

CANNABIS, XV. ¹ PREPARATION AND STABILITY OF Δ^9 -TETRAHYDROCANNABINOL- β -CYCLODEXTRIN INCLUSION COMPLEX

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ABSTRACT.—Investigation is made of the preparation of the inclusion complex of Δ^9 -tetrahydrocannabinol (**1**) with β -cyclodextrin (β -CD) in solid form by the precipitation method. In order to estimate the inclusion complex formation, various spectral data (uv, cd, and emission spectra) have been studied. It has been estimated that the inclusion complex is highly stable under various conditions [light of 10,000 lux, oxygen existence, and high temperature (120°)] compared to **1** alone. The extensive application for a drug delivery system is also discussed.

Current interest in the inclusion complex of cyclodextrins (CD) with various kinds of drugs has been centered on their chemical stability, solubility, and absorption properties. The major active principle of marijuana, Δ^9 -tetrahydrocannabinol, is a fragile compound with respect to light (1), auto-oxidation (2), high temperature (1,3), acidic medium (4), and free radical oxidation (5). The major pathway for auto-oxidation of **1** is summarized in figure 1. Compound **1** requires the presence of detergent in order to dissolve in water, thus creating problems in pharmacological studies.

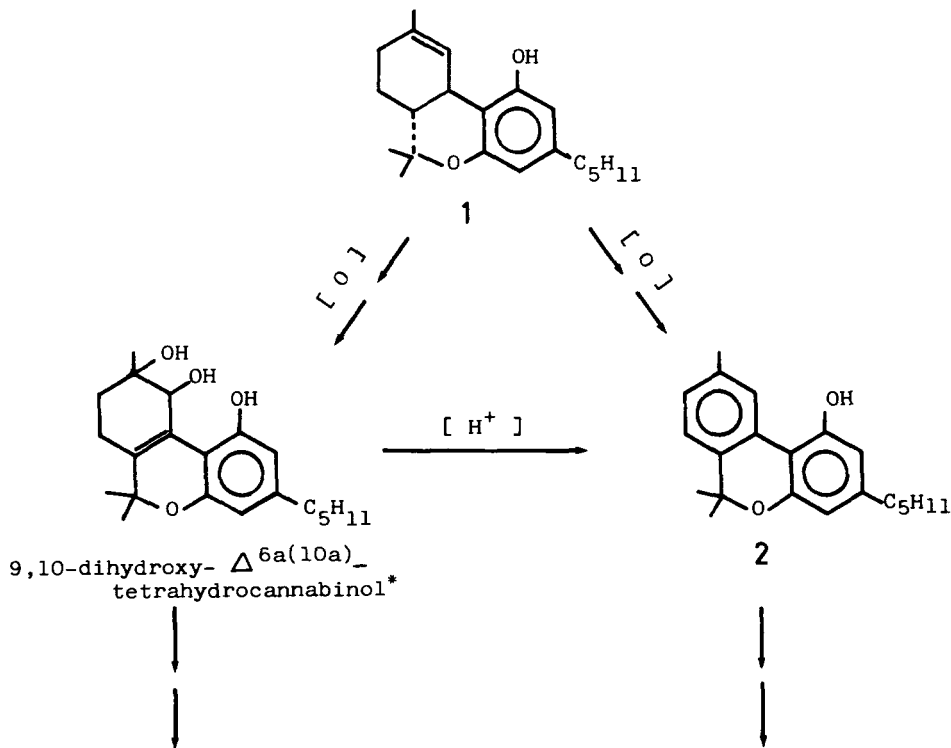


FIGURE 1. Pathway of autoconversion of **1**.

*M.A. Elsohly, F.S. El-Ferally and C.E. Turner, *J. Nat. Prod.* **40**, 275 (1977).

This paper presents the preparation of a Δ^9 -tetrahydrocannabinol- β -cyclodextrin complex (**1**- β -CD), a more stable compound when compared with **1** alone.

RESULTS AND DISCUSSION

In order to estimate the ability of inclusion complex formation, various spectra data have been studied. Figure 2 shows the uv spectral data of **1** alone and of **1**- β -CD in 0.1N NaOH. No variation between the **1** and **1**- β -CD spectral profile could be detected; however, the different spectrum in the similar spectra clearly showed the difference of intensity as indicated in Figure 3. The cd spectra of **1** alone and of **1**- β -CD are shown in Figure 4 to have similar maximum wave lengths; they differ from each other in intensity. Figure 5 illustrates the emission spectra of the **1**- β -CD system in 0.1N NaOH. These spectra indicate that **1** with β -CD increases the intensity and shifts the absorption wave length. These spectra support the existence of the formation of the inclusion complex between **1** and β -CD. The methods for preparation of the inclusion complex have previously been elucidated (6,7); in this study we used the precipitation method.

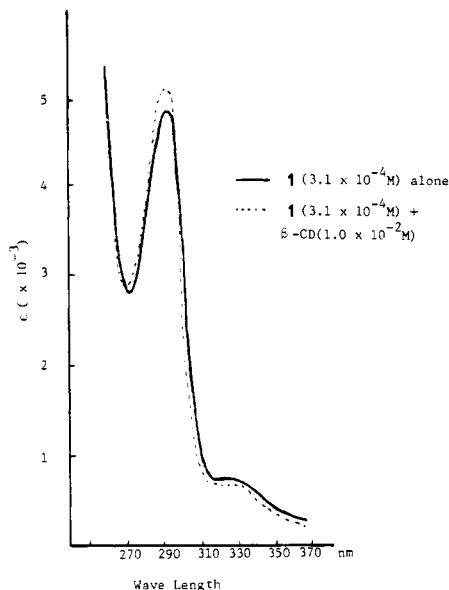


FIGURE 2. The uv spectra of **1** and **1**- β -CD inclusion complex in 1N NaOH.

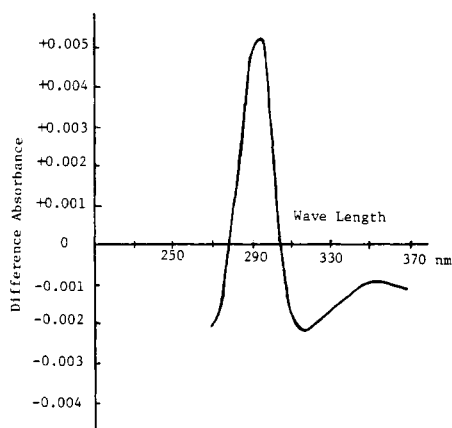


FIGURE 3. Difference spectrum between **1** and **1**- β -CD inclusion complex.

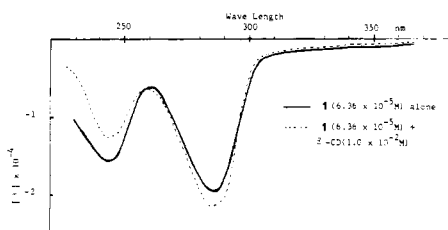


FIGURE 4. The cd spectra of **1**- β -CD system in 0.1N NaOH.

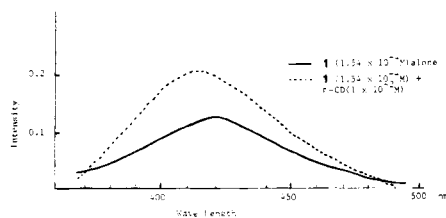


FIGURE 5. Emission spectra of **1**- β -CD system in 0.1N NaOH.

The inclusion complex of **1**- β -CD is a white powder, $C_{105}H_{170}O_{72}$, showing aromatic double bond absorbance (1622 and 1577 cm^{-1}) in the ir spectrum. ^1H -nmr spectrum shows aromatic protons at δ 5.57 and 5.90. However, it is difficult to confirm the effects of β -CD on each signal of **1**. The stoichiometry of the complex in the solid state was analyzed chemically, as indicated, and found to be 2:1 for the β -CD system.

Next, the stabilities of **1** and **1**- β -CD were determined. It has been demonstrated that the major artificial product of **1** is cannabinol (**2**) (8). The major route of **1** oxidation occurs by auto-oxidation under storage conditions. The influences of temperature, light, and the presence of oxygen have been determined (table 1). Degradation of the **1**- β -CD complex occurred only in experiment 4, which showed the appearance of **2** after storage for five months. Experiments 1 and 2 indicated the lack of effect by light, if the nitrogen was replaced. The presence of oxygen caused oxidative degradation of **1**, producing **2** and other products, as indicated in experiments 3 and 4. The amount of **2** produced varied, as indicated by these experiments. Moreover, the presence of light along with oxygen increased degradation to **2**. In addition to the experiments performed at 30° , the influence of oxygen also was observed at -10° (experiment 6). The amount of **1** present was 50.8% and that of **2** was 20.9%, thus indicating that the essential factor for storage of **1** was a decrease in the oxygen concentration. On the other hand, experiments performed at -10° and 30° (experiments 1 and 5) in the presence of nitrogen indicated little variation in the stability of **1**.

TABLE 1. Stabilities of **1**- β -CD^a and **1**^b alone under various conditions.

Experiment Number	Condition ^d	1 - β -CD ^a		1 ^b alone	
		1 ^b (%)	2 ^c	1 ^b (%)	2 ^c
1	$30^\circ\text{N}_2\text{D}$	100.0	0	76.6	11.4
2	$30^\circ\text{N}_2\text{L}$	100.0	0	74.5	10.0
3	$30^\circ\text{O}_2\text{D}$	98.2	0	2.7	30.0
4	$30^\circ\text{O}_2\text{L}$	86.1	2.0	3.3	17.4
5	$-10^\circ\text{N}_2\text{D}$	100.0	0	74.2	11.6
6	$-10^\circ\text{O}_2\text{D}$	98.2	0	50.8	20.9

^a Δ^9 -tetrahydrocannabinol- β -cyclodextrin inclusion complex.

^b Δ^9 -tetrahydrocannabinol.

^cCannabinol.

^d N_2 : replacement of air by N_2 , O_2 : air existence.

D=under dark, L=under light, storage period=5 months.

In order to confirm the stability of the **1**- β -CD under more stringent storage conditions, the effects of higher temperatures and darkness were studied to determine differences between the **1**- β -CD complex and the **1**- β -CD mechanical mixture. The amounts of **1** alone and of the **1**- β -CD mechanical mixture gradually decreased over time when heated to 120° in the dark. The explanation for this phenomenon may depend on the exposed surface areas of the different structures. That is, the decomposition of **1** may be increased by the porous surface of β -CD rather than **1** alone. The degradative product from **1** alone and from the **1**- β -CD mechanical mixture was **2** in both cases, as identified by glc; the amount of **2** present is listed in figure 6.

On the other hand, the **1**- β -CD inclusion complex does not degrade at 120° in the dark over a 24-h period. This finding suggests that the labile part, such as double bond and/or phenol is included by β -CD. Although the direct conformation of the **1**- β -CD inclusion complex in a solid state is not yet available, the results thus far support the existence of this structure.

The inclusion complex of **1** with β -CD may have wide applications. For example,

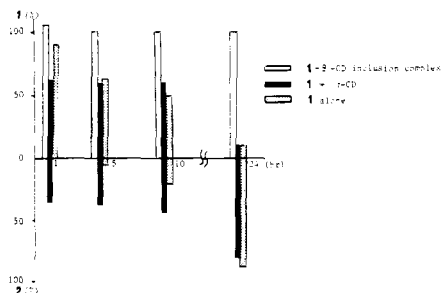


FIGURE 6. Stabilities of **1**- β -CD inclusion complex, **1**- β -CD mechanical mixture and **1** alone by heating at 120°.

the long-term storage of **1** with increased solubility in water will be possible. Also, CD complexation may be extensively applied to enhance the membrane permeability and bioavailability of slightly soluble **1**. Further studies on other cannabinoids are in progress. These results will be presented elsewhere in the near future.

EXPERIMENTAL

The uv spectra were obtained on a Hitachi Model 100-10 Spectrophotometer and the ir spectra were determined on a Nihonbunko Model DS-100 Spectrometer in KBr pellets. The ^1H -nmr spectra were recorded in 1 N NaOH on a JEOL PS-100 Spectrometer; chemical shifts are given on the ppm scale, and signal multiplicates are represented by s(singlet), d(doublet), t(triplet), q(quartet), m(multiplet), and br(broad). The cd spectra were measured on a JASCO J-20 Spectropolarimeter. The emission spectra were measured on a Hitachi MPF-2A Fluorescence Spectrophotometer. The glc analyses were determined on a Shimadzu Gas Chromatograph model GC-4BM under previously reported conditions (9).

Compound **1** and **2** were isolated from the Mexican cannabis as described previously (10).

PREPARATION OF 1- β -CD INCLUSION COMPLEX.—Compound **1** (0.4 g) dissolved in MeOH (8 ml) was added to 4.3% β -CD solution (400 ml) dropwise, while being stirred. After 1 h of stirring, the reaction mixtures were centrifuged (3000 rpm, 25 min). The precipitates were then washed twice with a small amount of water, dried on $\text{c.H}_2\text{SO}_4$, and finally washed twice more with a small amount of ether to remove free **1**. Finally, the white powder obtained was completely dried over P_2O_5 at 80° for 5 h. The inclusion complex was analyzed by uv and glc methods, as described below, to determine the ratio of **1**- β -CD (1:2). The **1**- β -CD complex was a white powder: uv λ max (H_2O) 276 nm; cd ($6.72 \times 10^{-5}\text{M}$ 0.1N NaOH) $[\theta]_{247} -1.43 \times 10^4$, $[\theta]_{291} -2.35 \times 10^4$; ir(KBr) ν max: 1622, 1577 cm^{-1} ; ^1H -nmr (1 N NaOH) δ 0.99(t, ω -Me), 1.33(s, C_6 -Me), 1.36(s, C_9 -Me), 1.90-2.30(m, β - CH_2), 3.35(t, glu_4 -H), 3.46(q, glu_2 -H), 3.74(m, $\text{glu}_{3,5}$ and C_6 -H), 4.86(d, glu_1 -H), 5.57(bs, arom H), 5.90(bs, arom H), 6.23(bs, C_{10} -H).
Anal. Calcd for $\text{C}_{105}\text{H}_{170}\text{O}_{72}$: C, 48.80; H, 6.58. Found: C, 48.64; H, 7.09.

ANALYTICAL SYSTEM.—The uv method: **1**- β -CD inclusion complex was dissolved in MeOH- H_2O (1:1) and analyzed by uv photometer at 270 nm to determine the content of **1** using the calibration curves. The glc method: Samples of inclusion complex (20 mg) were suspended in diluted MeOH (1 ml) under sonication. To the solution a drop of cyclohexylamine was added, then extracted with ether (5 ml) five times. After evaporation of ether, the residue was analyzed by glc as described previously (9).

STORAGE OF SAMPLES.—Samples (**1** alone, 10.68 mg; **1**- β -CD inclusion complex, 20 mg) were sealed in glass ampules with air or with replacement of air by N_2 and were stored either under light of 10,000 lux or in the dark for five months. The storage at 30° was undertaken at the Biotron Institute of this University.

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