United States Patent

Bullock

[15] **3,656,906**

[45] **Apr. 18, 1972**

EXHIBIT 20

[54] METHOD FOR DETECTING AND QUANTITATING THE PRESENCE OF CANNABINOIDS AND ANALOGS THEREOF IN BIOLOGICAL MATERIALS AND RESULTING PRODUCTS

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[22] Filed: **Apr. 13, 1970**

[21] Appl. No.: **28,015**

[52] **U.S. Cl.** .....**23/230 B**, 260/247.1, 260/247.5 B, 260/247.7 E, 260/293.57, 260/293.58, 260/326.5 B, 260/327 TH, 260/332.3, 260/340.9, 260/343.2 R, 260/345.3, 260/479 R, 260/520, 260/619

[51] **Int. Cl.** **G01n 31/22**

[58] **Field of Search**.....23/230 B, 230; 260/247.1, 247.5 B, 260/247.7 E, 293.57, 293.58, 326.5 B, 327 TH, 332.3, 340.9, 343.2 R, 345.3, 479 R, 520, 619

[56] **References Cited**

OTHER PUBLICATIONS

Irudayasamy, A. et al., Chem. Abstr. 71, 1969

Primary Examiner—Morris O. Wolk
Assistant Examiner—R. M. Reese
Attorney—Bessie A. Lepper

[57] **ABSTRACT**

A method for detecting and quantitating submicrogram quantities of cannabinoids and their analogs in biological materials including blood plasma and urine. Detection system is based on the condensation of the cannabinoids with a polycarboxylic acid to give a highly fluorescent derivative, the intensity of the fluorescence being maximized in the pH range of 9–11.

3 Claims, No Drawings

METHOD FOR DETECTING AND QUANTITATING THE PRESENCE OF CANNABINOIDS AND ANALOGS THEREOF IN BIOLOGICAL MATERIALS AND RESULTING PRODUCTS

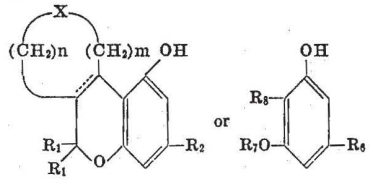
This invention relates to a method of detecting and quantitating traces of cannabinoids and their analogs, including marihuana constituents and certain of their derivatives, in biological materials including blood plasma and urine and to the novel compounds produced in the reactions which gives rise to the test.

There is a need for a qualitative and quantitative test for detecting the presence of marihuana and of related compounds and derivatives of marihuana in blood plasma and urine to determine whether or not an individual has consumed one of the marihuana related drugs in one form or another. Such a test must be reliable and relatively easy to perform.

It is therefore a primary object of this invention to provide a method of detecting and quantitating cannabinoids and particularly of detecting and quantitating these compounds in biological materials. It is another object to provide a method of the character described which is reliable and relatively simple to perform. It is another primary object of this invention to provide novel compounds which are derivatives of cannabinoids. Other objects of the invention will in part by obvious and will in part be apparent hereinafter.

The invention accordingly comprises the several steps and the relation of one or more of such steps with respect to each of the others, and the composition of matter possessing the characteristics, properties, and the relation of constituents which are exemplified in the following detailed disclosure, and the scope of the invention will be indicated in the claims.

The term "cannabinoid" is used hereinafter to designate compounds with a cannabinoid structure and their analogs and it includes those compounds which may be represented generally by formula I or formula II

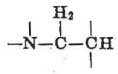


I II

wherein in formula I R_1 is lower-alkyl,

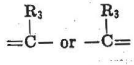
R_2 is alkyl, cycloalkyl-lower-alkyl or Alk —N=B where Alk is lower alkylene, and N=B is di-lower-alkylamino, N-morpholino, N-pyrrolidino or N-piperidino,

X is S and m is 1 or 2, n is 0 or 1 and $m+n$ is 2 or 3, X is



and m is 2 and n is 0,

X is



where R_3 is hydrogen or lower-alkyl and n is 0, 1, 2, or 3, m is 0, 1, 2, or 3 and $m+n$ is 2 or 3,

X is

where R_4 is hydrogen or lower alkyl and m is 0, 1, 2 or 3, n is , 1,2 or 3 and $m+n$ is 2 or 3,

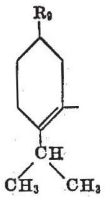
X is

where R_5 is hydrogen, alkyl, cycloalkyl-lower-alkyl, lower-alkanoyl, cycloalkyl-lower-alkanoyl, lower-alkenyl, lower-alkynyl, halo-lower-alkenyl, phenyl-lower-alkyl, phenyl-lower-alkenyl, phenyl-lower alkynyl, (the benzene ring of the phenyl being substituted by 1 to 3 members of lower-alkyl, lower-alkoxy, halo, nitro, lower-alkyl-mercapto, methylenedioxy, trifluoromethyl) and m is 1 or 2, n is 1 or 2 and $m+n$ is 2 or 3; and wherein formula II

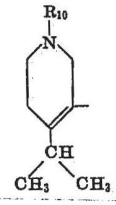
R_6 is alkyl containing from one to 10 carbon atoms,

R_7 is hydrogen or lower-alkanoyl,

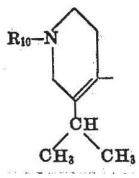
R_8 is 1-(2-isopropyl-5-lower-alkyl-1-cyclobexenyl) having the formula



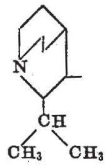
where R_9 is lower-alkyl; 3-(1-R_{10}-4-isopropyl-1,2,5,6-tetrahydropyridyl) having the formula



where R_{10} is hydrogen, lower-alkyl, or phenyl-lower-alkyl; 4-(1-R_{10}-3-isopropyl-1,2,5,6-tetrahydropyridyl) having the formula



where R_{10} has the meaning given above; or 3-(2-isopropyl-1,4-ethano-1,4,5,6-tetrahydropyridyl) having the formula



As used herein, the term "lower-alkyl" means saturated, monovalent aliphatic-radicals, including straight and branched-chain radicals of from one to six carbon atoms, as illustrated by, but not limited to methyl, ethyl, propyl, isopropyl, butyl, sec.- butyl, amyl, hexyl, and the like.

As used herein, the term "alkyl" means saturated, monovalent aliphatic radicals, including straight and branched-chain radicals of from one to 20 carbon atoms, as illustrated by, but not limited to methyl, n-amyl, n-hexyl, 2-heptyl, n-heptyl, 3-methyl-2-octyl, n-octyl, 2-nonyl, 2-tetradecyl, n-hexadecyl, 2-eicosanyl, and the like.

As used herein, the term "lower-alkenyl" means monovalent, aliphatic-radicals of from three to seven carbon atoms which contain at least one double bond, and are either straight or branched-chain, as illustrated by, but not limited to 1-(2-propenyl), 1-(3-methyl-2-propenyl), 1-(1,3-dimethyl-2-propenyl), 1-(2-hexenyl), and the like.

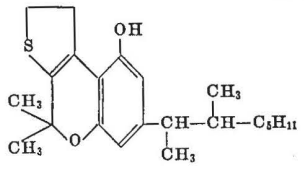
As used herein, the term "lower-alkynyl" means monovalent, aliphatic-radicals, of from three to seven carbon atoms which contain at least one triple bond, and are either straight or branched, as illustrated by, but not limited to 1-(2-propynyl, 1-(1-methyl-2-propynyl), 1-(2-heptynyl), and the like.

As used herein, the term "cycloalkyl" means cyclic, saturated aliphatic-radicals of from three to eight carbon atoms, as illustrated by, but not limited to cyclopropyl, cyclobutyl, 2-methylcyclobutyl, cyclohexyl, 4-methylcyclohexyl, cyclooctyl, and the like.

As used herein, the term "lower-alkanoyl" means saturated, monovalent, aliphatic radicals, derived from a monocarboxylic acid, including straight or branched-chain radicals of from one to six carbon atoms, as illustrated by, but not limited to formyl, acetyl, propionyl, a-methylpropionyl, butyryl, hexanoyl, and the like.

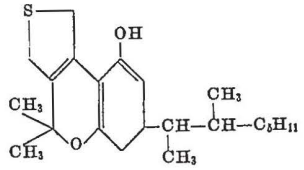
As used herein, the terms "phenyl-lower-alkyl," "phenyl-lower-alkenyl," and "phenyl-lower-alkynyl" means a monovalent radical consisting of a phenyl nucleus bonded to the rest of the molecule, respectively, through a divalent lower-alkylene radical of from one to four carbon atoms as illustrated by, but not limited to methylene, 1,1-ethylene, 1,2-ethylene, 1,3-propylene, 1,2-propylene, 1,4-butylene, and the like, or through a divalent lower-alkenylene radical of from two to four carbon atoms, as illustrated by, but not limited to 1,2-ethenylene, 1,3-(1-propenylene), 1,3-(1-butenylene), 1,4-(2-butenylene), and the like, or through a divalent lower-alkynylene radical of from two to four carbon atoms, as illustrated by, but not limited to 1,2-ethynylene, 1,3-propynylene, 1,3-(1-butynylene), and the like. Here and elsewhere throughout this specification, it will be understood the benzene ring of phenyl can bear any number and kind of substituents such as would occur to the man skilled in organic chemistry. Solely for illustration, and without limitation, such substituents include lower-alkyl, lower-alkoxy, halo (chloro, bromo, iodo, or fluoro), nitro, lower-alkylmercapto, and the like.

Exemplary of cannabinoids of formula I wherein X is S are



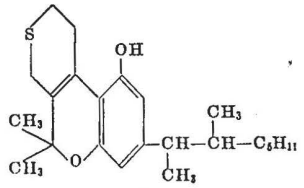
III

1,2-dihydro-4,4-dimethyl-9-hydroxy-7-(1,2-dimethylheptyl)-4H-thieno-[2,3-c] [1] benzopyran;



IV

1,3-dihydro-4,4-dimethyl-9-hydroxy-7-(1,2-dimethylheptyl)-4H-thieno-[3,4-c] [1] benzopyran; and

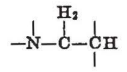


V

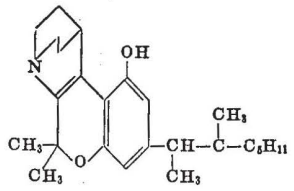
5,5-dimethyl-10-hydroxy-8-(1,2-dimethylheptyl)-1,2,4,5-tetrahydro-4H,5H-thiopyrano [3,4-c] [1]benzopyran.

Cannabinoids of the type illustrated by compounds III, IV and V are the subject of U.S. Pat. application, Ser. No. 852,928 filed Aug. 25, 1969, in the names of Raj K. Razdan and Harry G. Pars and assigned to the same assignee as the present application.

Exemplary of cannabinoids of formula I wherein X is



is

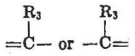


VI

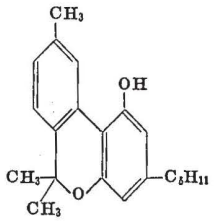
5,5-dimethyl-1,4-ethano-10-hydroxy-81(1,2-dimethylheptyl)-1,2,3,4-tetrahydro-5H-[1]benzopyrano[-c]pyridine.

Cannabinoids of the type illustrated by compound VI are the subject of U.S. Pat. No. 3,493,579.

Exemplary of cannabinoids of formula I wherein X is

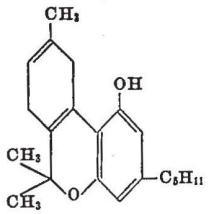


and R_3 is hydrogen or lower alkyl are

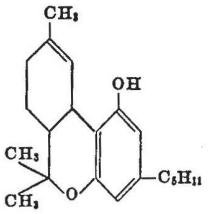


VII

cannabinol



and



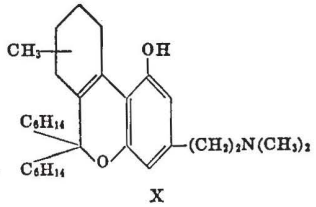
VIII IX

1-hydroxy-3-pentyl-6a,7,10,10a-tetrahydro-6,6,9-trimethyl-6H-dibenzo[b,d]pyran (VIII) and 1-hdyroxy-3-pentyl-6 a,7,8,10a-tetrahydro-6,6,9-trimethyl-6H-dibenzo[b,d]pyran (IX).

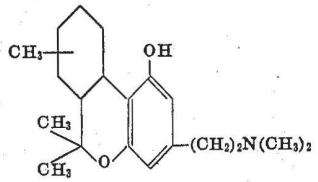
Compounds VI and VII are well known as components of the active ingredients of marihuana and compounds VIII and IX are the well known Δ^8 and Δ^9 tetrahydrocannabinols, respectively.

Exemplary of cannabinoids of formula I where X is

and R_4 is hydrogen or lower alkyl are

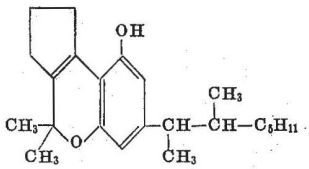


X



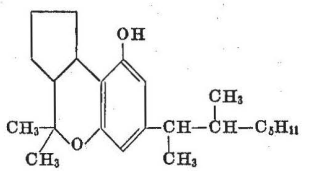
XI

3-(2-dimethylaminoethyl)-1-hydroxy-6,6-dihexyl-9-methyl-7,8,9,10-tetrahydro-6H-dibenzo[b,d]pyran (X) or 3-(2-dimethylaminoethyl)-1-hydroxy-6,6,9-trimethyl-7,8,9,10,11,12-hexahydro-6H-dibenzo[b,d]pyran (XI);



XII

and



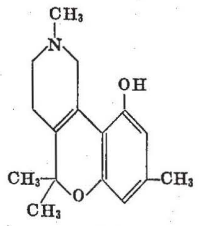
XIII

4,4-dimethyl-9-hydroxy-7-(1,2-dimethylheptyl)-1,2,3,4-tetrahydrocyclopenta[c][1]benzopyran (XII) or 4,4-di-(1-hexyl)-9-hydroxy-7-(1,2-dimethylheptyl)-1,2,3,4,12,13-hexahydrocyclopenta [c][1]benzopyran (XIII).

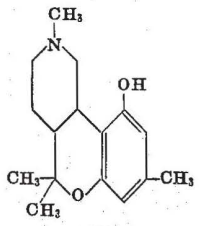
Cannabinoids of the type illustrated by compounds X and XI are the subject of U.S. Pat. application, Ser. No. 1,550, filed Jan. 8, 1970, in the names of Harry G. Pars and Felix E. Granchelli; and those of the type illustrated by compounds XII and XIII are the subject of U.S. Pat. application Ser. No. 642,192, filed May 2, 1967, in the names of Raj K. Razdan, Felix E. Granchelli and Harry G. Pars. Both of these applications are assigned to the same assignee as the present application.

Exemplary of cannabinoids of formula I wherein X is

are

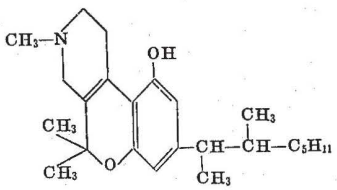


and



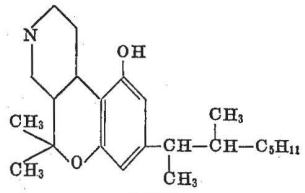
XIV XV

10-hydroxy-2,5,5,8-tetramethyl-1,2,3,4-tetrahydro-5H-[1]benzo-pyrano[3,4-d]pyridine (XIV) or 10-hydroxy-2,5,5,8-tetramethyl-1,2,3,4,13,14-hexahydro-5H-[1]-benzopyrano[3,4-d]pyridine (XV);



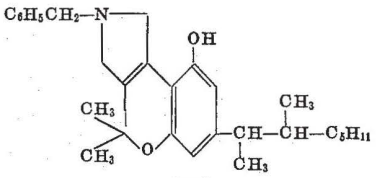
XVI

and



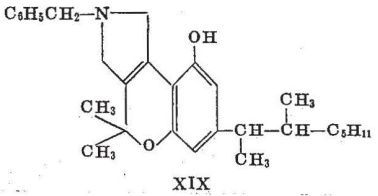
XVII

10-hydroxy81(1,2-dimethylheptyl)-3,5,5-trimethyl-1,2,3,4-tetrahydro-5H-[1]benzopyrane[3,4-c]pyridine (XVI) or 10-hydroxy-8(1,2-dimethylheptyl)-3,5,5-trimethyl 1,2,3,4,13,14-hexahydro-5H-[1]benzopyrano[3,4-c]pyridine (XVII); and



XVIII

and

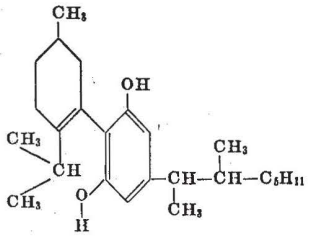


XIX

2-benzyl-4-dimethyl-9-hydroxy-7-(1,2-dimethylheptyl)-1,2,3,4-tetrahydro[1]benzopyrano[3,4-c]pyrrole (XVIII) or 2-benzyl-4-dimethyl-9-hydroxy-7-(1,2-dimethylheptyl)-1,2,3,4,11,12-hexahydro[1]benzopyrano[3,4-c]pyrrole (XIX).

Cannabinoids of the type illustrated by compounds XIV and XV are disclosed in U.S. Pat. No. 3,429,889; cannabinoids of the type illustrated by compounds XVI and XVII are the subject of U.S. Pat. application Ser. No. 642,223, filed May 29, 1967, **in the names of Harry G. Pars, Felix E. Granchelli and Raj K. Razdan; and cannabinoids of the type illustrated by compounds XVIII and XIX are the subject of U.S. Pat. application, Ser. No.** 842,690, filed July 17, 1969, in the names of Harry G. Pars and Raj K. Razdan. These two applications are assigned to the same assignee as the present application.

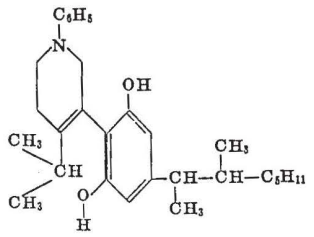
Exemplary of the cannabinoids of formula II wherein R_7 is hydrogen and R_8 is 1-(2-isopropyl-5-lower-alkyl-1-cyclohexenyl) is



XX

2-[1-(2-isopropyl-5-methyl-1-cyclohexenyl)]-5-(1,2-dimethylheptyl)resorcinol.

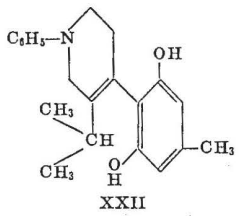
Exemplary of the cannabinoids of formula II wherein R_7 is hydrogen and R_8 is 3-(1-R_{10}-4-isopropyl-1,2,5,6-tetrahydropyridyl) is



XXI

2-[3-(1-benzyl-4-isopropyl-1,2,5,6-tetrahydropyridyl)]-5-(1,2-dimethylheptyl)resorcinol.

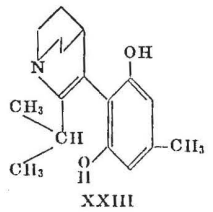
Exemplary of the cannabinoids of formula II wherein R_7 is hydrogen and R_8 is 4-(1-R_{10}-3-isopropyl-1,2,5,6-tetrahydropyridyl) is



XXII

2-[4-(1-benzyl-3-isopropyl-1,2,5,6-tetrahydropyridyl)]-5-methylresorcinol.

Exemplary of the cannabinoids of formula II wherein R_7 is hydrogen and R_8 is 3-(2-isopropyl-1,4-ethano-1,4,5,6-tetrahydropyridyl) is

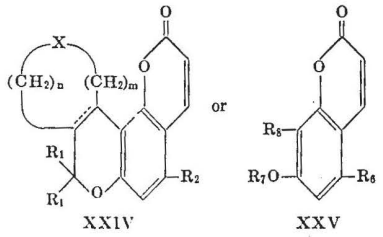


XXIII

2-[3-(2-isopropyl-1,4-ethano-1,4,5,6-tetrahydropyridyl)]-5-methylresorcinol.

Cannabinoids of the type illustrated by compounds XX - XXIII are the subject of U.S. Pat. application Ser. No. 811,701 filed Mar. 27, 1969, in the names of Raj K. Razdan, William R. Thompson, Felix E. Granchelli and Harry G. Pars and assigned to the same assignee as the present application.

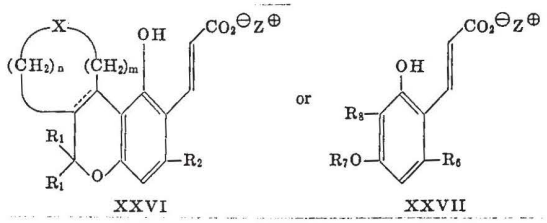
The invention sought to be patented in one of its composition aspects resides in the concept of a class of compounds represented by formulas XXIV and XXV



XXIV XXV

wherein R_1, R_2 and X have the same meanings as in the compounds of formula I and R_6R_7 and R_8 have the same meanings as in the compounds of formula II.

The invention sought to be patented in another of its composition aspects resides in the concept of a class of compounds represented by formulas XXVI and XXVII



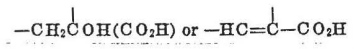
XXVI XXVII

wherein R_1, R_2 and X have the same meanings as in the compounds of formula I; R_6, R_7 and R_8 have the same meanings as in the compounds of formula II; and Z is a cation such as H^+, $Na,^+$ K^+ and the like.

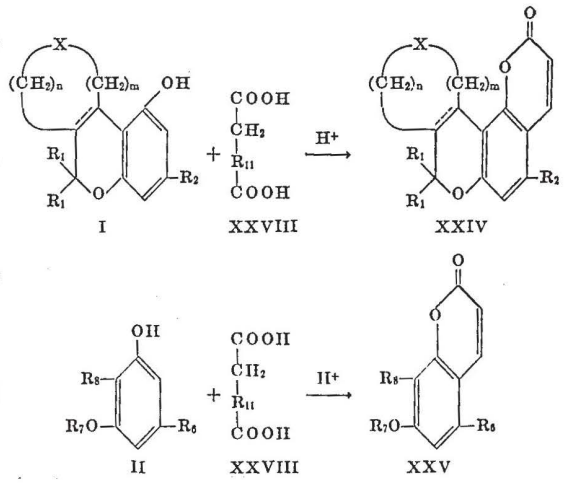
The invention sought to be patented in one method aspect resides in reacting a cannabinoid of the class defined by formulas I and II with a poycarboxylic acid of the general formula XXVIII

$$HOOC\text{-}CH_2\text{-}R_{11}\text{-}COOH, \qquad \text{XXVIII}$$

wherein R_{11} is CH_2,



to produce the compounds of the general formulas XXIV and XXV by the following reaction



I XXVIII XXIV

II XXVIII XXV

The invention sought to be patented in another method aspect resides in converting the compounds of of formulas XXIV and XXV to those of formulas XXVI and XXVII by the addition of an alkali to raise the pH to at least 9. The formation of compounds of the formulas XXVI and XXVII gives rise to a high degree of fluorescence emmission at 470 nm when excited at 380 nm. The occurrence of this fluorescence is indicative of the presence of a cannabinoid structure and hence serves as the basis for the detection of marihuana and marihuana derivatives as well as the cannabinoids and their analogs as represented by general formulas I and II.

The cannabinoids in amounts from 0.6 micrograms and greater have been detected by the method of this invention.

In reacting the cannabinoid with the polycarboxylic acid the hydrogen ion is furnished by an acid catalyst such as polyphosphoric acid, sulfuric acid or a mixture of sulfuric acid and acetic acid. The reaction is preferably carried out at somewhat elevated temperatures, typically in the range from about 80 to 90° C. and for at least 20 minutes.

The polycarboxylic acids of formula XXVIII are illustrated by malic acid, citric acid, isocitric acid, aconitic acid and succinic acid.

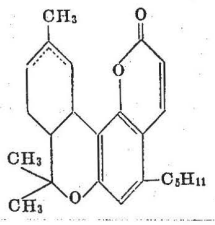
The composition and method aspects of this invention are illustrated by the following examples which are meant to be illustrative and not limiting.

EXAMPLE 1

1-Hydroxy-3-pentyl-6a,7,8,10a-tetrahydro-6,6,9-trimethyl-6H-dibenzo[b,d]pyran-2-acrylic Acid δ-lactone

Blood plasma containing Δ^9 tetrahydrocannabinol (1-hydroxy-3-pentyl-6a,7,8,10a-tetrahydro-6,6,9-trimethyl-6H, dibenzo[b,d]pyran) was prepared by the following procedure. A concentrated solution of the Δ^9 THC (prepared according to the procedure disclosed by D. Petrzilka, W. Haefliger and C. Sikemeier in *Helvetica Chemica Acta*, 52: 1102 (1969)) was made by dissolving 1 milligram of the Δ^9 THC in 1 milliliter of absolute ethanol. To 0.2 milliliters of this solution was then added 20 milliliters of human blood plasma. This blood plasma containing the THC was then diluted with additional fresh plasma to the desired concentration which in the following examples was 0.3 micrograms or more of THC/milliliter of plasma.

Two milliliters of the THC-containing plasma (equivalent to 0.6 **micrograms of** Δ^9 THC was diluted with an equal volume of pH 5 citrate buffer and the mixture was saturated with sodium chloride. It was then extracted twice with 5-milliliter portions of hexane containing 1.5% ethanol. The combined hexane layers were washed once by shaking with 5 milliliters of 0.1 normal sodium hydroxide solution. The hexane layer was separated and evaporated to dryness in a 15-milliliter graduated, stoppered conical centrifuge tube. 0.5 milliliter of an ethanolic solution of malic acid (1 mg/ml) was added and the ethanol evaporated by warming the tube in a water bath at 90°. The refluxing ethanol was allowed to wash the contents of the tube into the tip. After evaporation of the ethanol, 0.5 ml of a polyphosphoric acid mixture (prepared by mixing polyphosphoric acid and 85% phosphoric acid in a 2:1 volume ratio) was added; the tube was stoppered and heated at 90° for 20 minutes. The mixture was diluted with 1 milliliter of distilled water and extracted with 10 milliliters of hexane. The hexane layer was washed once by shaking with 5 milliliters of pH 5 citrate buffer. The aqueous layer was removed and there remained in the hexane layer the product 1-hydroxy-3-pentyl-6a,7,8,10a-tetrahydro-6,6,9-trimethyl-6-H-dibenzo[b,d]pyran-2-acrylic acid δ-lactone of the formula

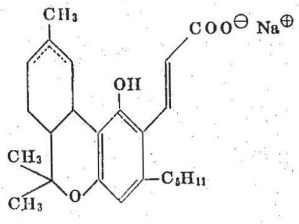


The formula for the resulting lactone is written in this manner since at least a part, if not all, of the Δ^9 THC is probably converted to the Δ^8 THC along with the formation of the δ-lactone in the polyphosphoric acid at 90° C.

EXAMPLE 2

1-Hydroxy-3-pentyl-6a,7,8,10a-tetrahydro-6,6,9-trimethyl-6H-dibenzo[b,d]pyran-2-sodium Acrylate

One milliliter of pH (0.2 molar) carbonate buffer was added to the hexane layer of Example 1 and it was shaken with the hexane solution for 30 minutes. The hexane layer was then aspirated off and discarded. The fluorescence of the buffer layer was then read using an excitation of 380 nm. The presence of



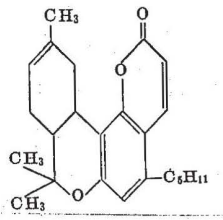
was indicated by a fluorescence emission at 470 nm which was significantly higher than that obtained by carrying 2 milliliters of control plasma through the procedures of Examples 1 and 2.

The production of the fluorescent acrylate (or the corresponding acrylic acid) is indicative of the presence of the cannabinoid structure as represented by formula I. It will be seen that this method of THC detection was capable of detecting the presence of as little as 0.6 micrograms of the THC.

EXAMPLE 3

1-Hydroxy-3-pentyl-6a,7,10, 10a-tetrahydro 6,6,9-trimethyl-6H-dibenzo[b,d]pyran-2-acrylic acid δ-lactone

The procedure of Example 1 was repeated by substituting a concentrated solution of Δ^8 THC (1-hydroxy-3-pentyl-6 a,7,10,10a-tetra-hydro-6,6,9-trimethyl-6H-dibenzo[b,d]p yran) for the Δ^9 THC Example 1. This resulted in the formation of the compound of the formula

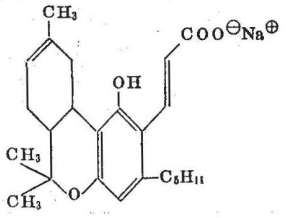


EXAMPLE 4

1-Hydroxy-3-pentyl-6a,7,10,10a-tetrahydro-6,6,9-trimethyl-6H-dibenzo[b,d]pyran-2-sodium Acrylate

The product lactone of Example 3 was subjected to the procedure detailed in Example 2. An intense fluorescence at the excitation of 380 nm was noted.

The acrylate salt

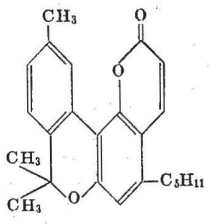


or of example 2 may easily be converted to the acrylic acid form by reaction with a suitable acid such as hydrochloric acid.

EXAMPLE 5

1-Hydroxy-3-pentyl-6,6,9-trimethyl-6H-dibenzo[b,d]pyran-2-acrylic Acid δ-lactone

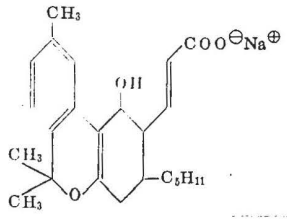
5 micrograms of cannabinol in 5 microliters of ethanol was added to a 15-milliliter conical centrifuge tube with 500 micrograms of malic acid and 0.5 milliliter of ethanol. The solvent was removed by warming at 60° C. and then 0.5 milliliter of the polyphosphoric acid mixture of Example 1 was added. The tube was stoppered and the contents heated at 90° C. for 20 minutes. After the contents were cooled, 1 milliliter of water was added and the mixture was extracted with 7 milliliter of hexane. The hexane layer was removed and washed with 5 milliliters of pH 5 citrate buffer to form the product having the formula



EXAMPLE 6

1-Hydroxy-3-pentyl-6,6,9-trimethyl-6H-dibenzo[b,d]pyran-2sodium acrylate

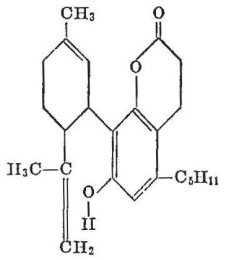
A 6-milliliter aliquot of the hexane layer of Example 5 treated with the pH 5 citrate buffer was then transferred to a dry centrifuge tube and shaken for 30 minutes with 2 milliliters of pH 10 buffer. The fluorescence of the aqueous phase was recorded (λ excitation 380 nm, λ emission 470 nm) and was significantly higher than that of a reagent blank carried through the procedures of Example 5 and this example. The intensity of the fluorescence was about one-third that obtained with an equivalent amount of Δ^9 THC. The acrylate salt formed had the formula



EXAMPLE 7

2,4-Dihydroxy-5-(6-isopropenyl-3-methyl-2-cyclohexenyl)-6-pentyl-phenylacrylic Acid 2-δ-lactone

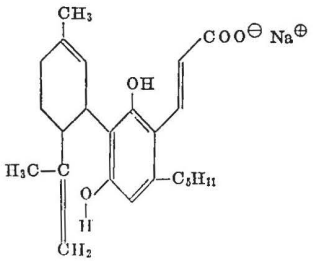
5 micrograms of cannabidiol in 5 microliters of ethanol was added to a 15-milliliter conical centrifuge tube with 500 micrograms of malic acid in 0.5 milliliter of ethanol. The solvent was removed by warming at 60° C. and then 0.5 milliliter of the polyphosphoric acid mixture of Example 1 was added. The tube was stoppered and the contents were heated at 90° C. for 20 minutes. After the contents were cooled, 1 milliter of water was added and the mixture was extracted with 7 milliliters of hexane. The hexane layer was removed and washed with 5 milliliters of pH 5 citrate buffer to form the product having the formula



EXAMPLE 8

2,4-Dihydroxy-5-(6-isopropenyl-3-methyl-2-cyclohexenyl)-6-pentyl-phenyl-1-sodium Acrylate

A 6-milliliter aliquot of the hexane layer of Example 7 was transferred to a dry centrifuge tube and shaken for 30 minutes with 2 milliliters of pH 10 buffer. The fluorescence of the aqueous phase was recorded (λ excitation 380 nm, λ emission 470 nm) and was significantly higher than that of a reagent blank carried through the same procedure. The intensity of the fluorescence obtained with the cannabidiol was about 3 times that obtained with Δ^9 THC and about 10 times that obtained with cannabinol. The intensely fluorescent compound can be represented by the formula



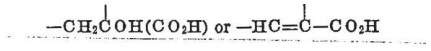
It will be seen from the above description and from the examples that there is here provided a method of identifying the presence of a cannabinoid, and that the novel reactions disclosed may be used as a test for the presence of a cannabinoid, e.g., one or more of the active ingredients of marihuana, in a body fluid. The novel acrylate salts of the cannabinoids as represented by formulas XXVI and XXVII have utility as standards in a test procedure for identifying cannabinoids and the novel δ-lactones of formulas XXIV and XXV have utility as intermediates in the preparation of compounds of the formulas XXVI and XXVII, respectively.

It will thus be seen that the objects set forth above, among those made apparent from the preceding description, are efficiently attained, and, since certain changes may be made in carrying out the above method and in the compositions set forth without departing from the scope of the invention, it is intended that all matter contained in the above description shall be interpreted as illustrative and not in a limiting sense.

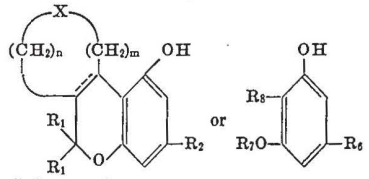
I claim:

1. A method of detecting the presence of a cannabinoid in a body fluid, comprising the steps of treating an extract of a body fluid containing a cannabinoid with a polycarboxylic acid in the presence of an acid catalyst thereby to condense the cannabinoid with said polycarboxylic acid to form a δ-lactone cannabinoid and then converting the δ-lactone form to the free acid or salt form which is intensely fluorescent and detecting said fluorescence as indicative of the presence of said cannabinoid.

2. A method in accordance with claim 1 wherein said polycarboxylic acid has the formula HOOC—$CH_2$13 R—COOH, wherein R is CH_2,



3. A method in accordance with claim 1 wherein said cannabinoid is represented by the formula

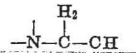


wherein R_1 is lower-alkyl;

R_2 is alkyl, cycloalkyl-lower-alkyl or Alk-N = B where Alk is lower alkylene, and N = B is di-lower-alkylamino, N-morpholino, N-pyrrolidino or N-piperidino;

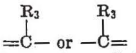
X is S and *m* is 1 or 2, *n* is 0 or 1 and *m* + *n* is 2 or 3;

X is



and *m* is 2 and *n* is 0;

X is



where R_3 is hydrogen or lower alkyl and *n* is 0, 1, 2 or 3, *m* is 0, 1, 2 or 3 and *m* + *n* is 2 or 3;

X is

where R_4 is hydrogen or lower-alkyl and *m* is 0,1,2 or 3, *n* is 0,1,2 or 3 and *m* + *n* is 2 or 3;

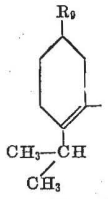
X is

where R_5 is hydrogen, alkyl, cycloalkyl-lower-alkyl, lower-alkanoyl, cycloalkyl-lower-alkanoyl, lower-alkenyl, lower-alkynyl, halo-lower-alkenyl, phenyl-lower-alkyl, phenyl-lower-alkenyl, phenyl-lower-alkynyl, (the benzene ring of the phenyl being substituted by 1 to 3 members of lower-alkyl, lower-alkoxy, halo, nitro, lower-alkyl-mercapto, methylenedioxy, trifluoromethyl) and m is 1 or 2, n is 1 or 2 and $m+n$ is 2 or 3;

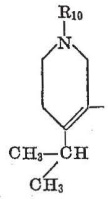
R_6 is alkyl containing from one to 10 carbon atoms;

R_7 is hydrogen or lower-alkanoyl; and

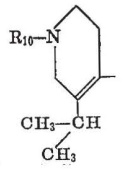
R_8 is 1-(2-isopropyl-5-lower-alkyl-1-cyclohexenyl) having the formula



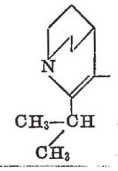
where R_9 is lower-alkyl; 3-(1-R_{10}-4-isopropyl-1,2,5,6-tetrahydropyridyl) having the formula



where R_{10} is hydrogen, lower-alkyl or phenyl-lower-alkyl; 4-(1-R_{10}-3-isopropyl-1,2,5,6-tetrahydropyridyl) having the formula



where R_{10} has the meaning given above; or 3-(2-isopropyl-1,4-ethano-1,4,5,6-tetrahydropyridyl) having the formula



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resulting products

Abstract

A method for detecting and quantitating submicrogram quantities of cannabinoids and their analogs in biological materials including blood plasma and urine. Detection system is based on the condensation of the cannabinoids with a polycarboxylic acid to give a highly fluorescent derivative, the intensity of the fluorescence being maximized in the pH range of 9-11.

Classifications

G01N33/948 Sedatives, e.g. cannabinoids, barbiturates

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Inventor: Francis J Bullock

Current Assignee : Arthur D Little Inc

Worldwide applications

1970 • US

Application events

1970-04-13 • Application filed by Arthur D Little Inc

1970-04-13 • Priority to US2801570A

1972-04-18 • Application granted

1972-04-18 • Publication of US3656906A

1989-04-18 • Anticipated expiration