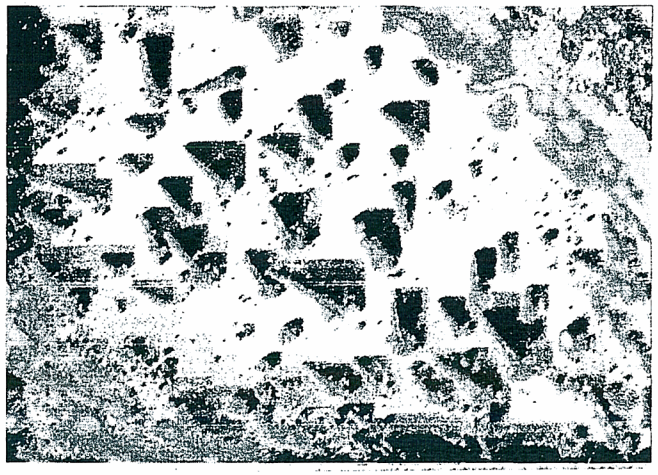


Fig. 23. Leaf hairs of *Petalonyx thurberi* showing minute barbs on the wall.

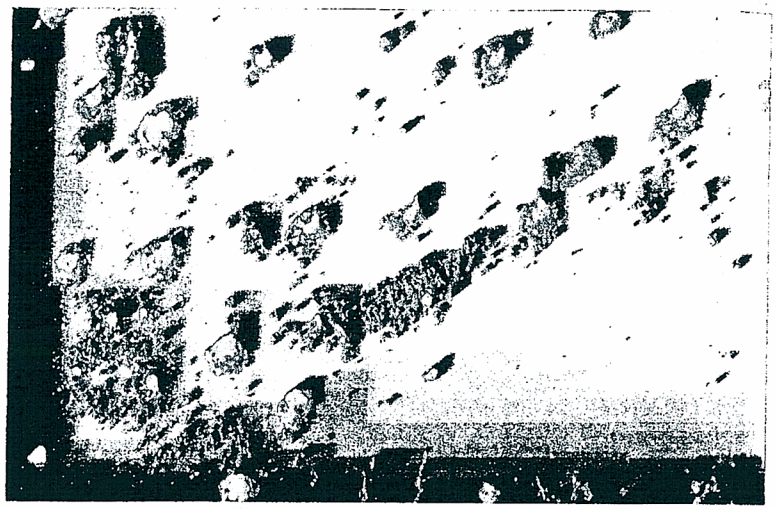


Fig. 24. Cystolith rosettes in the subsidiary cells of the leaf hairs of *Melothria gualapensis*, 60×.

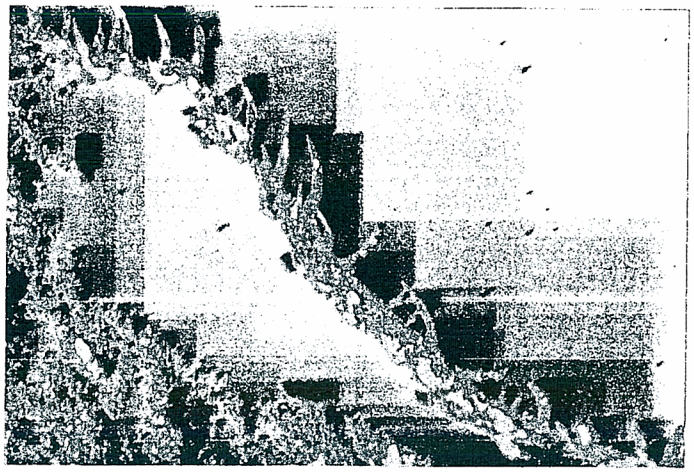


Fig. 25. Multicellular leaf hairs of *Acanthus mollis*, 60×. Note absence of cystolith in this species.

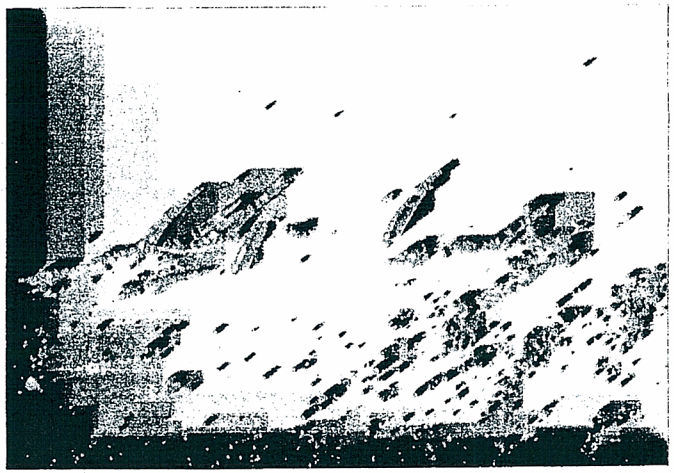


Fig. 26. Multicellular leaf hairs of *Campanula americana*, 60×. Hairs of this genus are commonly calcified or silicified.

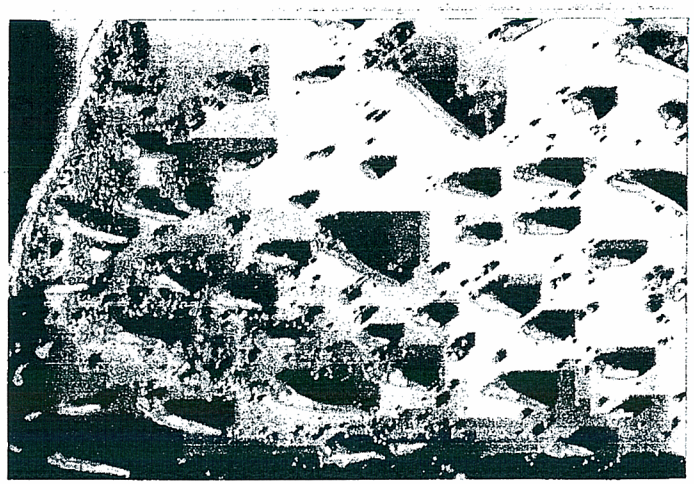


Fig. 27. Leaf hairs of *Lantana camara;* note cystoliths in subsidiary cells of hairs, 60×.