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ORIGINAL ARTICLE

Chemical transformation of cannabidiol into psychotropic cannabinoids under acidic reaction conditions: Identification of transformed products by GC–MS

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Abstract

Recently, cannabidiol (CBD), one of the major components of the *Cannabis* species, has been a focus in the cannabis industry due to its various pharmacological effects. Interestingly, CBD can be converted into several psychoactive cannabinoids, such as 9-tetrahydrocannabinol (Δ^9-THC) and its structural isomers, under acidic reaction conditions. In this study, chemical transformation of CBD in ethanol solution was conducted with variation in pH at 2.0, 3.5, and 5.0 by addition of 0.1 M hydrochloric acid (HCl). These resulting solutions were derivatized with trimethylsilyl (TMS) reagent and analyzed using GC/MS-scan mode. Time profiles of CBD degradation and transformation of products were examined according to variations in pH and temperature. Several transformed products produced after the acidic reaction of CBD were identified by matching retention times and mass spectra to authentic standards. Regarding the identification of products without authentic standards, the EI-mass spectra of such cannabinoid-OTMS derivatives were interpreted according to structural class, suggesting mass fragmentation pathways. From the GC/MS data, Δ^9-THC, CBC, and ethoxy-hexahydrocannabinol (HHC) analogs were shown to be major components, and THC isomers (Δ^8- and Δ^{10}-THCs) and 9-hydroxy-HHC were observed as minor components. Using time profile data, the acidity of the reaction solution was an important factor in degradation of CBD. Degradation of CBD and formation of THC rarely occurred at pH 5.0, even at 70 °C with a long process time of 24 h. In contrast, degradation of CBD occurred readily at pH 3.5 and 30 °C over a short process time and was further accelerated by lowering pH, increasing temperature, and lengthening the process time. Based on profile data and identified transformed products, formation pathways from the degradation of CBD under acidic reaction conditions are suggested. Among the transformed products, seven components are known to have psychoactive effects. Thus, industrial CBD manufacturing processes in food and cosmetic products should be carefully controlled. These results will provide important guidelines on the control of manufacturing processes, storage, fermentation processes, and new regulation in industrial applications of CBD.

Keywords: Degradation of cannabidiol, GC–MS, LC–MS/MS, Psychotropic substances, Transformation products

1. Introduction

Recently, many industrial applications of *Cannabis* have been centered around cannabinodiol (CBD), one of the major cannabinoids of *Cannabis*, due to its various pharmacological effects, resulting in many cosmetic products and supplements. There are various types of phytocannabinoids in the *Cannabis* species, and 100 cannabinoids have been disclosed. Among them, the major components are cannabidiol acid (CBDA), CBD, Δ^9-

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tetrahydrocannabinol (Δ^9-THC), and THC acid (THCA). Generally, *Cannabis* species contain psychotropic substances, including Δ^9-THC. Despite the psychoactive effects of Δ^9-THC, the cultivation of *Cannabis* species with low THC content (below 0.3%) is authorized in many countries for the purpose of manufacturing CBD as a therapeutic drug, food constituent, or cosmetic product.

Most previous studies have focused on transformation of acidic cannabinoids in *Cannabis* by thermal conversion of CBDA and THCA to CBD and Δ^9-THC *via* decarboxylation according to variation in temperature and time to find the optimum conditions for complete decarboxylation [1,2]. Recently, the conversion of CBD has been studied in the literature at acidic conditions using H_2SO_4, HCl, and acetic acid [3–5]. The chemical transformation of CBD is mainly triggered by the presence of acids and results in several products. Acids can be used to catalyze the reaction converting CBD into psychotropic transformed substances, such as Δ^9-THC, Δ^8-THC, and hexahydrocannabinols (HHCs) [6]. Other than these major transformed products, hydroxy-CBD derivatives and hydroxy-HHC derivatives are observed under acid treatment of CBD [3,4]. Most of these transformed products are psychotropic substances. Typically, THC isomers, HHC derivatives, and CBN are classified as Schedule 1 according to the Controlled Substance Act [7]. It has been reported that transformed products formed during the processing, degradation, and storage of CBD-based products may have psychotropic effects [8,9]. Therefore, it is necessary to elucidate the transformed products and investigate their formation pathways during chemical reactions pertinent to CBD.

Gas chromatography–mass spectrometry (GC–MS) and liquid chromatography–tandem mass spectrometry (LC–MS/MS) have been most popularly used for identification and quantification of cannabinoids in *Cannabis* species and acid reaction products of CBD [10–12]. LC–MS/MS techniques provide high sensitivity as well as high specificity and are sophisticated analytical tools in the quantification and qualification of cannabinoids using multiple-ion reaction monitoring (MRM) and product ion scanning modes, respectively. However, some cannabinoids often cannot be successfully separated on a conventional reverse-phase column. Especially, isobaric cannabinoids, such as CBD, THC isomers, and CBC, are not easily separated on LC columns [refer Fig. S1-a (https://www.jfda-online.com/cgi/viewcontent.cgi?filename=0&article=3452&context=journal&type=additional)] and produce identical signal in MS measurements, providing ambiguous analytical results. Moreover, it was reported that CBD could be partially transformed into THC using an acidic mobile phase during electrospray ionization (ESI) [13]. Thus, LC–MS/MS techniques seem to be an unappreciated tool in the identification of unpredictable transformed products, such as isobaric and unsuccessfully separable analytes.

GC–MS and GC–MS/MS techniques have several advantages, such as high separation capacity, high sensitivity and selectivity, selected ion monitoring or MRM mode, and access to a mass spectral library database. Especially, GC–MS can provide higher separation capability for similar structural analogs than LC-MS [refer to Fig. S1-b (https://www.jfda-online.com/cgi/viewcontent.cgi?filename=0&article=3452&context=journal&type=additional)] and reproducible spectral fragment patterns for analytes in electron ionization (EI) mode. However, one of the disadvantages of GC-based methods is that acidic cannabinoids can be converted into their corresponding neutral forms through decarboxylation due to the high temperature of the injection port and column oven, producing unreliable qualification and quantitation results [14,15]. Cannabinoids with carboxylic acid and/or hydroxyl groups have poor chromatographic properties, such as low peak intensity and peak tailing, in GC analysis. Trimethylsilylation (TMS) is widely applied to enhance GC chromatographic properties and overcome these analytical problems, as well as to protect against decarboxylation of acidic cannabinoids in the high temperatures of the GC system. In addition, cannabinoid-OTMS derivatives produce specific fragment ions based on their structural similarity with abundant molecular ions (M^+) and $[M\text{-}15]^+$ and structurally characteristic ions, enabling their clear structural elucidation.

In this study, transformation products of CBD and cannabinoids in *Cannabis* were studied under acidic reaction conditions by applying GC–MS combined with TMS derivatization. Their mass spectral fragmentation was interpreted to identify characteristic ions based on structural similarity to further identify transformed products. In addition, the conversion of CBD and cannabinoids in *Cannabis* extract according to variation in pH and temperature was investigated to obtain time profiles of transformed products. Based on time profile data and identification results, plausible product formation pathways are suggested. These results provide important guidelines on the control of manufacturing processes, storage, fermentation processes, and new regulation in industrial applications of CBD.

2. Materials and methods

2.1. Materials

Analytical grade methanol (MeOH), ethanol (EtOH), acetonitrile (ACN), and ethyl acetate (EtOAc) were purchased from J. T. Baker (Phillipsburg, NJ, USA). Deionized water (DW) was obtained using a Milli-Q purification system (Millipore Co., Bedford, MA, USA). A reference standard mixture of 8 neutral cannabinoids (purity $\geq$ 99.0%) [cannabidivarin (CBDV), tetrahydrocannabivarin (THCV), cannabidiol (CBD), cannabinol (CBN), delta-9-tetrahydrocannabinol (Δ^9-THC), delta-8-tetrahydrocannabinol (Δ^8-THC), cannabichromene (CBC), and cannabigerol (CBG)] were purchased from Cerilliant (Round Rock, TX, USA). A standard mixture of 6 acidic cannabinoids (purity $\geq$ 98.5%) [cannabichromenic acid (CBCA), cannabidivarinic acid (CBDVA), cannabidiolic acid (CBDA), cannabigerolic acid (CBGA), tetrahydrocannabivarinic acid (THCVA), and tetrahydrocannabinolic acid-A (THCA-A)] and isotopically labeled internal standards, such as Δ^9-THC-d_3, CBD-d_3, CBDA- d_3, and THCA-d_3 (purity $\geq$ 99.9%), were also purchased from Cerilliant (Round Rock, TX, USA). Analytical-grade formic acid (purity $\geq$ 98%), acetic acid, and hydrochloric acid were purchased from Sigma–Aldrich (St. Louis, MO, USA). The trimethylsilyl (TMS) derivatization reagent *N,O-bis*(-trimethylsilyl)-trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) (purity $\geq$ 99%) was purchased from Sigma–Aldrich (St. Louis, MO, USA) and used in GC–MS analysis. Inflorescence and leaves of *Cannabis* were sonicated for 30 min in a Branson 5510 water bath (Branson Ultrasonics, Danbury, CT, USA). *Cannabis* samples, inflorescence, and leaves of two strains were provided by Nongboomind Company (Seoul, Korea). *Cannabis* samples used in this study were produced by blending *Cannabis sativa*, Cherry Blossom hybrid, and White Widow hybrid. After drying until the weight was reduced by 30%, the inflorescence and leaves were air-dried at room temperature in a dark environment for approximately 72 h. Dried cannabis samples were vacuum-packed and stored in the freezer at −20 °C.

2.2. Isolation of CBD from Cannabis inflorescence

Cannabis samples were air-dried at room temperature in a dark environment. Fifty grams of *Cannabis* were ground and added to 500 mL ethanol, and extraction of cannabinoids was performed by ultra-sonication for 30 min in duplicate. The extract was filtrated with Whatman (Maidstone, UK) filter paper no. 1 before the solvent was evaporated under reduced pressure at 35 °C. Dried residue (7.9 g) was subjected to Sephadex LH-20 column chromatography (35 mm × 500 mm) and was eluted with $CHC1_3$–MeOH based on previous reports [16,17]. Fractions were monitored using TLC silica gel 60 F254 (Merck, Søborg, Denmark). Then, all fractions containing CBD were pooled together and finally purified through silica gel 60 column chromatography (30 mm × 400 mm) using petroleum ether-ethyl acetate to obtain 85 mg of isolated CBD, confirmed by exact mass measurement and matching of retention time and mass spectrum to an authentic standard in both GC/MS and LC-MS/MS analyses. CBD purity was checked in GC/MS scan mode and was approximately 95% [Fig. S2 (https://www.jfda-online.com/cgi/viewcontent.cgi?filename=0&article=3452&context=journal&type=additional)]. The isolated CBD powder was stored in an amber vial at −20 °C until use.

2.3. Sample preparations

Isolated CBD (25 mg) was dissolved in 25 mL of 95% ethanol solvent in a 30 mL test tube. To investigate the effect of pH, we adjusted CBD solution (in ethanol and methanol) to pH 2.0, 3.5, and 5.0 by addition of 0.1 M HCl. The reaction solution contained 1 mg of CBD in 1 mL of 95% ethanol and was incubated at 30, 50, and 70 °C under adjusted pH conditions for 1, 2, 3, 5, 10, 16, and 24 h. After the acidic reaction, 0.1 mL of the reaction solution was collected and evaporated until dry under a nitrogen stream. Regarding GC–MS analysis, the dried sample was derivatized in a 1-mL reaction vial with 50 μL BSTFA and 1% TMCS at 70 °C for 40 min. For LC-MS/MS analysis, we evaporated 50 μL of diluted sample under a nitrogen stream. The resulting solution was re-dissolved in LC mobile phase. The overall analytical procedure for CBD degradation and formation of transformed products is depicted in Fig. 1.

2.4. GC/MS conditions

GC–MS analysis was performed using an Agilent 6890N gas chromatograph combined with an Agilent-5975C mass spectrometer equipped with electron ionization (EI) and a quadrupole analyzer. Separation was performed using an Agilent Technologies DB-5MS column (30 m × 0.25 mm i.d., 0.25 μm film thickness; J&W Scientific, USA). Sample (1 μL) was injected into the injection port, heated at 280 °C in split mode (5:1). As a carrier gas, helium

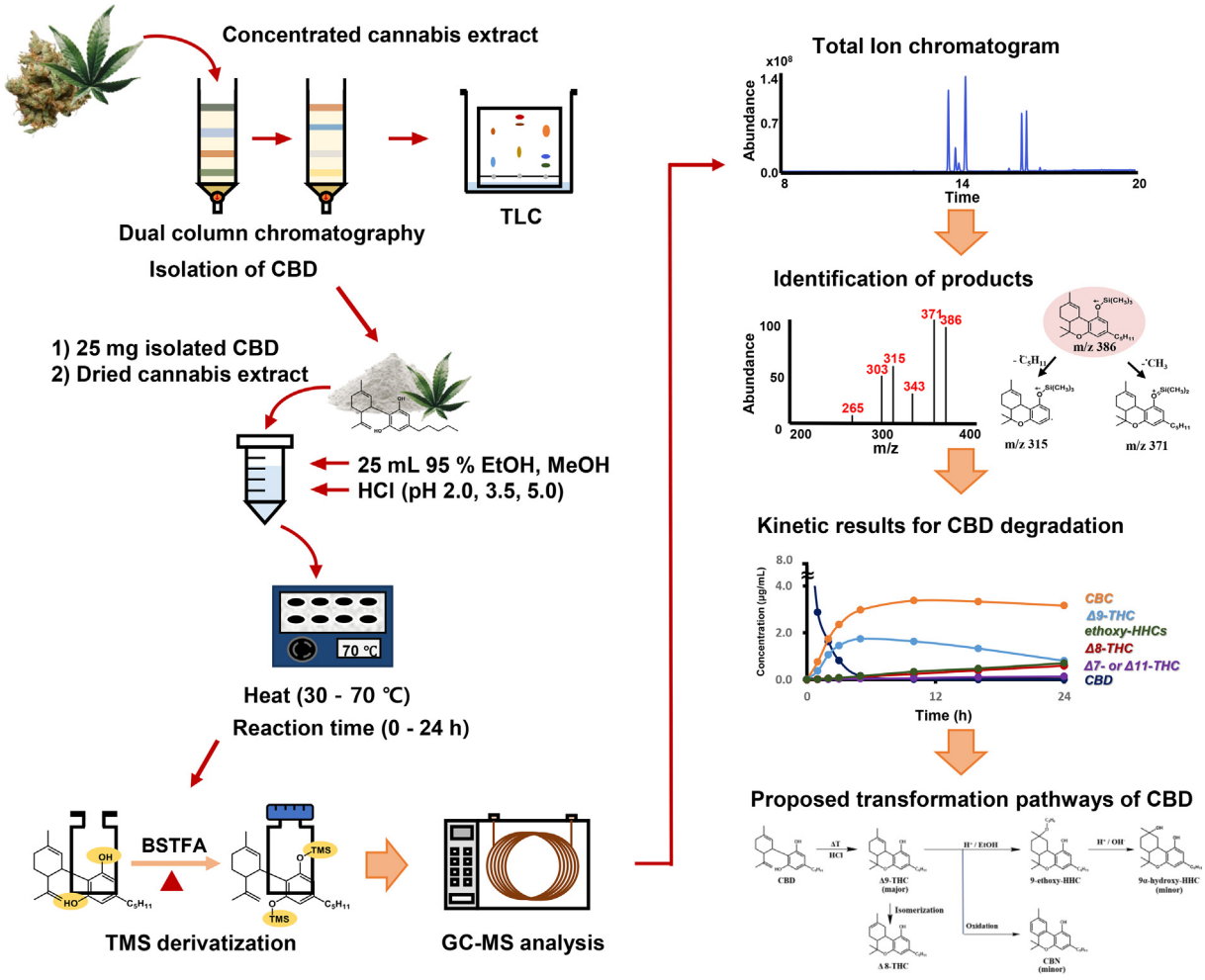


Fig. 1. Overall analytical procedure for transformed products of CBD under acidic conditions.

(purity: 99.999%) was set at a flow rate of 1 mL/min. The oven temperature program was controlled as follows: 150 °C (0.5 min) to 200 °C (1 min) at 20 °C/min, ramped to 240 °C (1 min) at 5 °C/min, and increased to 300 °C (2 min) at 20 °C/min. For characterization of EI mass fragmentation patterns of conversion products of CBD, the mass spectrometer was operated in scan mode (*m/z* 100–650) with electron impact ionization energy at 70 eV and ion source temperature at 230 °C. The transfer line between GC and MS was set at 280 °C.

3. Results and discussion

3.1. Identification of transformed products of CBD and Cannabis extract under acidic reaction conditions

Individual reactant solutions were derivatized with BSTFA and analyzed in GC–MS scan mode to identify the conversion products of CBD and *Cannabis* extract under acidic reaction conditions. Typical total ion chromatograms (TICs) of conversion products of CBD, acid-treated *Cannabis* extract, and untreated *Cannabis* extract are shown in Fig. 2. A total of 8 transformed products were observed after treating CBD in acidic ethanol solution (pH 2.0) at 70 °C for 24 h. The products transformed in CBD reaction solution mainly underwent cyclization, oxidation, cyclization/ring fusion, and ethoxylation of CBD. As shown in Fig. 2-a, CBC (peak 2), Δ^9-THC (peak 5), and 9- and iso 8-ethoxy-HHCs (peaks 8 and 10) were detected as major components. Some of the minor components were Δ^8-THC (peak 3), Δ^{10}-THC (peak 4), CBN (peak 7), and 9-hydroxy (OH)-HHC (peak 11). THC isomers, CBC, and CBN were clearly identified by matching of retention time (RT) and mass spectra of authentic standards. On the other hand, ethoxy HHC isomers and 9-OH-HHC were tentatively identified by GC retention behavior and interpretation of EI-mass spectral patterns due to the lack of authentic standards. These compounds

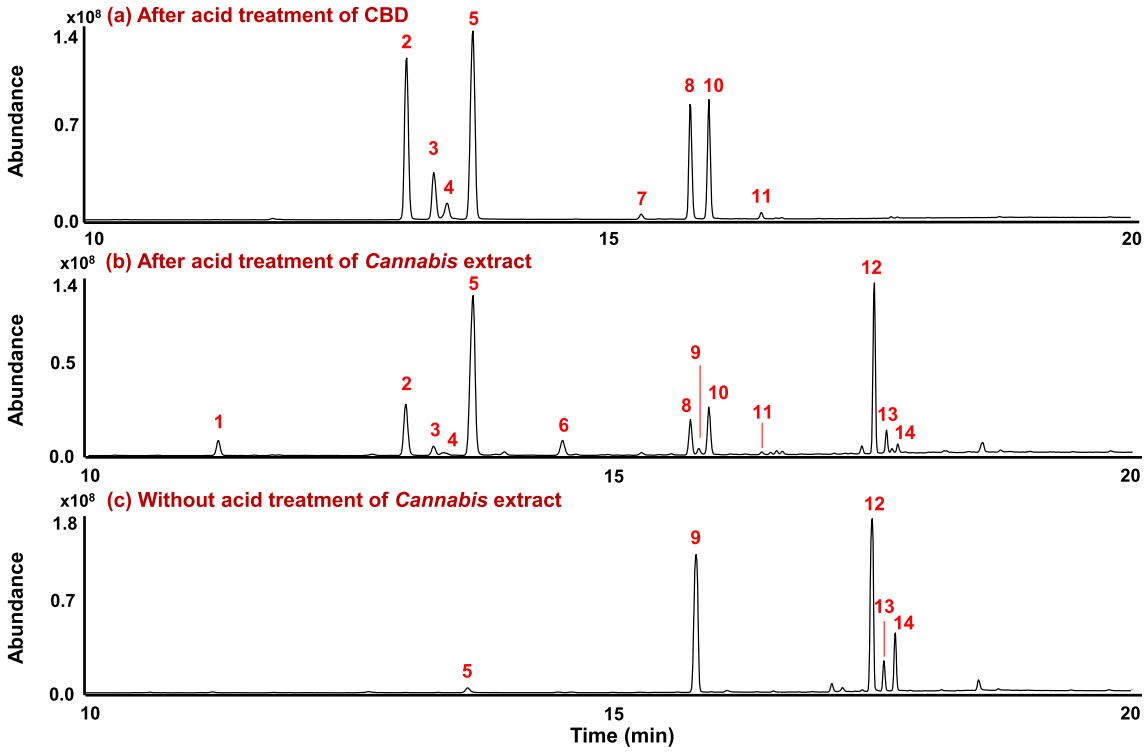


Fig. 2. TICs of transformed products of (a) isolated CBD, (b) Cannabis extract in ethanol at pH 2.0 and 70 °C for 24 h (c) without acid treatment of Cannabis extract. Peak identities as follows: (1) CBD, (2) CBC, (3) Δ^8-THC, (4) Δ^{10}-THC, (5) Δ^9 -THC, (6) CBG, (7) CBN, (8) 9-ethoxy-HHC, (9) CBDA, (10) iso-8-ethoxy-HHC, (11) 9-OH-HHC, (12) THCA, (13) CBCA, (14) CBGA, (15) 8-methoxy-HHC and (16) 9-methoxy-HHC.

were also observed in the literature [3,18]. Ethoxy-HHC isomers were expected to be formed by addition of ethanol to THC isomers during acidic treatment of CBD. Although 9-OH-HHC was detected as a minor component, it was known to be a potent psychoactive substance [6]. Therefore, the occurrence of these compounds should be carefully monitored and controlled during the processing of *Cannabis*-derived products.

To investigate the transformed cannabinoid profiles of *Cannabis* extract, *Cannabis* extracts before and after acid treatment were analyzed and compared with GC/MS [Fig. 2-b and c]. After acid treatment of *Cannabis* extract at pH 2.0 and 70 °C for 24 h, acidic cannabinoids such as CBDA, THCA, CBCA, and CBGA were partly converted into their corresponding neutral cannabinoids CBD, THC, CBC, and CBG through decarboxylation. These compounds were identified by matching retention times and mass spectra to authentic standards. Most of the CBDA in *Cannabis* extract was converted into CBD and transformed to Δ^9-THC, as demonstrated by a significant increase in its amount (peak 5 in Fig. 2-b). CBGA was also readily decarboxylated into CBG, exhibiting a reaction yield of 94%. However, only 19% of THCA in the extract was converted into THC based on the peak area of THCA in the TICs shown in Fig. 2b and c, indicating that decarboxylation of THCA did not readily occur under our experimental conditions. This is consistent with previous study [19] that decarboxylation of THCA occurs at higher temperatures than that of CBDA and does not readily occur at temperatures below 100 °C. CBN was not detected in a TIC of *Cannabis* extract but was observed in acid-treated *Cannabis* extract. This can be explained through conversion of Δ^9-THC into Δ^8-THC through isomerization and subsequent oxidative transformation to CBN by loss of two H_2 molecules in the cyclohexene ring to form a stable aromatic ring [20,21]. Similar to the acidic reaction of CBD, CBC was also observed under acidic treatment of *Cannabis* extract and likely is formed through an acidic reaction with CBD from *Cannabis* extract. Interestingly, CBC was not observed in previous studies, and its formation mechanism will be explained in Sec 3.4.

All EI-mass spectra of the transformed products, neutral cannabinoids, and acidic cannabinoids obtained in this study are presented in Fig. S3 (https://www.jfda-online.com/cgi/viewcontent.cgi?filename=0&article=3452&context=journal&type=additional). The mass spectrum of CBN-OTMS

showed low intense M^+ ion at *m/z* 382, an intense $[M-15]^+$ ion at *m/z* 367, and less fragment ions due to its aromatic structure. Also, characteristic ion at *m/z* 310 was produced by loss of C_5H_{12} molecule of the branched alkane chains from M^+ ions. Several fragment ions below *m/z* 310 might be formed by successive losses of CH_2 or CH_3 radicals at branched chain [Fig. S3-g (https://www.jfda-online.com/cgi/viewcontent.cgi?filename=0&article=3452&context=journal&type=additional)].

For elucidation of tentatively identified ethoxy-HHC isomers, CBD was dissolved in ethanol and methanol, respectively, and reacted for 24 h under acidic conditions. Chemical structures of these HHC analogs are very similar to those of THC isomers, and only the double bond of the cyclohexene ring is replaced with a hydrogen atom and a methoxy or ethoxy group. The presence of methoxy-HHC isomers is an important piece of evidence that the reaction solvent participates in the acidic reaction of CBD. As indicated in Fig. 3, 9- and iso-8-ethoxy-HHC isomers and 9-methoxy-HHC (peak 15) were observed as major compounds under the reaction of ethanol solution and methanol solution, respectively. Their formation mechanisms are likely very similar. Interestingly, mass fragmentation patterns below *m/z* 386 are identical to those of THC, and only M^+ ions are different at increments of 32 Da (*m/z* 418) and 46 Da (*m/z* 432) due to respective addition of CH_3O^-/H^+ or $C_2H_5O^-/H^+$ to THC. These additional groups are easily lost as methanol or ethanol molecules from their corresponding M^+ ions during EI fragmentation to form identical M^+ ion structures of THC isomers. However, mass spectral patterns of ethoxy-HHC isomer (peak 10) showed slightly different mass fragmentations, compared to other HHC analogs. 9-Hydroxy-HHC-OTMS derivative [Fig. 3d] produced $[M\text{-}TMSOH]^+$ ions in the same manner to form *m/z* 386 ions, the M^+ ion structure of THC. Interestingly, Δ^9-THC was observed as a major product in ethanol solution, whereas it was observed at less than 1% in acidic methanol solution.

3.2. Electron ionization mass fragmentation of transformed cannabinoid-TMS derivatives

Most cannabinoids exhibited generally poor chromatographic properties in GC–MS analysis due to their high boiling point and poor detection

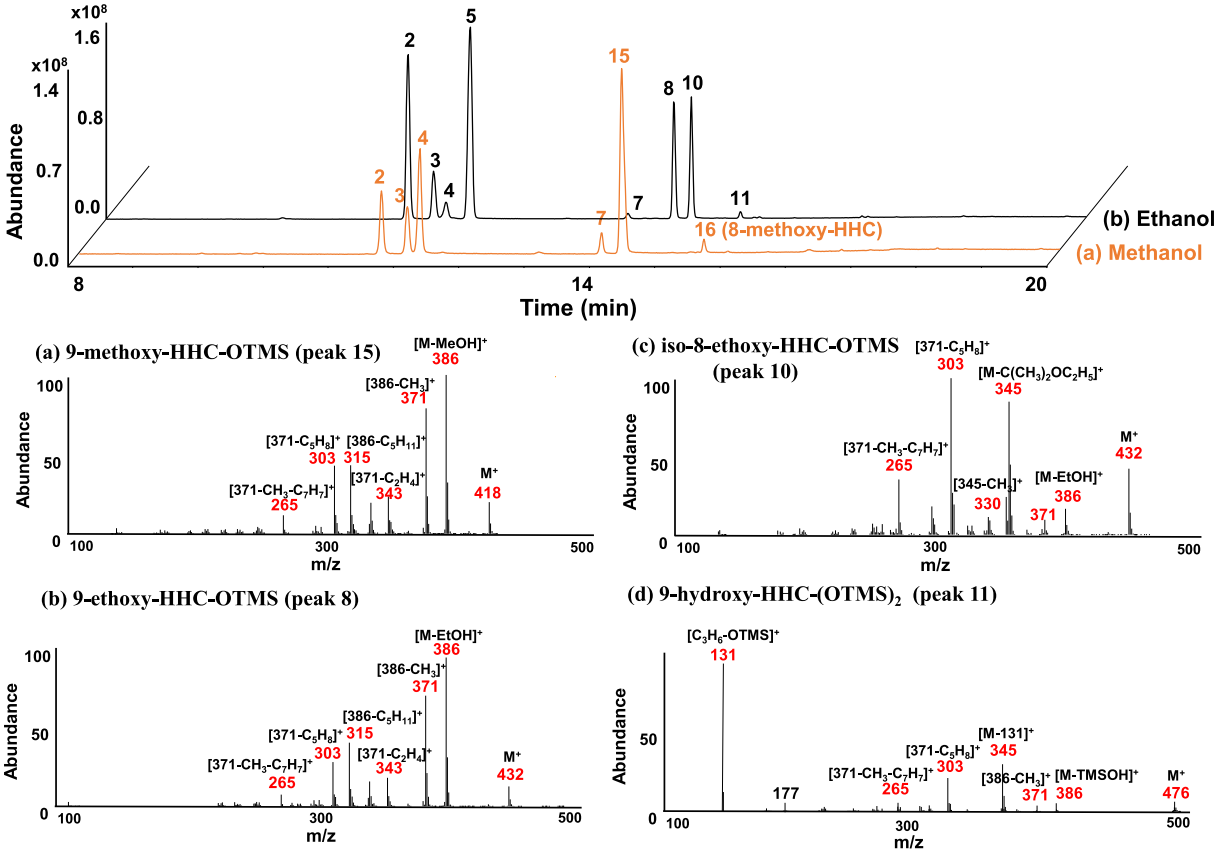
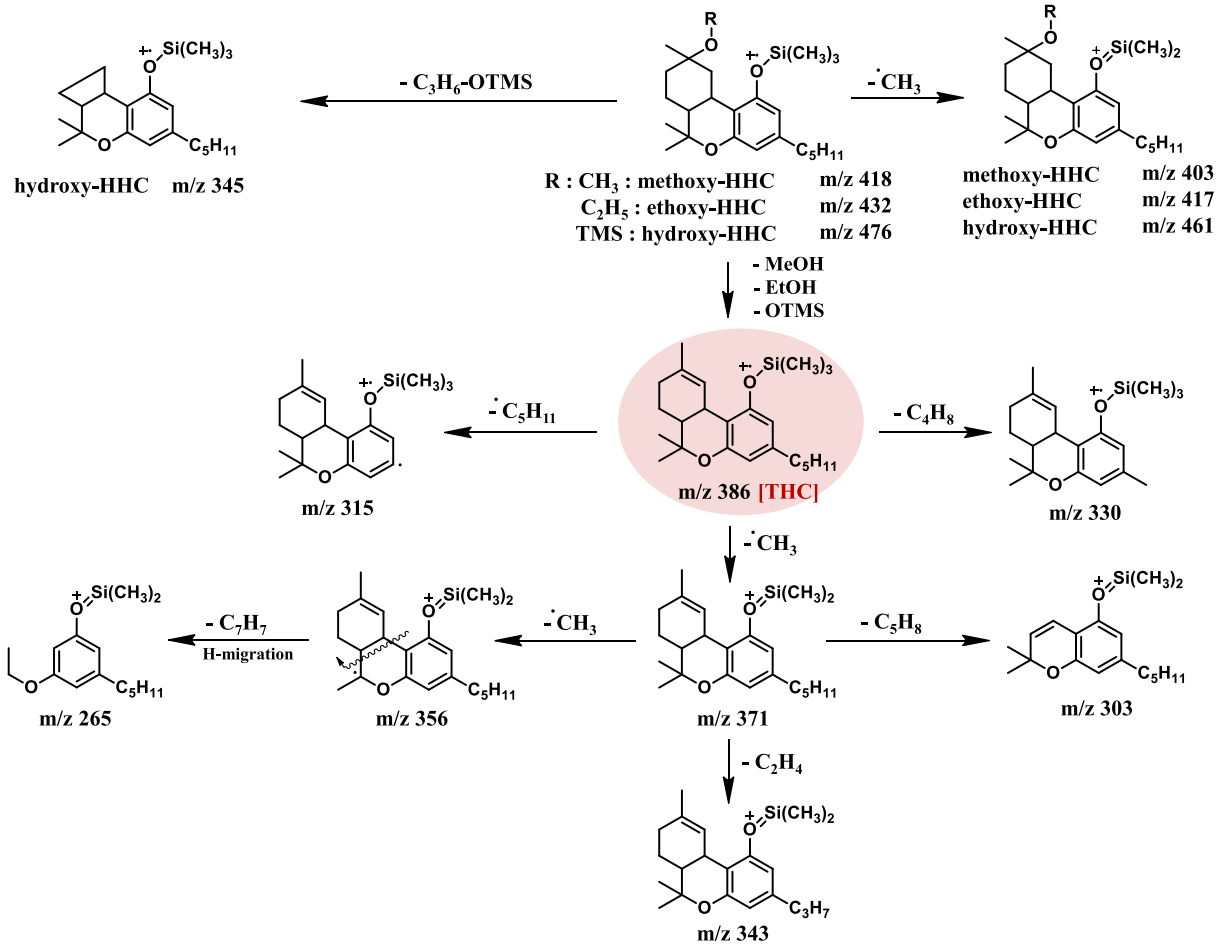


Fig. 3. TICs of transformed products of CBD in (a) ethanol and (b) methanol at pH 2.0 and 70 °C for 24 h. Mass spectra of (a) 8-methoxy-HHC-OTMS, (b & c) 9- & iso-8-ethoxy-HHC-OTMS, and (d) 9-OH-HHC-(OTMS)$_2$.

sensitivity in EI. Decarboxylation of underivatized acid cannabinoids occurred at the high temperatures of the GC system, causing conversion into their corresponding neutral cannabinoids and producing ambiguous qualitative/quantitative results also depicting their transformed products. TMS derivatization of involatile analytes with polar groups could significantly improve detection sensitivity and chromatographic properties [15]. Peak shapes of cannabinoid-OTMS derivatives were sharp, and sensitivity was greatly improved compared to the corresponding free forms. Furthermore, TMS derivatives provided several characteristic ions for identification of polar analytes in EI mass spectra. Typically, the presence of M^+ and $[M-15]^+$ ions with strong intensity could be used to identify the molecular weights, providing the number of active functional groups in the transformed cannabinoids. Polar cannabinoids with hydroxyl and/or carboxyl groups could be easily identified by addition of TMS groups to their mass and the resulting characteristic fragment ions.

EI-mass fragmentations are interpreted in this study to further characterize the transformed products. Most observed transformed products have closely related chemical structures, particularly THC-like structures or CBC/CBN structures. Some of the transformed products, such as THC isomers and CBN, showed intense M^+ ions and/or $[M-15]^+$ ions due to increased structural rigidity compared to that of CBD. CBC-OTMS produced weak M^+ ions at *m/z* 386 and $[M-15]^+$ ions and exclusively showed an intense $[M-83]^+$ ion at *m/z* 303 due to the loss of C_6H_{11} at the branched chain of the pyran ring. Regarding THC-like structures, such as HHC analogs, mass spectra of these transformed products exhibited common fragment ions of THC M^+ ions, as shown in Scheme 1. These specific fragments at *m/z* 343, 330, 315, 303, and 265 can be used as structural diagnostics of THC isomers and HHC analogs. Proposed EI-mass fragmentation pathways of THC-OTMS derivatives and methoxy-, ethoxy-HHC-, and OH-HHC-OTMS derivatives are summarized in Scheme 1. Mass fragmentations of



Scheme 1. EI mass fragmentation pathways of TMS derivatized–THC isomers and HHC derivatives.

iso-8-ethoxy-HHC-OTMS is slightly different from those of other analogs due to different structure and exhibited a specific fragment at *m/z* 345 formed by the loss of $C(CH_3)_2OC_2H_5$ from molecular ion [refer Fig. S3-j (https://www.jfda-online.com/cgi/viewcontent.cgi?filename=0&article=3452&context=journal&type=additional)].

Pertinent to ethoxy-HHC derivatives, their M^+ ions and $[M\text{-}15]^+$ ions were observed, and intense $[M\text{-}C_2H_5OH]^+$ ions were produced to form *m/z* 386 ions, which are as same as the M^+ ions of THC derivatives. Thus, fragment ions of ethoxy-HHC derivatives below *m/z* 386 were the same as those of THC derivatives. In a similar way, methoxy-HHC derivatives also produced intense $[M\text{-}CH_3OH]^+$ ions to form *m/z* 386 and then exhibited similar fragmentation patterns and characteristic ions of THC, such as *m/z* 371, 343, 358, 330, 315, 303, and 265. Especially, a characteristic ion at *m/z* 303 was formed by loss of methylbutadiene (C_5H_8) from *m/z* 371 through ring fusion of methylcyclohexene. Especially, Δ^8-THC and 8-methoxy-HHC TMS derivatives produced the most abundant ion at *m/z* 303 in their mass spectra, due probably to retro-Diels Alder (RDA) dissociation. Also, among THC isomers, Δ^8-THC produced specific fragment ion at *m/z* 318 $[M-C_5H_8]^+$ *via* RDA dissociation, enabling discrimination of other THC isomers. Another characteristic ion at *m/z* 265 might be formed by loss of a stable tropylium radical (C_7H_7) accompanied by hydrogen migration from an *m/z* 358 ion *via* fusion of a pyran ring. As depicted in Fig. 3-a–c, mass fragmentation patterns of 9-ethoxy-HHC were the same as those of 9-methoxy-HHC. However, mass fragmentation patterns of 9-ethoxy-HHC were slightly different from those of iso-8-ethoxy-HHC due to the presence of an abundant characteristic ion at *m/z* 345. Structural diagnostic ion *m/z* 345 of iso-8-ethoxy-HHC might be formed by loss of a stable tertiary $C(CH_3)_2OC_2H_5$ radical from the cyclohexane ring of the M^+ ion.

The base peak of the 9-OH-HHC-$(OTMS)_2$ derivative was observed at *m/z* 131, and the $[C_3H_6\text{-}OTMS]^+$ ion was formed by two bond cleavages of the cyclohexane in the C9-OTMS moiety. Other characteristic ions, such as M^+ ion *m/z* 476 and $[M\text{-}TMSOH]^+$ at *m/z* 386, were observed. Another characteristic ion at *m/z* 345 corresponding to the $[M\text{-}131]^+$ ion was also observed. These two ions were complementary in the mass spectrum of the 9-OH-HHC-$(OTMS)_2$ derivative [Fig. 3-d]. Characteristic ion *m/z* 147, which corresponds to $[(CH_3)_3Si{-}O{=}Si(CH_3)_2]^+$, is diagnostic for derivatives with more than two TMS groups, such as acidic cannabinoids, CBD, and 9-OH-HHC [refer Fig. S3 (https://www.jfda-online.com/cgi/viewcontent.cgi?filename=0&article=3452&context=journal&type=additional)]. Proposed EI-mass fragmentation pathways of THC-OTMS derivatives and methoxy-, ethoxy-HHC-, and hydroxy-HHC-OTMS derivatives are summarized in Scheme 1.

3.3. Time profiles of CBD degradation and THC formation according to variations of pH and temperature

In this study, CBD degradation into psychotropic compounds was examined under acidic conditions. It has been reported that cyclization of CBD readily occurs under acid-catalyzed activation of the double bond in the branched propylene group, which then intra-molecularly reacts with phenol to form a dihydrobenzopyran ring, yielding the psychotropic compound Δ^9-THC [22]. The pH of ethanolic CBD solution was adjusted to 2.0, 3.5, and 5.0 by addition of 0.1 M HCl. Also, the influence of reaction temperature on the formation of psychotropic compounds from CBD under acidic reaction conditions adjusted to 30, 50, and 70 °C were investigated. Time profiles of CBD degradation and THC formation are indicated in Fig. 4. According to previous study on the kinetics of CBD degradation [23], an Arrhenius plot for CBD degradation against 1/T (T: absolute temperature, Kelvin) is depicted in Fig. S4 (https://www.jfda-online.com/cgi/viewcontent.cgi?filename=0&article=3452&context=journal&type=additional). As expected, the kinetic rate constant (k) increased when the reaction temperature increased. Thus, the main factors of CBD degradation are temperature and acidity of the reaction system.

As shown in Fig. 4a and b, degradation of CBD and formation of THC were not greatly affected at pH 5.0 even when the respective reaction temperature and time increased to 70 °C and 24 h. From this observation, CBD degradation is significantly affected by the acid concentration of the reaction solution. In other words, no degradation of CBD occurred in ethanol solution above pH 5. However, the amount of CBD exponentially decreased at pH 3.5 and 2.0, and that of Δ^9-THC was steeply or gradually increased as the reaction time increased to 5 h at pH 2.0 and to 10 h at pH 3.5. Thus, degradation of CBD and formation of THC were more significantly affected by the acidity of the reaction solution than reaction temperature or time. Degradation of CBD rarely occurred below the threshold proton concentration of the reaction solution, pH 5.0. Degradation of CBD was initialized to form a stable tertiary carbocation by acid-catalyzed activation of a double bond and then mainly underwent

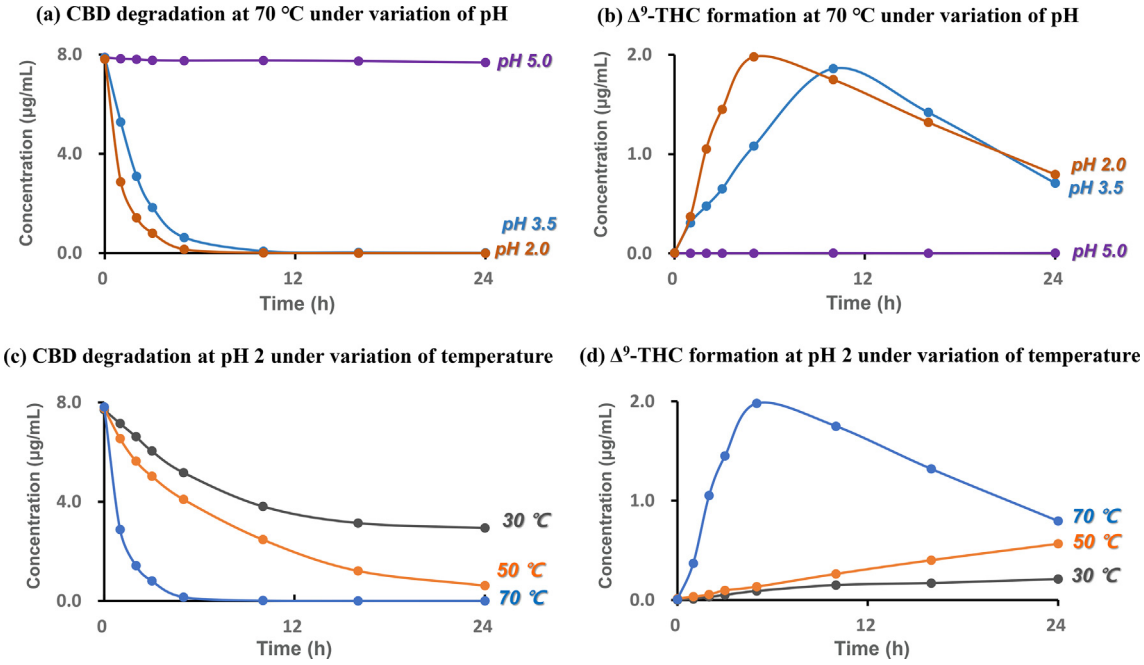


Fig. 4. Time profiles of CBD degradation and THC formation over a range of pH and temperature.

an intra-molecular phenol reaction to yield THC. As the acidity of the reaction solution decreased, the degradation rate of CBD and formation rate of THC exponentially increased in the range of 5–10 h. The formation mechanism of THC from CBD is indicated in Scheme S1-a (https://www.jfda-online.com/cgi/viewcontent.cgi?filename=0&article=3452&context=journal&type=additional). The amount of THC was gradually decreased when the reaction went longer than 5 h at pH 2.0.

Considering a pH 2.0 reaction solution, the influence of temperature on degradation of CBD and formation of THC was examined at 30, 50, and 70 °C. As shown in Fig. 3c and d, degradation of CBD occurred even at 30 °C, and its rate increased as temperature increased. At 70 °C, CBD was degraded by approximately 95% by 5 h, and no CBD was observed when the reaction continued for 10 h. In contrast, the amount of Δ^9-THC linearly increased at 30 °C and 50 °C when the reaction time was 24 h. At 70 °C, the amount of Δ^9-THC increased rapidly up to 5 h and then gradually decreased until 24 h. This indicates that Δ^9-THC starts to be converted into other transformed products after 5 h.

3.4. Formation pathways of transformed products from CBD

Individual peak areas of the transformed products in all TICs were integrated based on CBD time profile data. Semi-quantitation of some transformed products was performed under the assumption that their MS response factors were the same as those of CBD and Δ^9-THC due to the absence of authentic standards. Based on their identification and semi-quantitation results, time profiles of products transformed at pH 2.0 and 70 °C are shown in Fig. 5. The amounts of ethoxy-HHC isomers and Δ^8- and Δ^{10}-THC isomers linearly increased with a slight difference in slope as the reaction time increased to 24 h. These transformed products might be formed through the degradation of Δ^9-THC by addition of ethanol and isomerization. The amount of 9-OH-HHC was relatively small and was not included in the formation profile data. Interestingly, the amount of CBC increased exponentially during 5 h of reaction, but showed no significant increase thereafter. After 10 h of reaction, the amount of CBC showed no significant change until 24 h. CBC is one of the major transformation products, and its formation mechanism is expected to be a complicated serial process in which cyclohexene ring opening initially occurs through formation of allylic and benzylic carbocations, and double bond migration would form allylic and tertiary carbocations, resulting in the formation of a pyran ring. The formation mechanism of CBC from CBD under acidic conditions is depicted in Scheme S1-b (https://www.jfda-online.com/cgi/viewcontent.cgi?filename=0&article=3452&context=journal&type=additional). Moreover, this compound was the first observed under the acidic reaction of CBD.

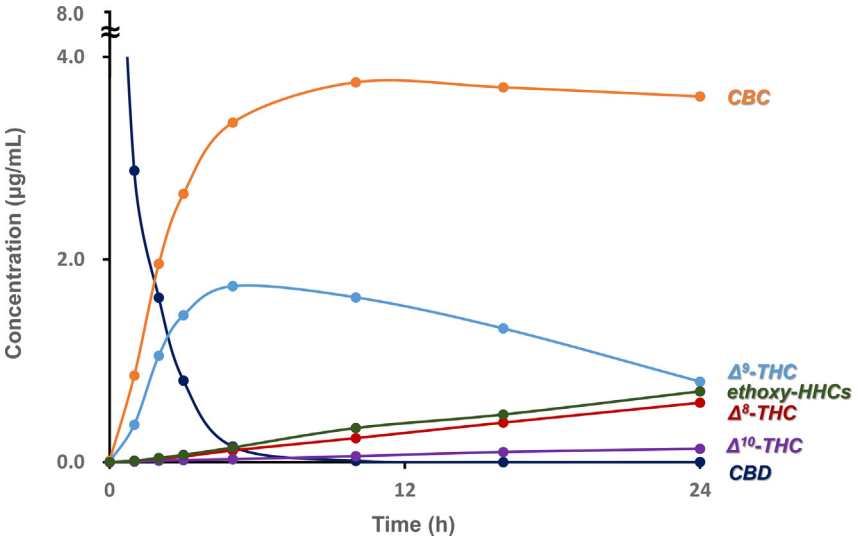


Fig. 5. Time profiles of CBD degradation and formation of transformed products at pH 2.0 and 70 °C.

In this study, kinetic results of CBD degradation and formation of transformed products at pH 2.0 and 70 °C were determined *via* plotting concentration (C) and ln(C) against reaction time range, as illustrated in Fig. S5 (https://www.jfda-online.com/cgi/viewcontent.cgi?filename=0&article=3452&context=journal&type=additional). The linear equations and correlation coefficients were calculated according to the least squares method [24]. Correlation coefficients of transformed products ranged from 0.9211 to 0.9993 within the given reaction time range, indicating good linearity [Table S1 (https://www.jfda-online.com/cgi/viewcontent.cgi?filename=0&article=3452&context=journal&type=additional)]. These formation reactions were characteristic of zero-order kinetics over the time range 0–24 h, except for THC. Regarding CBD degradation, its reaction exhibited first-order kinetics within the reaction time range of 0–5 h.

Based on the identified transformed products and their time profiles, the transformation pathways during acid treatment of CBD are suggested in Fig. 6. Formation of the major product, Δ^9-THC, was explained by initial formation of a tertiary

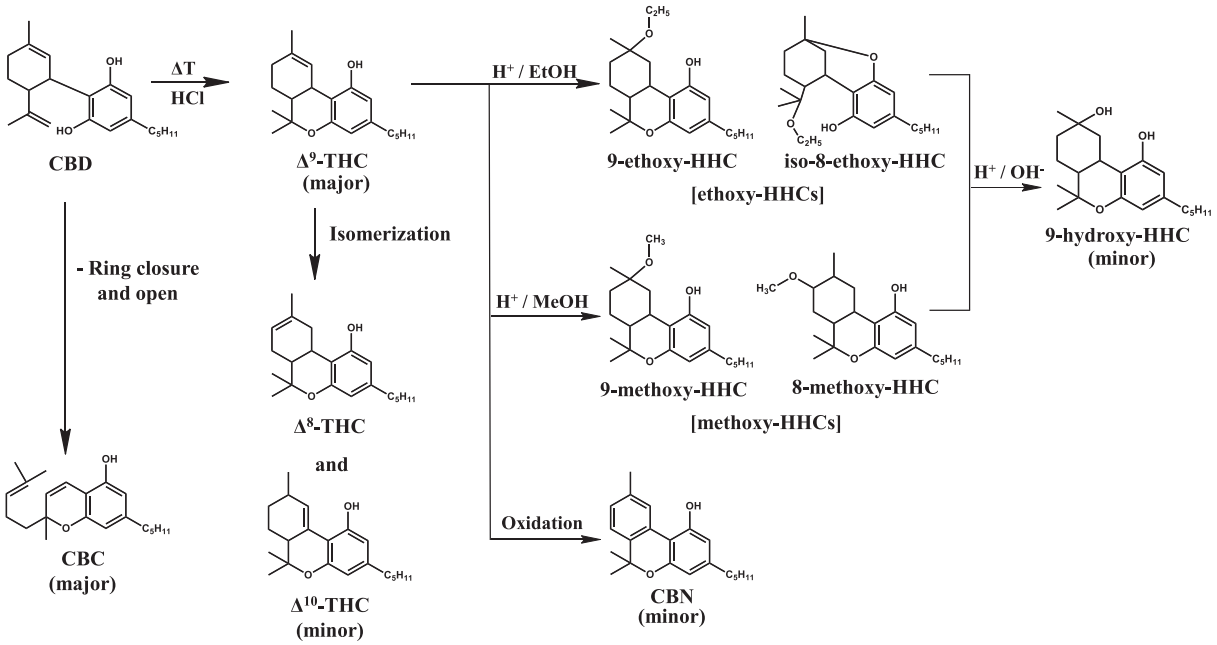


Fig. 6. Proposed pathways of CBD conversion into transformed products under acidic reaction conditions.

carbocation at the ethene group with protonation and then ring closure through intramolecular reaction. It was reported previously that Δ^9-THC could be partly isomerized into Δ^8-THC and other THC isomers [25,26]. From Δ^9-THC, 9- and iso-8-ethoxy-HHCs were formed through protonation at the double bond of the cyclohexene ring to form a stable tertiary carbocation, followed by addition of ethanol and deprotonation. Further reaction at the ethoxy group of ethoxy-HHC with H_2O might lead to formation of 9-OH-HHC. The mechanism of ethoxy-HHC and OH-HHC formation from THC is suggested in Scheme S1-c (https://www.jfda-online.com/cgi/viewcontent.cgi?filename=0&article=3452&context=journal&type=additional). Among the transformed products identified in this study, some substances, such as Δ^9-THC, Δ^8-THC, 9-OH-HHC, and CBN, have been reported to have psychoactive effects [6,7,27]. Ethoxy-HHC derivatives have not been fully studied and are still controversial regarding their psychoactive effects. It is necessary to carefully monitor all transformation products that occur during CBD processing in medical applications. The psychotropic effects of transformed substances formed during the processing and manufacture of CBD in pharmaceutical, cosmetic, and supplement industries need to be carefully conducted.

4. Conclusions

In this study, chemical transformation of CBD under acidic conditions was investigated according to variation in reaction solution pH, reaction temperature, and reaction time. GC/MS combined with TMS derivatization was successfully applied to obtain the mass spectra of the transformed products of CBD and *Cannabis* extract under acidic reaction conditions. Based on mass spectral interpretation, several types of transformed products were clearly identified, and a total of 16 cannabinoids, including 8 suspected psychoactive THC isomers, CBN, methoxy-HHC analogs, and ethoxy-HHC analogs, as well as OH-HHC were observed. In this study, the formation of CBC was observed for the first time in acidic reaction of CBD, and its formation mechanism was suggested. EI-mass fragmentations of these TMS derivatives were plausibly proposed based on structural characteristic ions. EI-mass fragmentations will be useful for identification of related cannabinoids and newly transformed cannabinoids during the storage and fermentation of *Cannabis* to produce various types of dietary supplements, drugs, and cosmetic products.

Based on identification of the transformed products and their time profile data, formation pathways and formation mechanisms under acidic reaction conditions are suggested. Formation pathways and mechanisms of transformed products are pertinent to safety management and quality guidelines in the processing of CBD-based products.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgments

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