

STEREOCHEMICAL ASSIGNMENTS FOR THE TWO ENANTIOMERIC
PAIRS OF 9,10-DIHYDROXY- $\Delta^{6a(10a)}$ -TETRAHYDROCANNABINOLS.
X-RAY CRYSTAL STRUCTURE ANALYSIS OF
(\pm)-TRANS-CANNABITRIOL

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ABSTRACT.—Single-crystal X-ray analysis of (\pm)-cannabitriol (**1**), isolated from Panamanian *Cannabis*, confirmed the structure of previously isolated (+)- and (−)-cannabitriol and defined the relative stereochemistry at the asymmetric centers. Based on the results obtained from this study, all compounds previously named cannabitriol [(+), (−), or (\pm)] should henceforth be designated *trans*-cannabitriol with appropriate sign of optical rotation, the other enantiomeric pair of (\pm)-9,10-dihydroxy- $\Delta^{6a(10a)}$ -tetrahydrocannabinols being (\pm)-*cis*-cannabitriol (**4**).

In previous publications (1-5), we have reported on the isolation of polyhydroxylated cannabinoids from *Cannabis sativa* L. (+)-Cannabitriol was isolated (**1**) together with (−)-10-ethoxy-9-hydroxy- $\Delta^{6a(10a)}$ -tetrahydrocannabinol (**2**) from an ethanolic extract of *Cannabis* provided by the National Institute on Drug Abuse (NIDA). On the other hand, (−)-cannabitriol has been isolated from Jamaican (**6**) and Japanese (**7**) variants of *Cannabis* and characterized (**7**). In addition, cannabitriol was reported (**8**) to be the alcohol component of the ester of cannabidiolic acid (**3**). At a later date, ElSohly *et al.* (**2**) isolated two new polyhydroxylated compounds, namely, (\pm)-9,10-dihydroxy- $\Delta^{6a(10a)}$ -tetrahydrocannabinol (**4**) and (\pm)-8,9-dihydroxy- $\Delta^{6a(10a)}$ -tetrahydrocannabinol (**5**), from an Indian *Cannabis* variant.

We describe here the isolation (see Experimental section) of crystalline (\pm)-cannabitriol from a Panamanian variant of *C. sativa* L. and present the results of a single-crystal X-ray analysis, which not only confirm the previously reported structure (**1**, **7**) but also define the relative stereochemistry at the two asymmetric centers, C-9 and C-10.

The crystal structure of **1** was solved by direct methods (**9**). Least-squares refinement of atomic positional and thermal parameters converged to $R=0.059$ over 2535 reflections measured by diffractometer. Non-hydrogen atom fractional coordinates are listed in Table 1. A view of the solid-state conformation of one enantiomer, with the atom numbering scheme, is provided in Figure 1.

Bond lengths in the fused ring system are all in accord with expected values, but the C-C single bonds in the fully extended *n*-pentyl side-chain are progressively foreshortened to a greater degree the further they are from the phenyl ring. This is attributed to lack of corrections for the effects of thermal motion associated with this conformationally flexible moiety. The cyclohexene ring approximates to a half-chair form with the C_2 symmetry axis passing through the mid-points of the C(6a)-C(10a) and C(8)-C(9) bonds. In the 1,2-pyran ring, two of the torsion angles are constrained to lie close to 0° , and this ring adopts a 1,3-diplanar form with the C_2 symmetry axis passing through the

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TABLE 1. Non-Hydrogen Atom Fractional Coordinates, with Standard Deviations in Parentheses

Atom	x	y	z
C-1	15840(19)	20958(20)	3337(24)
C-2	22473(22)	26853(24)	-1366(28)
C-3	31493(22)	24402(29)	1733(33)
C-4	33634(21)	15775(29)	9299(35)
C-4a	26984(21)	9852(24)	13877(28)
O-5	29704(14)	1633(17)	21718(22)
C-6	23742(21)	-7315(23)	21623(30)
C-6a	14263(19)	-3586(21)	22631(25)
C-7	8208(23)	-11028(22)	28874(29)
C-8	-1586(20)	-7896(21)	27016(26)
C-9	-2670(19)	3796(21)	28684(24)
C-10	2127(18)	9665(20)	18774(22)
C-10a	11542(18)	5692(20)	17768(22)
C-10b	17818(18)	12322(21)	11394(24)
O-11	7100(13)	23878(14)	269(17)
C-12	38805(26)	31174(34)	-2701(42)
C-13	44452(31)	36503(41)	7951(57)
C-14	39424(34)	44010(44)	15379(64)
C-15	44594(39)	49829(57)	25670(68)
C-16	39901(51)	57368(69)	32281(89)
C-17	24263(24)	-13507(30)	9412(36)
C-18	27625(26)	-13643(29)	33106(37)
C-19	-12499(23)	6841(28)	28176(33)
O-20	1879(15)	6777(15)	40797(17)
O-21	-3148(13)	8720(16)	6588(16)

mid-points of the C(5)-C(6) and C(10a)-C(10b) bonds.² Molecules of **1** are associated in the crystal by extensive O-H . . . O hydrogen bonding.³

Although the relative stereochemistry of the hydroxy groups at C-9 and C-10 in **1** is here established unequivocally as *trans*, there still remains to be defined which of the two enantiomers is dextrorotary and which is levorotatory. On the basis of the results of the present X-ray analysis, **1** should be designated (\pm)-*trans*-cannabitrinol; consequently, the pair of enantiomers with *cis*-related C-9 and C-10 hydroxy substituents constitute (\pm)-*cis*-cannabitrinol. These configurational assignments are in accord with the findings of ElSohly *et al.* (2) in which the enantiomeric pair (**4**) afforded an acetonide upon reaction with Me₂CO-perchloric acid whereas (-)-cannabitrinol did not (7).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The melting point was determined in an open, glass capillary tube and is uncorrected. The ¹H-nmr spectrum was recorded in CDCl₃ on an EM-390 90 MHz instrument using TMS as internal standard. The mass spectrum was taken with a Finnigan 3200, MS/DS system. The optical rotation was measured on a Perkin-Elmer 141 polarimeter.

PLANT MATERIAL.—A Panamanian variant of *C. sativa* L. grown in Mississippi was used in this study. Herbarium specimens are deposited in the Herbarium, Research Institute of Pharmaceutical Sciences, University of Mississippi.

²Endocyclic torsion angles (ω_{ij} , $\sigma \pm 0.2-0.3^\circ$) in one enantiomer follow: $\omega_{6a,7}$ 10.4, $\omega_{7,8}$ -42.5, $\omega_{8,9}$ 61.2, $\omega_{9,10}$ -47.6, $\omega_{10,10a}$ 16.2, $\omega_{10a,6}$ 3.0 in the cyclohexene ring; $\omega_{4a,5}$ 33.1, $\omega_{5,6}$ -47.2, $\omega_{6,6a}$ 31.0, $\omega_{6a,10a}$ 0.0, $\omega_{10a,10b}$ -17.2, $\omega_{10b,4a}$ 0.6.

³Hydrogen bonded distances follow: O(11) . . . O(21) 2.615 Å (intramolecular); O(20) . . . O(20) 2.707 Å and O(21) . . . O(21) Å between molecules related by crystallographic centers of symmetry; O(20) . . . O(11) 2.721 Å between molecules related by the *c*-glide plane. The disposition of molecules around the crystallographic centers of symmetry demands that the hydrogen atoms involved in the intermolecular hydrogen bonds are disordered over two sites.

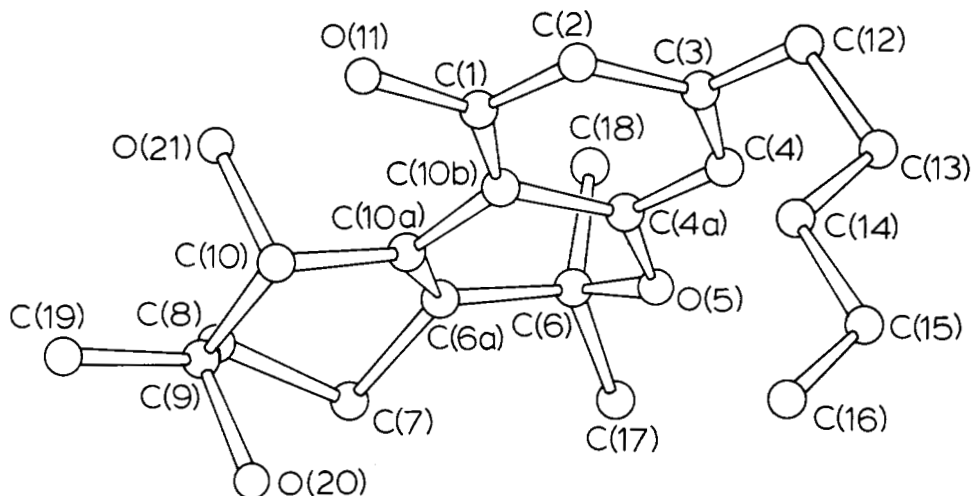
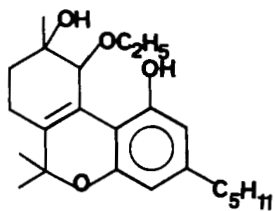
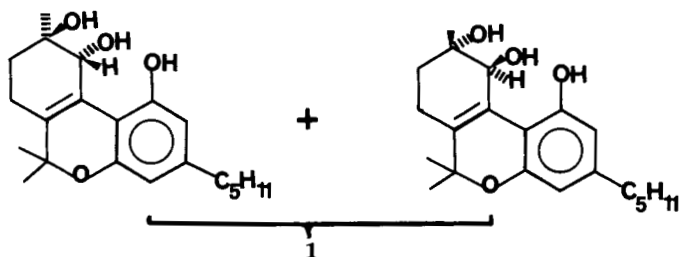
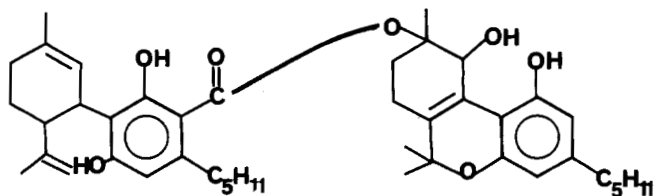


FIGURE 1. Atom numbering scheme and solid-state conformation of one enantiomer of **1**; hydrogen atoms have been omitted for clarity.

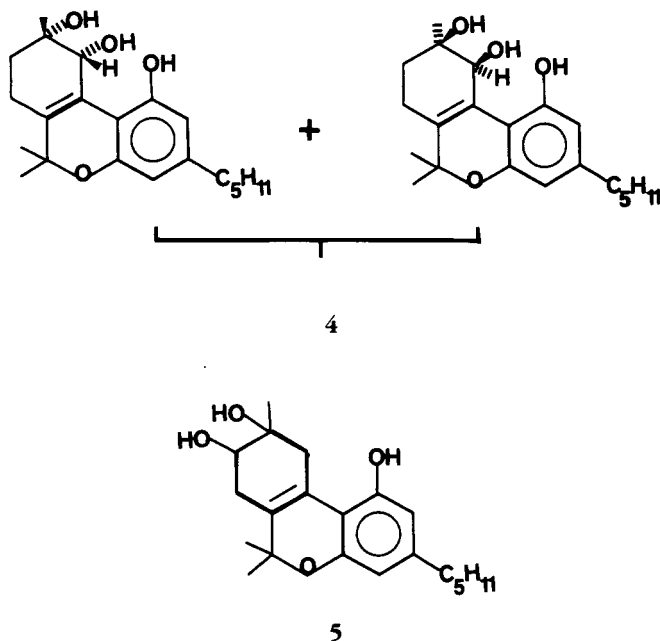
EXTRACTION AND FRACTIONATION.—Powdered leaves (7 kg) were extracted by percolation with 95% EtOH (72 gallons), and the solvent was evaporated to yield a residue (1.8 kg, extract A). About 800 g of extract A was partitioned between CHCl_3 (fraction B) and H_2O (fraction C). Fraction B was further partitioned between *n*-hexane (fraction D) and ice-cold 3 N NaOH (fraction E). Fraction E was rendered acidic with HCl and extracted with Et_2O to yield a polar acidic fraction (fraction F, 35.2 g). Fraction F (16 g) was



2



3



further fractionated on a polyamide⁴ column (320 g, 4.6×90 cm) packed in EtOH-H₂O (6:4). The concentration of EtOH was gradually increased and nine fractions were collected. Fraction 1 eluted with 1.5 liters of EtOH-H₂O (6:4) (6.4 g) was rechromatographed on a silica gel G column (600 g, 4.6×83 cm) packed in hexane-Et₂O (6:4), and 500 fractions, each of 20 ml, were collected.

ISOLATION OF (±)-CANNABITRIOL.—Fractions 221-256, eluted with hexane-Et₂O (1:1), were combined and the solvent evaporated to yield a brown residue (237 mg). This residue was subjected to preparative tlc using precoated silica gel G UC-254 plates⁵ (20×20 cm, 0.25 mm thickness) and 10% EtOAc in CH₂Cl₂. The partially purified material was then chromatographed on reversed phase KC-18 plates⁶ (5×20 cm, 0.2 mm thickness) using MeOH-H₂O (8:2). Crystallization of the residue obtained from the reversed phase plates (Me₂CO-hexane) afforded needle crystals of **1** (19 mg) mp 171°, [α]_D²⁵ = 0.00, identical with previously isolated **1** material (¹H-nmr and ms).

CRYSTAL DATA.—(±)-Cannabitriol, C₂₁H₃₀O₄, *M* = 346.47, Monoclinic, *a* = 14.993(8) Å, *b* = 12.712(6) Å, *c* = 10.603(5) Å, β = 96.05(1)°, *U* = 2009.6 Å³, *Z* = 4, *D*_{calcd} = 1.145 g cm⁻³. Absorption coefficient for Cu-K_α radiation (λ = 1.5418 Å), μ = 6.3 cm⁻¹. Space group *P*2₁/*c* (*C*_{2h}⁵) established uniquely by the systematic absences: *OkO* when *k* ≠ 2*n*, *bOℓ* when *ℓ* ≠ 2*n*.

CRYSTALLOGRAPHIC MEASUREMENTS.—Preliminary unit-cell parameters and space group information having been obtained by photographic methods, the intensities of 3573 unique reflections with θ < 67° from a crystal of dimensions *ca.* 0.14×0.36×0.80 mm were recorded on an Enraf-Nonius CAD-3 automated diffractometer (Ni-filtered Cu-K_α radiation; θ-2θ scans) by procedures described in detail elsewhere (10). Refined unit-cell parameters were derived by least-squares treatment of the diffractometer setting angles for 40 reflections widely separated in reciprocal space. A total of 2535 reflections with *I* > 2.0σ(*I*)[σ²(*I*) = scan count + total background count] were judged to be observed and were retained for the structural analysis. The data were corrected for the usual Lorentz and polarization effects but not for the small effects of absorption.

STRUCTURE ANALYSIS.—The structure was solved by direct methods with the aid of the MULTAN76 (9) suite of programs. Full-matrix, least-squares adjustment of non-hydrogen atom positional and anisotropic thermal parameters, with hydrogen atoms included at their calculated positions in the later iterations, converged to *R* = 0.059. Atomic scattering factors for carbon and oxygen were taken from reference (11), and for hydrogen from reference (12). In the least-squares calculations, Σ*w*Δ² (Δ = ||*F_o*|| - ||*F_c*||)

⁴Machery Nagel—polyamid SC6.

⁵Brinkman Instruments, Inc.

⁶Whatman.

was minimized, with weights w assigned according to the scheme $\sqrt{w}=1$ for $|F_o| \leq 14.0$, and $\sqrt{w}=14.0/|F_o|$ for $|F_o| > 14.0$. Final non-hydrogen atom parameters are in Table 1.

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