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Microbiological Transformations of $\Delta^{6a,10a}$-Tetrahydrocannabinol

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A screening program was conducted to find microorganisms that catalyze transformation reactions with cannabinoids. Three hundred fifty-eight cultures, consisting of 97 bacteria, 175 actinomycetes, and 86 molds, were incubated in media containing 0.5 mg of $\Delta^{6a,10a}$-tetrahydrocannabinol ($\Delta^{6a,10a}$-THC) per ml. After 120 h of cultivation, ethyl acetate extracts of the cultures were examined by thin-layer chromatography (TLC) for transformation products. About 18% of the cultures modified $\Delta^{6a,10a}$-THC. The ability to modify the substrate did not predominate among any particular group of microorganisms. After purification, the products from three cultures were analyzed by high-resolution mass spectrometry, 100-mHz proton magnetic resonance spectrometry, ultraviolet spectrometry, and infrared spectrometry. These spectral data indicated that a *Mycobacterium* sp. oxidized $\Delta^{6a,10a}$-THC to cannabinol and a diastereomeric pair of 6a-hydroxy-$\Delta^{10,10a}$-THC isomers; a *Streptomyces* sp. and a *Bacillus* sp. oxidized $\Delta^{6a,10a}$-THC to 7-keto-$\Delta^{6a,10a}$-THC and 4'-hydroxy-$\Delta^{6a,10a}$-THC, respectively. The occurrence of these products and the presence of others that have not yet been isolated or identified indicate that microbial transformation may be a useful tool for the preparation of new cannabinoids that have desirable pharmacological properties.

The naturally occurring cannabinoid Δ^9-tetrahydrocannabinol (Δ^9-THC) (the dibenzopyran numbering system is used; see Fig. 1 for positions and numbers) is believed to be the principle psychotomimetic constituent of the marijuana plant, *Cannabis sativa* L. (6, 7, 14, 15). A wide variety of pharmacological activities has been attributed to Δ^9-THC. Tachycardia and euphoria are believed to be the major effects in man, whereas animal models suggest that Δ^9-THC and other cannabinoids may also possess blood pressure-lowering, antianxiety, and antidepressant properties. In attempts to separate the desirable activities from other pharmacological effects, new cannabinoids have been synthesized de novo and by chemical modifications of naturally occurring compounds (13). These efforts have led to new groups of synthetic cannabinoids that show promise as clinically useful agents (3, 12, 19).

Microbial transformation provides an alternative mechanism for generating new and interesting cannabinoids that may be difficult to prepare by conventional chemical methods. Previous studies have shown that certain microorganisms hydroxylate the *n*-pentyl side chain of cannabinoids at the 2'- or 4'-position (17; E. E. Fager and N. E. Wideburg, U.S. Patent 3,808,234, 1974; Schering AG, German Patent 2,335,135, 1975) and hydroxylate the cyclohexene ring of Δ^8-THC at the allylic 7-position (Schering AG, German patent 2,335,135, 1975).

Our studies were initiated to assess the ability of microorganisms to modify the synthetic cannabinoid $\Delta^{6a,10a}$-THC (see Fig. 1). This compound was selected because it (i) can be readily synthesized and thus was available in relatively large amounts, (ii) has very little pharmacological activity in animals at doses at which Δ^8- or Δ^9-THC is very active, and (iii) contains most of the structural features of the natural biologically active cannabinoids.

MATERIALS AND METHODS

Microorganisms. The microorganisms tested in the screening program were randomly selected cultures from the Lilly Research Laboratories Culture Collection or were randomly selected soil isolates. The microorganisms that produced the transformation products described in this study are *Mycobacterium rhodochrous* ATCC 19068 (Lilly culture no. A36437), an unidentified *Streptomyces* species designated A41596, and a strain of *Bacillus cereus* NRRL B-8172, designated A36659. Stock cultures of *M. rhodochrous* were grown on Trypticase soy agar slants (BQ, BBL, Cockeysville, Md.) at 25 to 30°C. *Streptomyces* A41596 and *B. cereus* A36659 were cultivated at 30°C on agar slants of Bennett modified medium

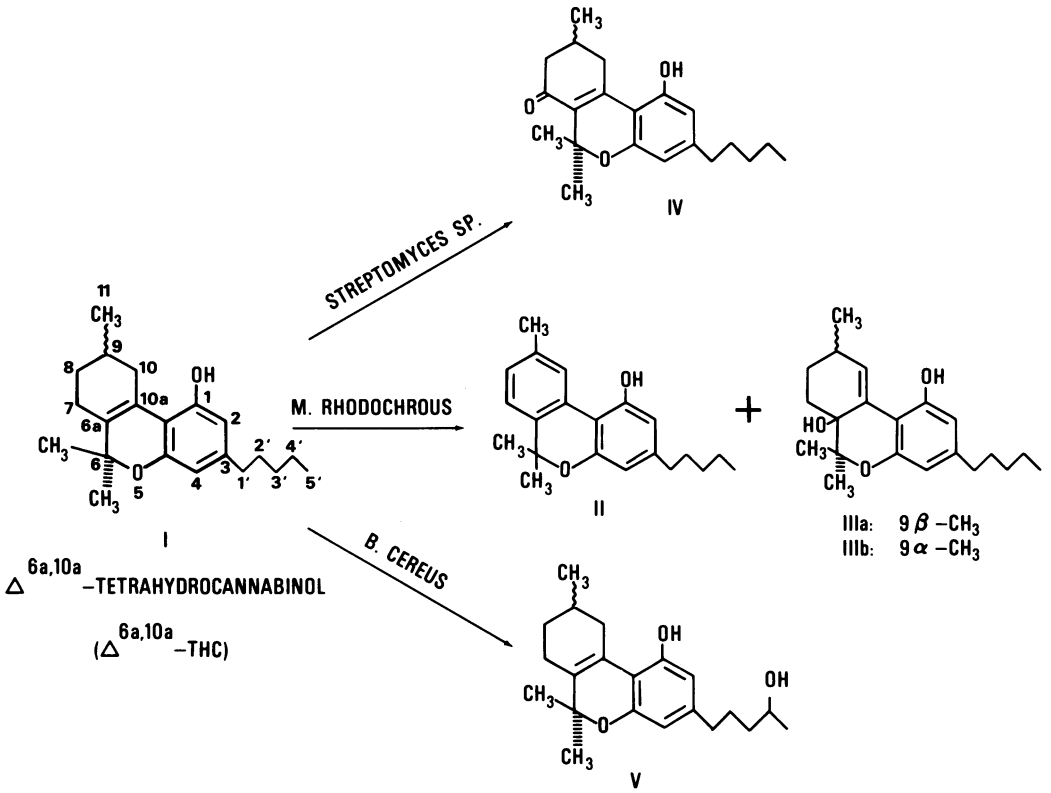

FIG. 1. *Biotransformation products of* $\Delta^{6a,10a}$-THC.

(9) and peptone-beef extract medium (10), respectively, and stored at 4°C. Permanent preservations of the microorganisms were made by lyophilization of cell suspensions washed from the agar slants with calf serum (North American Biologicals Incorporated, Miami, Fla.).

Screening program. A total of 358 randomly selected microorganisms that included bacteria, actinomycetes, and fungi were tested for their ability to modify $\Delta^{6a,10a}$-THC.

Bacteria. The bacteria were transferred from a slant to 10 ml of P-1 mineral salts medium (1) in a 50-ml Erlenmeyer shake flask. Glucose was sterilized separately and added to the medium as the sole carbon source (final concentration 3%). The inoculated flasks were incubated at 30°C on a rotary shaker (250 rpm, 2.5-inch stroke). After 24 h, 0.1 ml of an ethanol solution containing 50 mg of $\Delta^{6a,10a}$-THC per ml was added to the cultures. These cultures were incubated an additional 72 h and then extracted to recover the transformation products.

Actinomycetes. Actinomycetes were transferred from slants into 10 ml of medium in 50-ml Erlenmeyer shaker flasks. The medium contained cornsteep liquor, starch, and soybean grits (8). After 48 h of incubation on the rotary shaker at 30°C, 0.75 ml of culture was transferred to 15 ml of a peptone-molasses medium (5) in a 50-ml shake flask. Immediately after inoculation, 0.15 ml of a 50-mg/ml ethanol solution of $\Delta^{6a,10a}$-THC was added to each culture flask. The culture was incubated at 30°C on the rotary shaker for 96 h and then extracted to recover the transformation products.

Fungi. Fungi were transferred from slants into 15 ml of peptone-molasses medium (5) in a 50-ml shake flask and incubated at 25°C on a rotary shaker. After 48 h, 0.75 ml of culture was transferred to 15 ml of peptone-molasses medium (5) in a 50-ml shake flask and 0.15 ml of a 50-mg/ml ethanol solution of $\Delta^{6a,10a}$-THC was added. The culture was incubated an additional 96 h and then extracted to recover transformation products.

Detection, isolation, and purification of products. Cannabinoid transformation products were recovered by extracting the culture three times with a 1:1 volume of ethyl acetate. The extracts were combined, washed with deionized water, and dried with Na_2SO_4, and the ethyl acetate was removed by evaporation in vacuo. The concentrated extracts were applied to silica gel 60 F254 (E. Merck, Darmstadt, Germany) thin-layer chromatography (TLC) plates. The plates were developed in a solvent system consisting of either benzene, benzene-ethyl acetate (9:1), or benzene-ethyl acetate (7:3). Cannabinoids were detected by observing the plates under short-wave ultraviolet (UV) lamp or by spraying the plates with Fast Blue B (*O*-dianisidine, tetrazotized, Sigma Chemical Co.).

To obtain further confirmation that the compounds observed on TLC plates were cannabinoid

transformation products, control flasks were prepared. Control and experimental flasks were prepared by similar procedures except that the control flasks were autoclaved 48 h after inoculation (two sterilization cycles at 121°C for 20 min). The cannabinoid substrate was then added to the sterilized cultures and the flasks were incubated for an additional 96 h on the rotary shaker. Control flasks were also prepared by not adding $\Delta^{6a,10a}$-THC to viable cultures. Control and experimental flasks were extracted and analyzed by similar procedures.

Larger quantities of the transformation products were obtained by using resting cell suspensions. The microorganism was grown for 24 to 48 h in 500-ml shake flasks containing 100 ml of medium without cannabinoid. Ten to thirty flasks were prepared. After cultivation, the contents were combined, and the cells were recovered by centrifugation at 8,000 × *g* for 15 min. The cells were washed with 0.1 M phosphate buffer (pH 7.0) and suspended in a similar buffer solution. The concentration of cells in the buffer suspension was fivefold greater than in the fermentation medium from which they were recovered. The cannabinoid substrate (0.5 mg/ml final concentration) was added to the buffer/cell suspension and incubation was continued under the conditions used with the original fermentation medium. The flask contents were then extracted with ethyl acetate to recover the transformation products.

An extract (1.5 g[dry weight]) from cultures of *M. rhodochrous* ATCC 19067, containing cannabinol (product II) and 6a-hydroxy-$\Delta^{10,10a}$-THC (products IIIa and IIIb), was applied to a Woelm silica gel activity I (ICN Pharmaceuticals, Cleveland, Ohio) glass column (2.5 cm by 66 cm) equilibrated with heptane. The column was eluted with a stepwise gradient of solvent mixtures containing heptane and increasing amounts of ethyl acetate. The column was operated at a flow rate of 2 to 3 ml/min, and 10-ml fractions were collected. Every tenth fraction was concentrated in vacuo and the residue was examined by TLC to monitor the purification. Fractions containing the products were combined and concentrated to dryness. Final purification of the 6a-hydroxy-$\Delta^{10,10a}$-THC diastereomers (products IIIa and IIIb) was achieved by preparative TLC on silica gel 60 F254 plates (EM Laboratories, Inc., Elmsford, N.Y.). About 20 mg of extract was applied per plate. The plates were developed with a solvent mixture of benzene-ethyl acetate (9:1).

Culture extracts (1.2 g [dry weight]) of *Streptomyces* sp. A41596 containing 7-keto-$\Delta^{6a,10a}$-THC (product IV) were partially purified by silica gel column chromatography using the procedure described above. Further purification was achieved by high-performance liquid chromatography (HPLC) using a stainless-steel column (4 feet by 0.38 inch [about 1.2 m by 1 cm]) packed with Porasil A (Waters Associates, Milford, Mass.), mesh size 37 to 75 μm. The column was equilibrated with heptane-benzene (1:1), and then 40 mg (dry weight) of extract was applied. The product was eluted using a step-wise solvent gradient of increasing polarity. The column was operated at a flow rate of 3 to 7 ml/min, and 7-keto-$\Delta^{6a,10a}$-THC (product IV) was eluted with benzene. Final purification was accomplished with preparative TLC using a solvent system of benzene-ethyl acetate (9:1).

Culture extracts (500 mg) of *B. cereus* NRRL B-8172, containing 4'-hydroxy-$\Delta^{6a,10a}$-THC (product V), were partially purified by HPLC as described above. Final purification was achieved by preparative TLC using a solvent of benzene-ethyl acetate (7:3).

Gas-liquid chromatography. The amounts of the transformation products in the culture extracts were estimated by gas-liquid·chromatography using a Hewlett-Packard model 5700A gas chromatograph with a flame ionization detector. A 1-μl heptane solution of the extracts was injected into a stainless-steel column (6 feet by 0.13 inch [about 1.83 m by 0.33 cm]) packed with 2% OV-17 on 80/100 Gas-Chrom Q (McFaul Co., Cincinnati, Ohio). The column temperature was isothermal at 240°C. Detector and injector temperatures were 250°C. Nitrogen was used as a carrier gas at a flow rate of 60 ml/min. The peak areas of the products and the substrate ($\Delta^{6a,10a}$-THC) were calculated by multiplying the peak height by the peak width at one-half the peak height. The amounts of the products were expressed as the percentage of their peak areas relative to the peak area of the substrate.

Physical/chemical analyses. Low-resolution mass spectra were obtained from a CEC 21-110 spectrometer (Consolidated Electrodynamics Corp., Monrovia, Calif.) at an ionizing voltage of 70 eV. For exact mass determinations, a Varian Mat 731 spectrometer (Varian Associates, Palo Alto, Calif.) was used. Proton magnetic resonance (PMR) spectra were measured with Varian Associates HA-100 and HA-200 spectrometers; chemical shifts are expressed in parts per million (δ) downfield from the internal standard tetramethylsilane. Infrared spectra were obtained using a Perkin-Elmer (Norwick, Conn.) model 457 diffraction grating spectrophotometer. UV spectra were measured with a Cary 15 spectrophotometer (Varian Associates). Optical rotations were determined with a Perkin-Elmer 241 polarimeter.

$\Delta^{6a,10a}$-THC. The substrate ($\Delta^{6a,10a}$-THC) was prepared by the procedure of Adams and Baker (2). The compound has the following physical and chemical characteristics: UV (EtOH) λ_{max} 228 and 274 nm (ϵ = 35,300 and 15,200); ^{1}H nuclear magnetic resonance (NMR) ($CDCl_3$) δ6.31, 6.12 (2d, 1H each, J = 1.8 Hz, H_4 and H_2), 4.75 (s, 1H, exchanges with D_2O), 2.75 to 0.80 (27H) especially 2.67 (brd, 1H, J = 17 Hz, H_{10}), 2.43 (t, 2H, benzyl-CH_2-), 1.40 (s, 3H, 6β-CH_3), 1.20 (s, 3H, 6α-CH_3), 1.00 (d, 3H, J = 6.7 Hz, C9 CH_3), and 0.87 ppm (t, 3H, ω-CH_3); mass spectrum, *m/e* 314 (M^+).

RESULTS

Three hundred fifty-eight microorganisms, representing 97 bacteria, 175 actinomycetes, and 86 molds, were screened for their ability to oxidize $\Delta^{6a,10a}$-THC. About 18% of these cultures appeared to modify the cannabinoid as indicated by the presence of new spots on thin-layer plates that were visible under a UV lamp

and stained by Fast Blue B. Cannabinoid transformation did not seem to predominate among any particular groups of microorganisms. Cannabinoid transformation was detected in 15.4% of the bacterial cultures, 17.7% of the actinomyces cultures, and 19.7% of the mold cultures. Most of the transforming cultures appeared to produce more than one transformation product. Three cannabinoid-modifying cultures were selected for further studies. These cultures were selected primarily because they appeared to produce larger quantities of the transformation products. Based on the amounts of the products isolated and on quantitative estimates by gas chromatography, it appeared that all of the products were produced in yields between 4 and 7% (based on the amount of $\Delta^{6a,10a}$-THC added to the cultures).

Structure assignment. TLC (benzene) of the *M. rhodochrous* culture extracts revealed the presence of a product with an R_f value (0.32) that differed from the R_f value of the substrate (I: R_f = 0.47). Approximately 90 mg (dry weight) of the new product (II), shown in Fig. 1, was isolated from 1.5 g of culture extract. Identification of II as cannabinol was based upon a comparison of PMR and mass spectral data with those previously reported (16) for this compound. Product II could arise from air oxidation of I; however, substantially larger amounts of II were found in viable cultures compared to controls. Thus, product II appeared to be produced from I primarily by enzymatic oxidation by *M. rhodochrous*.

The presence of two additional microbial conversion products in culture extracts of *M. rhodochrous* was indicated on TLC (benzene-ethyl acetate, 9:1) by the appearance of spots at R_f = 0.25 and 0.200 versus 0.57 for I. Isolation and purification of these products were accomplished by a combination of HPLC and preparative TLC. The assignment of the 6a-hydroxy structures, IIIa and IIIb (Fig. 1), to these products is based upon the following interpretations of spectral data.

High-resolution mass spectra of IIIa and IIIb exhibited molecular ions at *m/e* 330.221 and 330.219, respectively. The empirical formula ($C_{21}H_{30}O_3$) obtained from these exact mass determinations indicated that both products were monohydroxylated derivatives of I.

The PMR spectra of IIIa and IIIb are quite similar (see Table 1 for a summary of recorded PMR data for compounds I, IIIa, IIIb, IV, and V). Additionally, the chemical shifts and spin multiplicities for H_2, H_4, 6β-CH_3, 6α-CH_3, 9-CH_3, Ph-CH_2-, and Ph$(CH_2)_4$-CH_3 are essentially the same for all three compounds (I, IIIa, IIIb). This observation, coupled with the absence of resonances in the spectra of IIIa and IIIb for any protons on carbon bearing oxygen, establishes that the hydroxy group introduced by microbial conversion is on a tertiary carbon atom, i.e., at position *6a*, *10a*, or *9*.

The appearance of signals (not present in the spectrum of I) at δ6.64 for IIIa or δ6.75 for IIIb clearly establishes the presence of a proton on a double bond that is further deshielded by being in the proximity of some electronegative center. In Δ^9-THC the H_{10} proton is deshielded by the proximity of the 1-phenolic hydroxyl and appears at δ6.33 (4). Thus, the signals at δ6.64 and δ6.75 are assigned to a H_{10} proton on a double bond.

These assignments are supported by decoupling experiments. Application of a secondary radiofrequency field at δ2.22, the approximate resonance position of H_9 in the spectrum of IIIa, caused a sharpening of the broad singlet at δ6.64 (H_{10}) and a collapse of the doublet at δ1.14 (9-CH_3) to a broad singlet. Similarly, application of a secondary radiofrequency field at δ2.56, the approximate resonance position of H_9 in the spectrum of IIIb, caused a collapse of the

TABLE 1. *Proton magnetic resonance data[a] for Δ^{6a-10a}-THC (I) and microbial conversion products, IIIa, IIIb, IV, and V*

Resonance	I	IIIa	IIIb	IV	V
OH	4.75 brs	5.42 brs	5.28 brs		5.90 brs
H_4	6.31 d	6.30 d	6.30 d	6.30 d	6.26 d
H_2	6.12 d	6.21 d	6.15 d	6.15 d	6.10 d
H_9		2.22 m	2.56 m		
H_{10}	2.67 brd	6.64 brs	6.75 d	3.05 brd	2.65 brd
6β-CH_3	1.40 s	1.42 s	1.42 s	1.71 s	1.40 s
6α-CH_3	1.20 s	1.20 s	1.22 s	1.37 s	1.20 s
9-CH_3	1.00 d	1.14 d	1.06 d	1.07 d	1.16 d
Ph-CH_2-	2.43 t	2.45 t	2.45 t	2.46 t	2.43 t
Ph$(CH_2)_4$-CH_3	0.87 t	0.88 t	0.89 t	0.87 t	1.01 d

[a] The table reports shift values (δ) measured in $CDCl_3$ solution with tetramethylsilane as an internal reference. Abbreviations: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad.

doublets at δ6.75 (H_{10}) and δ1.06 (9-CH_3) to broad singlets.

The above assignments and decoupling experiments require that both IIIa and IIIb contain a $\Delta^{10,10a}$ double bond and a proton at C9. The only position remaining for placement of a tertiary hydroxyl group, provided that no gross structural arrangement has occurred during the microbial fermentation, is the *6a* position. The observation that neither IIIa nor IIIb is optically active, coupled with the above arguments, leads us to the conclusion that IIIa and IIIb are diastereomers of 6a-hydroxy-$\Delta^{10,10a}$-tetrahydrocannabinol.

The assignment of stereochemistry at positions 6a and 9 of structures IIIa and IIIb follows from further analysis of their PMR spectra. The chemical shifts of the 6α- and 6β-methyl group are the same for compounds I, IIIa, and IIIb (see Table 1). Thus, the conformation of ring B is fixed and the same for all three compounds.

Although the resonance for H_{10} appears as a broad singlet in the spectrum of IIIa, it is clearly a doublet (J = 6.8 Hz) in the spectrum of IIIb. Such a large coupling could arise in the spectrum of IIIb only if the dihedral angle between H_9 and H_{10} were approximately 0 to 10° (11). Examination of Dreiding models shows that the required angle between H_9 and H_{10} in IIIb can occur if H_9 is in the quasi-equatorial beta configuration of the cyclohexene C-ring. Conversely, the dihedral angle between the quasi-axial alpha hydrogen at C9 of IIIa and H_{10} would be approximately 90° and thus would lead to a small coupling between these two protons.

Smaller unresolved splittings, which cause broadening of the H_{10} doublet of IIIb and singlet of IIIa, originate from the coupling of H_{10} to the quasi-equatorial proton at C8. Such coupling is known to occur when the arrangement of carbon atoms separating the two protons in question is in a W-form with both protons nearly coplanar (18). The observation of this coupling in the spectra of both IIIa and IIIb after spin decoupling of H_{10}-H_9 aids in establishing the conformation of ring C in both compounds.

Heat-inactivated control flasks of *M. rhodochrous* cultures contained small amounts of IIIa and IIIb, suggesting that these compounds may also arise via air oxidation. The amounts of IIIa and IIIb in viable cultures were 5 to 10 times greater than in control flasks. Thus these compounds appeared to be derived primarily by enzymatic oxidation of I. Products IIIa and IIIb could also be prepared by oxidizing I with *m*-chloroperbenzoic acid. The yields in the latter reaction were low, and several side-products were also produced.

Product IV (Fig. 1) was isolated from culture extracts of *Streptomyces* A41596. On silica gel TLC plates developed with a solvent mixture of benzene-ethyl acetate (7:3), product IV moved with an R_f value of 0.32 ($\Delta^{6a,10a}$-THC, R_f = 0.70). Isolation and purification of IV were accomplished by silica gel column chromatography followed by HPLC on Porasil A. Final purification was achieved by preparative TLC. The assignment of the 7-keto-$\Delta^{6a,10a}$-THC structure, IV, to this product is based upon the following interpretation of spectral data.

A high-resolution mass spectrum of IV exhibited a molecular ion at *m/e* 328.2076 ($C_{21}H_{28}O_3$), indicating the addition of an oxygen to and loss of two hydrogens from the parent compound, I. Thus, the conversion product is a keto derivative of the parent compound I. The infrared spectrum of IV contains no absorption for a saturated cyclic or acyclic ketone but does have a band of 6.06 μ, which suggests the presence of an α,β-unsaturated ketone. The α,β-unsaturated ketone function is further indicated by the presence of a shoulder at 228 nm in the UV spectrum of IV.

A comparison of PMR data for IV and I obtained from 220-MHz spectra is shown in Table 1. The absence of resonances which would be consistent with protons on the double bond of an α,β-unsaturated ketone or olefin establishes the $\Delta^{6a,10a}$ structure of IV. Since the *n*-pentyl side chain methylene (δ2.46) and ω-methyl (δ0.87) are present in the spectrum of IV, the ketone part of the α,β-unsaturated ketone function must be at either position 7 or 10.

Placement of the ketone at position 7 is supported by the following PMR spectral evidence. (i) The methyl doublet (δ1.07) for the 9-methyl group is at approximately the same resonance as the 9-methyl group in the starting material I. However, both 6α- and 6β-methyl groups are shifted to lower fields in IV in comparison to I (such a shift would be consistent with a carbonyl function at C7). (ii) The H_{10} proton is still present as a broad doublet at δ3.05.

The product obtained from cultures of *B. cereus* was assigned the 4′-hydroxy-$\Delta^{6a,10a}$-THC structure, V (Fig. 1), on the basis of the following interpretation of spectral data.

The high-resolution mass spectrum of V exhibited a molecular ion at *m/e* 330.219 ($C_{21}H_{30}O_3$), indicating a monohydroxylation of the starting material I. Examination of the PMR spectra of V in comparison to I (see Table 1) clearly establishes that the point of hydroxylation is at the 4′ position on the *n*-pentyl side chain. For instance, the spectra are essentially identical except for a doublet at δ1.01 in the spectrum of V and an absence of the ω-CH_3

triplet at δ0.87. The appearance of a new quartet at δ3.69 thus confirms the presence of the hydroxy at the 4′ position. Since the microbial fermentation product, V, is optically active ($[\alpha]_D$ = +18.3°), the hydroxylation of I by *B. cereus* occurs stereospecifically.

Summary of physical/chemical data: "Cannabinol": 6,6,9-trimethyl-3-pentyl-6H-dibenzo-[b,d]pyran-1-ol (II). ^{1}H NMR ($CDCl_3$) δ8.2 (s, 1H, H_{10}), 7.12 (s, 2H, H_7 and H_8), 6.43, 6.25 (2d, 1H each, J = 1.5 Hz, H_4 and H_2), 4.83 (brs, 1H, exchanges with D_2O), 2.80 to 0.80 (20 H) especially 2.49 (t, 2H, benzyl-CH_2-), 2.38 (s, 3H, C9 CH_3), 1.6 (s 6H, C6 CH_3's), and 0.88 ppm (t, 3H, ω-CH_3); mass spectrum, *m/e* 314 (M+). These spectral data are identical to those reported for cannabinol (2).

"9β-Methyl-6aβ-hydroxy-$\Delta^{10,10a}$-tetrahydrocannabinol": (±)-6a,7,8,9-Tetrahydro-6,6,9β-trimethyl-3-pentyl-6H-dibenzo[b,d]pyran1,6aβ-diol (IIIa). ^{1}H NMR ($CDCl_3$) δ6.64 (brs, 1H, H_{10}), 6.30, 6.21 (2d, 1H each, J = 1.6 Hz, H_4 and H_2), 5.42 (brs, 1H, exchanges with D_2O), 2.60 to 0.80 (26H) especially 2.45 (t, 2H, benzyl-CH_2-), 2.22 (m, 1H, H_9), 1.42 (s, 3H, 6β-CH_3), 1.20 (s, 3H, 6α-CH_3), 1.14 (d, 3H, J = 7 Hz, C9 CH_3), and 0.88 ppm (t, 3H, ω-CH_3); an exact mass determination gave *m/e* 330.221 (calculated for $C_{21}H_{30}O_3$, 330.219).

"9α-Methyl-6aβ-hydroxy-$\Delta^{10,10a}$-tetrahydrocannabinol": (±)-6a,7,8,9-Tetrahydro-6,6,9α-trimethyl-3-pentyl-6H-dibenzo[b,d]pyran-1,6aβ-diol (IIIb). UV (EtOH) λ_{max} 211 (shoulder), 228, and 265 nm (ε = 26,600, 22,380, and 16,800); ^{1}H NMR ($CDCl_3$) δ6.75 (d, 1H, J = 6.8 Hz, H_{10}), 6.30, 6.21 (2d, 1H each, J = 1.6 Hz, H_4 and H_2), 5.28 (brs, 1H, exchanges with D_2O), 2.70 to 0.80 (26H) especially 2.56 (m, 1H, H_9), 2.45 (t, 2H, benzyl-CH_2-), 1.42 (s, 3H, 6β-CH_3), 1.22 (s, 3H, 6α-CH_3), 1.06 (d, 3H, J = 7 Hz, C9 CH_3) and 0.89 ppm (t, 3H, ω-CH_3); an exact mass determination gave *m/e* 330.219 (calculated for $C_{21}H_{30}O_3$, 330.219).

"7-Keto-$\Delta^{6a,10a}$-tetrahydrocannabinol": (±)-7,8,9,10-Tetrahydro-1-hydroxy-6,6,9-trimethyl-6H-dibenzo[b,d]pyran-7-one (IV). UV (EtOH) λ_{max} 208, 228 (shoulder) and 338 nm (ε = 11,345, 6,201, and 6,352); infrared ($CHCl_3$) 6.06 μ (α,β-unsaturated C=O); ^{1}H NMR ($CDCl_3$) δ6.30, 6.15 (2d, 1H each, J = 2 Hz, H_4 and H_2), 3.20 to 0.80 (26H) especially 3.05 (brd, 1H, J = 17 Hz, H_{10}), 2.46 (t, 2H, benzyl-CH_2-), 1.71 (s, 3H, 6β-CH_3), 1.37 (s, 3H, 6α-CH_3), 1.07 (d, 3H, J = 6 Hz, C9 CH_3), and 0.87 ppm (t, 3H, ω-CH_3); an exact mass determination gave *m/e* 328.2036 (calculated for $C_{21}H_{28}O_3$, 328.2038).

"4′-Hydroxy-$\Delta^{6a,10a}$-tetrahydrocannabinol": (±)-7,8,9,10-Tetrahydro-3-(4-hydroxypentyl)-6,6,9-trimethyl-6H-dibenzo[b,d]pyran-1-ol (V). $[\alpha]^{20}D$ +18.3° [*c*l, $CHCl_3$] ^{1}H NMR ($CDCl_3$) δ6.26, 6.10 (2d, 1H each, J = 2 Hz, H_4 and H_2), 5.90 (brs, 1H, exchanges with D_2O), 3.69 (q, 2H, J = 6 Hz, $H_{4'}$), 2.80 to 0.90 (25H) especially 2.65 (brd, 1H, J = 17 Hz, H_{10}), 2.43 (t, 2H, benzyl-CH_2), 1.40 (s, 3H, 6β-CH_3), 1.20 (s, 3H, 6α-CH_3), 1.16 (d, 3H, J = 6 Hz, C5′ CH_3) and 1.01 ppm (d, 3H, J = 7 Hz, C9 CH_3); an exact mass determination gave *m/e* 330.219 (calculated for $C_{21}H_{30}O_3$, 330.219).

DISCUSSION

Our studies show that microorganisms are capable of transforming $\Delta^{6a,10a}$-THC into cannabinol (II), a side-chain hydroxylated derivative (V), a C7 oxygenated compound (IV), and a pair of diastereomeric tertiary hydroxyl compound (IIIa and IIIb). Other reactions may also occur since many compounds, with R_f values different from those of the isolated products, were detected in the screening program. In addition, it is possible that microorganisms that modify $\Delta^{6a,10a}$-THC will catalyze similar types of reactions with other synthetic and natural cannabinoids. Thus, microorganisms may generate a myriad of products from cannabinoids, some of which may prove to have desirable pharmacological effects.

Microbial transformation may also augment the chemical modification efforts on the cannabinoids. Some of the transformation products may serve as useful intermediates for further chemical modification. Studies on mammalian metabolism of cannabinoids have led to the identification of many metabolities. Microorganisms may provide a means for producing some of these metabolites in larger quantities. The availability of larger amounts will facilitate unambiguous structure determinations and provide an opportunity to test the metabolite for its contribution to the overall pharmacological effects of the parent compound.

The yields of the products isolated in this study were between 4 and 7% based on the amount of $\Delta^{6a,10a}$-THC added to the cultures. No attempts were made to increase these yields. Considering the water insolubility of the cannabinoids, the addition of surfactants or organic solvents to the fermentation media may significantly enhance yields. Also, a systematic study of other fermentation variables should lead, as it has with steroid transformations, to higher-yield cannabinoid transformations.

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