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Δ^1-3,4-*CIS*-TETRAHYDROCANNABINOL IN *CANNABIS SATIVA*

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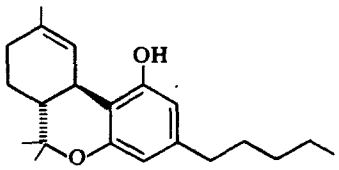
Key Word Index—*Cannabis sativa*; Cannabinaceae; marijuana; cannabinoids; Δ^1-3,4-*cis*-tetrahydrocannabinol identification.

Although the major psychomimetic principle of marijuana (*Cannabis sativa*), Δ^1-3,4-*trans*-tetrahydrocannabinol (1, *trans*-THC), was isolated from plant samples several years ago [1], the corresponding *cis*-isomer (2; *cis*-THC), which is not psychoactive [2], has been known only from laboratory syntheses of 1 [3]. Recently, however, we have found that 2 occurs as a major contaminant in samples of 1 obtained during routine analysis of some confiscated marijuana.



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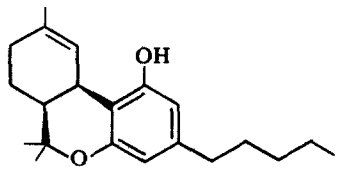

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Spectral and chromatographic data on material isolated from these samples and purified by repeated TLC and HPLC were totally compatible with those obtained from authentic *cis*-THC prepared in our laboratory[3]. Of particular importance was the PMR spectrum of this material, which also matched that published for *cis*-THC [3]. In addition, the low resolution MS of our material and of synthetic 2 were nearly identical with that of *trans*-THC [4], except in the case of 2 there were minor enhancements in the relative intensities of the peaks at *m/e* 221 and 231. This spectrum for 2 is consistent with that which we expected for this compound, but it does differ significantly from that reported by Vree *et al.* [5]. In view of our complete spectral characterization of *cis*-THC, we must conclude that these authors were in error in their GC–MS identification of 2. This is substantiated by the presence of a relatively large peak at *m/e* 246 in their spectrum, which cannot be adequately explained by the structure of 2 [6]. Further, treatment of the isolated material with *p*-toluenesulfonic acid in C_6H_6 under reflux gave primarily $\Delta^{4(8)}$-*iso*-THC, which was identical by GC–MS to that obtained from synthetic *cis*-THC under the same conditions [7].

Because of the small amounts of material isolated, ORD data was necessarily limited. However, the CD curve of this material in the region 245–350 nm (in $CHCl_3$) was quite similar in shape and intensity to that obtained from *trans*-THC. This indicates that the isolated *cis*-THC has the absolute configuration shown [1, 8].

Since the natural occurrence of *cis*-THC had not been reported earlier, and since our own experience had indicated that this compound was not a common constituent of all marijuana samples, we became curious about the distribution of *cis*-THC in marijuana. Cannabinoid assay of numerous samples by extraction, preparative-TLC and GLC indicated that the only marijuana plants showing significant quantities of *cis*-THC were those which simultaneously exhibited high ratios of cannabidiol (CBD) to total THC (the phenotype ratio [9]). In typical cases, samples having CBD–THC ratios of about 16:1 had *trans*-THC–*cis*-THC ratios of about 1:1 or 2:1, whereas samples having phenotype ratios less than 1 had *trans*-THC–*cis*-THC $\gg$ 10:1.

Marijuana samples with high phenotype ratios have been noted previously [9, 10], and have been characterized by Small and Beckstead [9] as 'Phenotype III' plants (having poor psychomimetic, but good fiber, properties). In contrast, 'normal' marijuana (Phenotype I) is characterized by high concentrations of *trans*-THC and/or cannabinol relative to cannabidiol [9]. Phenotype III plants generally have been considered to originate in more northerly climates, and information received with the samples we examined indicated that some, if not most, of these plants were grown locally.

Although the relative amounts of *cis*- and *trans*-THC were quite sensitive to changes in the relative cannabidiol concentration, the actual amount of *cis*-THC present in most of these samples was not significantly affected by the phenotype ratio. Instead, the concentration of *cis*-THC in samples having a phenotype ratio greater than *ca* 2 was relatively constant at *ca* 0.04% of the dry plant wt. Plants having lesser relative amounts of cannabidiol also had lesser amounts of *cis*-THC, with plants showing little or no cannabidiol having no detectable amounts of *cis*-THC.

Since all of the samples which we examined were from mature or nearly mature plants (all samples contained germinatable seeds), it appears that Phenotype III marijuana is unable to convert significant quantities of cannabidiol to *trans*-THC as 'normal' marijuana plants do. This suggests that the presence of *cis*-THC in these samples may be related to the blockage of this biosynthetic path, allowing material which normally would be channeled into eventual conversion to *trans*-THC to be diverted more easily at some early point in the biosynthetic sequence toward the production of *cis*-THC than in Phenotype I plants.

EXPERIMENTAL

Dried plant material from seized contraband (460 g) was sieved to remove seeds and stalks, and the remaining material (240 g) extracted thoroughly with 3 l. of petrol. The residue (3.9 g) after flash evaporation of the solvent was taken up in a minimal vol. of $CHCl_3$, to which was added a large excess of MeOH. The resulting mixture was filtered to remove insoluble waxes, and solvent removed by flash evaporation. The oily residue (3 g) was passed through a small Florisil column [1], and the cannabinolic fractions (monitored by GLC) thus obtained were combined and subjected to repeated preparative-TLC on Si gel using C_6H_6. The band corresponding to *trans*-THC was visualized by spraying a small portion of the plate with aq. Fast Blue B, and then removed and extracted with $CHCl_3$–Me_2CO (1:1). After 3 developments 86 mg of residue was obtained, which by GLC was found to consist primarily of 3 compounds, cannabidiol, *trans*-THC, and a compound having a R_f midway between those of cannabidiol and *trans*-THC. Final purification was effected by subjecting the residue from TLC to repeated HPLC using a chromatograph fitted with a variable wavelength detector (243 nm). The separation was carried out on a 0.5 m × 2.6 mm i.d. Sil-X or Phenyl Sil-X column at 25–42° using 2.5% $CHCl_3$, 2.5% *iso*-propyl ether, and 0.05% MeOH in hexane with a flow rate of 0.5 ml/min. Three passages through the column were necessary to give *ca* 1 mg (0.0002% overall yield) of a colorless oil which was at least 95% pure by GLC. GLC R_t 240°, 2m 3% OV-101 on HP Chromosorb W, 0.89 relative to *trans*-THC; IR (neat), 3410 (OH), 2930, 1624 (—C=C—), 1580 (—C=C—), 1510 (aromatic), 1428 (—CH_2—), 1160, 1055, and 1039 cm^{-1}; MS (GC–MS; 70 eV); *m/e* (rel. intensity), 314 (48), 299 (52), 271 (30), 258 (22), 243 (43), and 231 (100); PMR ($CDCl_3$), δ6.23 (*br*-2H; olefinic and aromatic H), δ6.13 (*br*-1H; aromatic), δ4.76 (*s*-1H; phenol), δ3.55 (*br*-1H; C-3 methine), δ1.69 (*s*-3H; olefinic CH_3), δ1.39 (*s*-3H) and δ1.27 (*s*-3H; *gem*-diMe), and δ0.88 (*t*-3H; ω-Me) [11].

Plant assays. Ca 100 mg samples of dry, unmanicured plant material from 20 different sources were soaked in $CHCl_3$ (1 ml) and the relative concns of the pentyl cannabinoids in these solns were determined by GLC (conditions as above). Although this procedure readily yielded relative concns of cannabidiol and total THC, those samples containing *cis*-THC generally had such high concns of cannabidiol that determining the relative amounts of *cis*- and *trans*-THC by this method proved impractical. In these cases *ca* 1 g samples of plant material were extracted with $CHCl_3$ and the extract subjected to preparative-TLC (*vide supra*). The band corresponding to *trans*-THC was then removed and examined by GLC as above.

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FERULATES FROM CORK LAYERS OF *SOLANUM TUBEROSUM* AND *PSEUDOTSUGA MENZIESII*

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INTRODUCTION

The process of natural wound healing of the cut surface of potato tubers has been well documented [1, 2]. The initial step in wound healing is the suberization of a layer of intact cells at the surface of the wound. Any agent