
Ali R. Banijamali *, Cornelis J. Van der Schyf * and Alexandros Makriyannis*

Contribution from the Departments of Pharmaceutical Science and Molecular and Cell Biology and the Institute of Materials Science, The University of Connecticut, Storrs, CT 06269-3136

SUMMARY

Addition of HCl gas to $(-)\Delta^8$, $(-)\Delta^9$, or $(-)\Delta^{9,11}$-THC solutions at -60°C results in the formation of $9\alpha$- and $9\beta$-chlorohexahydrocannabinol (Cl-HHC). The addition appears to involve initial protonation of the double bond in the form of a bridged hydrogen cation followed by attack of the chloride anion at the most substituted 9-position. For both steps in the addition reaction the stereochemistry is dependent on the double bond position in the THC isomer. Elimination of HCl from each of the two addition products using potassium-tert-amylate leads exclusively to $9\alpha$-THC in the case of $9\alpha$-Cl-HHC and to $9\alpha\cdot11$-THC in the case of $9\alpha$-Cl-HHC. The individual addition products can be separated and used to obtain regio- and stereochemically $^2$H-isotopically labeled tetrahydrocannabinols which can be used in biophysical and biochemical studies.

KEYWORDS: Stereospecific Deuterium labeled THCs, $(-)\Delta^8$-THC, $(-)\Delta^9$-THC, $(-)\Delta^{9,11}$-THC

INTRODUCTION

The addition of HCl to $(-)\Delta^8$-tetrahydrocannabinol ($\Delta^8$-THC) at 25°C and subsequent elimination by using a bulky base is a well-known method for the synthesis of $\Delta^2$-THC (1-3) the least stable of the three possible tetrahydrocannabinol isomers, having a double bond in the 9-position (2,3). Previously, little attention was given to the intermediate or intermediates obtained after the addition of HCl (2-4). We were interested in studying these intermediates as an avenue to other synthetic routes for both $\Delta^2$-THC and $\Delta^{9,11}$-THC (1). A crucial aspect in this reaction is the stereochemistry of the tricyclic ring system, which apparently also contributes to the higher stability of $\Delta^8$-THC compared to $\Delta^9$-THC and $\Delta^{9,11}$-THC (3-5). However, our primary incentive was the labeling of the cannabinoids by utilizing $^2$HCl to introduce deuterium regio- and stereo-specifically in the C-ring of all three THC isomers. Although deuterium labeling procedures for cannabinoids have been developed earlier (6-9), no attempt had been made prior
to this work to control the stereochemistry of deuteration. As an avenue for obtaining the selectively labeled tetrahydrocannabinols we have explored the mechanism of HCl addition/elimination reaction. These regio- and stereoselectively labeled cannabinoid analogs can be used in biophysical experiments involving $^2$H NMR spectroscopy. They would also be of considerable potential utility in studies involving the enzymatic biotransformation of these compounds. Furthermore, the above approach can be applied toward the synthesis of [3H]-radiolabeled analogs.

**RESULTS AND DISCUSSION**

Addition of HCl gas to any of the three tetrahydrocannabinol isomers in solution at -60°C, gave mixtures of the same two addition products, namely 9α- and 9β-chlorohexahydrocannabinol (9α-Cl-HHC; 9β-Cl-HHC) in different proportions (as indicated from the $^1$H NMR spectra) depending on the THC isomer used. At 0 °C, 9β-Cl-HHC becomes the exclusive reaction product while at higher temperatures both addition compounds are obtained. The two 9-Cl-HHC isomers were separated and purified using HPLC and their conformations in solution were determined using $^1$H NMR in order to obtain additional insights into the stereochemistry of HCl addition/elimination.

![Chemical Structures](image)

**Conformational Analysis.** The $^1$H chemical shifts of the two HCl addition isomers were obtained from the respective 1D- and COSY spectra. Coupling constants were estimated from the experimentally obtained spectra and were then refined by spectral simulation and iteration as described earlier (14) using the ITRCAL program. This program is an implementation of the LAOCN3 algorithm on a minicomputer. Due to spectral overlap, the spectral portions of C7 of the C-ring were not amenable to detailed analysis. Individual chemical shifts could be extracted.
only from the COSY spectrum. The preferred conformations of the two chloro compounds were
determined using a similar approach as described in an earlier publication (14) from the C-ring
proton coupling constants which, together with the calculated dihedral angles, are listed in
Table I.

**TABLE I**

<table>
<thead>
<tr>
<th>Type of Coupling</th>
<th>Designation</th>
<th>$\eta_\text{J}$a (Hz)</th>
<th>Dihedral anglesb in degrees</th>
<th>$\eta_\text{J}$ (Hz)</th>
<th>Dihedral anglesb in degrees</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geminal</td>
<td>7α-7β a-e</td>
<td>c</td>
<td>d (106.816)</td>
<td>-10.9</td>
<td>d (106.378)</td>
</tr>
<tr>
<td></td>
<td>8α-8β e-a</td>
<td>-12.9</td>
<td>d (106.171)</td>
<td>-8.2</td>
<td>d (106.532)</td>
</tr>
<tr>
<td></td>
<td>10α-10β e-a</td>
<td>-12.8</td>
<td>d (105.893)</td>
<td>-14.2</td>
<td>d (105.713)</td>
</tr>
<tr>
<td>Vicinal</td>
<td>6α-7α a-a</td>
<td>c</td>
<td>d (177.962)</td>
<td>11.0</td>
<td>160 (178.294)</td>
</tr>
<tr>
<td></td>
<td>6α-7β a-e</td>
<td>c</td>
<td>d (63.9374)</td>
<td>2.2</td>
<td>67 (63.7746)</td>
</tr>
<tr>
<td></td>
<td>6α-10α a-a</td>
<td>11.0</td>
<td>160 (171.73)</td>
<td>11.0</td>
<td>160 (171.743)</td>
</tr>
<tr>
<td></td>
<td>7α-8α a-e</td>
<td>4.3</td>
<td>57 (58.8543)</td>
<td>2.6</td>
<td>65 (58.1436)</td>
</tr>
<tr>
<td></td>
<td>7α-8β a-a</td>
<td>c</td>
<td>d (172.415)</td>
<td>c</td>
<td>d (173.743)</td>
</tr>
<tr>
<td></td>
<td>7β-8α e-e</td>
<td>4.3</td>
<td>55 (59.6449)</td>
<td>2.6</td>
<td>63 (60.052)</td>
</tr>
<tr>
<td></td>
<td>7β-8β e-a</td>
<td>c</td>
<td>d (55.9159)</td>
<td>c</td>
<td>d (58.1436)</td>
</tr>
<tr>
<td></td>
<td>10α-10α a-e</td>
<td>2.6</td>
<td>65 (62.1835)</td>
<td>2.6</td>
<td>65 (62.5297)</td>
</tr>
<tr>
<td></td>
<td>10α-10β a-a</td>
<td>11.6</td>
<td>164 (178.716)</td>
<td>11.0</td>
<td>160 (178.481)</td>
</tr>
</tbody>
</table>

*a Determined from spectra obtained at 200 and 500 MHz and spectral simulations using ITRCAL; n denotes the number of bonds through which coupling occurs; gem. n = 2, vic. n = 3.

*Calculated using the equation $\phi = \cos^{-1} \sqrt{\eta_\text{J}/K}$; where $K_{s-a} = 12.5$, $K_{s-e} = 14.3$ and $K_{e-e} = 12.0$ Hz. $\phi$ is the dihedral angle, K values calculated from 1,3,5-trimethyl-cyclohexane.

*Coupling constants could not be determined due to overlapping of signals (see text).
Our conformational analysis indicates that both C-rings exist in undistorted chair conformations. The two isomers could easily be identified by virtue of the nOe measurements (Table II) which indicate a large nOe for 10α upon irradiation of the 9-methyl protons in 9β-Cl-HHC, but no nOe for the same experiment in 9α-Cl-HHC. The NMR results were confirmed using MM2 force field calculations to determine the preferred conformation for 9β-Cl-HHC and 9α-Cl-HHC as described in an earlier publication (15). For these calculations crystal structure of Δ²-THC acid (16) was used as the starting geometry for both HHC analogs.

**TABLE II**

<table>
<thead>
<tr>
<th>Proton(s) irradiated (δ, ppm)*</th>
<th>Proton observed (δ, ppm)*</th>
<th>% area increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>6αCH₂ (1.113)</td>
<td>10α (3.033)</td>
<td>8.0</td>
</tr>
<tr>
<td>9αCH₃ (1.647)</td>
<td>10α (3.033)</td>
<td>13.8</td>
</tr>
<tr>
<td></td>
<td>10α (3.423)</td>
<td>7.9</td>
</tr>
<tr>
<td>1°CH₂ (2.408)</td>
<td>2 (6.059)</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>4 (6.233)</td>
<td>3.5</td>
</tr>
</tbody>
</table>

*In C₂HCl₃

**Mechanism and Stereochemistry of Addition.** Although several interpretations of the mechanism of the addition of hydrohalogens to carbon-carbon double bonds exist, especially with regard to the influence of stereochemical orientation and reactivity (17), we found the addition of HCl to the THC isomers presented here, to be of particular interest due to the avenues it opens for the synthesis of isotopically labeled analogs difficult to obtain by other methods. Addition of HCl gas to the THC isomers was carried out in a number of aprotic and non-protonatable solvents including petroleum ether and dichloromethane and followed by ¹H NMR. Optimal results were obtained in dichloromethane. However, the reaction does not proceed in solvents which can be protonated in the presence of HCl gas such as diethyl ether and dioxane. The reaction also appears to be highly influenced by the diffusion of HCl gas in the solvent. The best results were thus obtained by using a high efficiency gas dispersion tube that serves to increase the area of the gas : solution interface.
Addition proceeded fastest with Δ⁹-THC followed by Δ⁹,₁₁-THC while Δ⁸-THC had the slowest reaction rate. Under the conditions described in the experimental section, the reaction time at which 50% of the starting material was consumed, varied between 25 and 45 minutes for the three THC isomers treated. With both Δ⁸- and Δ⁹-THC, 9α-Cl-HHC was the predominant product while 9β-isomer was the minor product. Conversely, Δ⁹,₁₁-THC gave a final reaction product composition favoring of 9β-Cl-HHC.

To obtain more information on the stereochemistry of the addition of HCl to the double bond, we used ³HCl and determined the relative positions of the ³H-labels in each of the products from the ¹H NMR spectra of the individual reaction mixtures. From the products obtained we were able to propose the following mechanisms:

Δ⁹-THC. The ¹H NMR spectra of both reaction products show that deuterium is present exclusively in the axial 10β position indicating preferential ²H⁺ addition from the β (top) face of the molecule (Figure 1A). This is probably because attack from the α (bottom) face is sterically hindered by the axial H10a. Furthermore, there is a strong steric interaction between the H10 vinylic proton and the phenolic hydroxyl group as shown in previous NMR studies (14) and X-ray data (18) of similar compounds. This introduces an additional disincentive for an α face ²H⁺ attack. Formation of the α-chloro isomer can be explained by invoking the same bridged H⁺ cation described in the previous example. The chloride anion will then attack from the opposite side leading to a trans diaxial addition product.

![Figure 1A. The Mechanism of HCl Addition to Δ⁹-THC](image-url)
To explain the formation of the β-chloro isomer through a syn addition we can invoke first addition of $H^+$ with the formation of an ion pair with the $Cl^-$ group. Collapse of this ion pair leads to the cis addition product.

$\Delta^8$-THC. Addition of the deuterium cation to the double bond occurs from either the α or β face of the molecule to give the respective bridged cations as intermediates (Figure 1B). Subsequently, the β adduct undergoes chloride attack at the 9-position from the opposite side resulting in the expected trans diaxial addition product. Conversely, the α-bridged cation undergoes chloride attack from the less hindered β side to give a trans diequatorial product. It is also possible to invoke here the formation of an ion pair of the chloride ion from the β face of the molecule with the tertiary carbocation. The proton NMR spectra of the products clearly indicate that no cis addition occurs.

**Figure 1B. The Mechanism of HCl Addition to $\Delta^8$-THC.**

$\Delta^8,11$-THC. As with $\Delta^9$-THC, both products involve $^2$HCl addition for the β side of the molecule (Figure 1C). Formation of the α-chloro isomer would again involve a β-bridged cation followed by chloride attack from the opposite side. On the other hand, the β-chloro product can be explained by invoking the syn addition of HCl to the double bond through the formation of an ion pair.
Preparation of Cannabinoids

Figure 1C. The Mechanism of HCl Addition to Δ⁹,¹¹-THC.

²H labeled Δ⁹- and Δ⁹,¹¹-THCs. As we described earlier (1), these can be obtained through the elimination of HCl from the stereospecifically deuterated 9-Cl-HHC addition products described above using potassium-tert-amylate. Elimination of HCl from 9β-Cl-HHC produced Δ⁹-THC exclusively. Here the bulky base directs itself toward the phenolic hydroxyl, to form a phenoxide anion which can in turn abstract the proximal 10α hydrogen. This results in the formation of Δ⁹-THC through a concerted diequatorial HCl elimination.

Figure 2. The Mechanism of HCl Elimination from 9β-Cl-HHC and 9α-Cl-HHC.

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Elimination of HCl from 9α-Cl-HHC under the same reaction conditions gave Δ9,11-THC exclusively. In this case, the "correct" geometry for a trans diaxial elimination to occur is available in the 9α-Cl-HHC molecule through proton abstraction from a "properly" oriented 11-methyl group. Although the opportunity also exists for a trans diaxial elimination involving the 10 proton, such a reaction does not occur as indicated from the absence of Δ9-THC as an elimination reaction product. Presumably this is because the equatorial 11-methyl group sterically hinders abstraction of the 10β-axial proton by the bulky base. The combined stereospecific 3HCl addition/elimination to the three tetrahydrocannabinol isomers can thus provide us with (-)-Δ9-THC specifically 3H-labeled at the H8β, H10, H11 positions and Δ9,11-THC labeled at the H8α, H10β and H11 positions.

EXPERIMENTAL SECTION

Commercially available reagents were used throughout without further purification, and solvents were dried by standard methods. HPLC was performed on reverse phase column (μ Bondapak, C18) using the instrument and technique described in reference 11.

(-)Δ9-THC, (-)Δ9-THC and (-)Δ9,11-THC were obtained from the National Institute on Drug Abuse (Bethesda, MD).

NMR. 1H NMR spectra were obtained on a home built FT NMR spectrometer operating at 500 MHz in the quadrature detection mode using a variable temperature 1H probe. Samples were prepared as 0.01M solutions in CDCl3 and were fully degassed to remove all oxygen. TMS was used as an internal reference.

1D spectra were obtained using a 4000 Hz spectral width and 8 k points. 1H difference nOe spectra were acquired using sequential acquisition and subsaturating preirradiation (1 sec) before acquisition. Preirradiation was gated off 2 msec before acquisition to eliminate decoupler turn off artefacts. A 10 sec. recycle delay was included after each transient to re-establish spin equilibrium. Eight FID’s were acquired and stored before moving to the next irradiation frequency. This cycle was repeated 100 times to give 800 transients per spectrum. nOe’s were quantitated by measuring the areas of peaks in both perturbed (with nOe) and control spectra. The nOe enhancement is reported as the percentage increase in peak area. 2D COSY spectra using the 90°(φ2)-t1-90°(φ2)-t2 pulse sequence (19,20) at 500 MHz. Spectral width in f1 and f2 was 3000 Hz and recycle delay, 1 sec. at 295 °K. The data were 512 w x 512 w in f1 and f2.
Preparation of 9-Chlorohexahydrocannabinols. Dry HCl or $^2$HCl gas was added through a gas dispersion tube to a solution of $\Delta^8$-THC (200 mg) in 20 ml of CH$_2$Cl$_2$ containing anhydrous ZnCl$_2$ (100 mg) and kept at $-60$ °C. The addition was allowed to proceed under dry conditions with continuous stirring for 2 hr, after which the mixture was quenched with a 10% Na$_2$CO$_3$/H$_2$O solution (10 ml) and allowed to warm to 25 °C. The organic layer was separated, washed with water and dried over anhydrous Na$_2$SO$_4$. Removal of the solvent gave quantitative recovery of a mixture of 9$\alpha$- and 9$\beta$-Cl-HHC as a pale yellow oil. $^1$H NMR spectra (CDCl$_3$) showed a ratio of 3:2 for 9$\alpha$-Cl-HHC and 9$\beta$-Cl-HHC respectively. Addition of HCl or $^2$HCl to $\Delta^8$-THC and $\Delta^{8,11}$-THC proceeded in a similar way and yielded the respective products as described elsewhere. The position and relative stereochemistry of the individual $^2$H-labels in each molecule were obtained by comparing their $^1$H NMR spectra with those of the respective HCl adducts. Changes in the $^1$H NMR spectra of the $^2$H-labeled analogs involved the disappearance of the proton in question as well as the simplified coupling patterns of vicinal protons. The $^1$H NMR spectra also allowed us to estimate the level of $^2$H-isotope ratio which was invariably greater than 90%.

Isomer Separation. 100 mg of the above sample was dissolved in 1 ml of acetonitrile and subjected to HPLC separation. Elution with a ternary solvent system water/acetonitrile/tetrahydrofuran of volume ratio 50/35/15 respectively and at a flow rate of 3 ml/min gave a near baseline separation of the two isomers after 120 min. Eluents representing individual peaks were collected and the organic solvents were removed. The isomers where then extracted from the remaining aqueous phase using methylene chloride. The extract was dried over anhydrous Na$_2$SO$_4$ and evaporated to give the pure 9$\alpha$- Cl-HHC and 9$\beta$-Cl-HHC.

Conversion of 9-Chlorohexahydrocannabinols to Tetrahydrocannabinols. 9$\alpha$-Cl-HHC or 9$\beta$-Cl-HHC (100 mg) was dissolved in dry benzene (3 ml) and cooled to 0 °C under a nitrogen atmosphere. Freshly prepared potassium-tert-amylate (1 ml of a 1 M solution in benzene) was added and the mixture stirred for 30 min. The mixture was then allowed to reach 25 °C, diluted with benzene and neutralized to pH 7 using dry ice. It was then extracted with ether (2 x 5 ml), washed with water and then with aqueous Na$_2$CO$_3$ and evaporated under vacuum to give $\Delta^{8,11}$-THC or $\Delta^8$-THC respectively, as the exclusive reaction products in quantitative yields.
REFERENCES AND NOTES

† Present address: Uniroyal Chemical Company, Inc. Middlebury, CT 06749.
‡ On sabbatical leave from the Department of Pharmaceutical Chemistry, Potchefstroom University for Christian Higher Education, Potchefstroom, South Africa.