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Hydrogen Bonding between Tetrahydrocannabinol and Vitamin E Acetate in Unvaped, Aerosolized, and Condensed Aerosol e-Liquids

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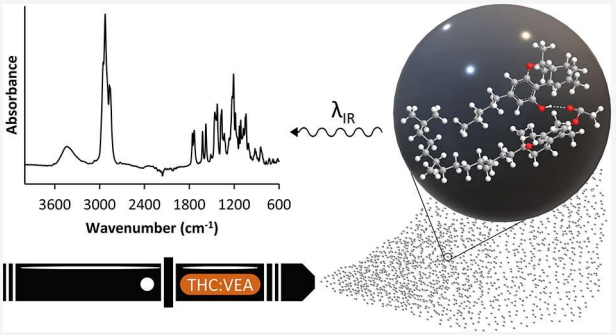
ABSTRACT: Vitamin E acetate (VEA) has recently been identified as a potential chemical of concern in the investigation of e-cigarette, or vaping, product use associated lung injury (EVALI). According to the Centers for Disease Control and Prevention (CDC), most patients report a history of using tetrahydrocannabinol (THC)-containing e-cigarette or vaping products; approximately 50% of the THC-containing vaping products examined by the Food and Drug Administration (FDA) in this investigation have been found to contain VEA. This letter demonstrates that THC and VEA exist in the unvaped e-liquids, vaped e-liquids, and the aerosol produced from vaporizing the mixture, as a hydrogen bonded THC/VEA complex linked by the THC hydroxyl and VEA carbonyl groups. Additional work should therefore be considered to investigate what happens to this complex in the lungs.



As of January 7, 2020, 2 602 cases and 57 deaths related to e-cigarette, or vaping, product use associated lung injury (EVALI) have been reported in the United States by the CDC.[1,2] Clinicians first linked this illness to vaping in September 2019,[3,4] but there has been debate whether these patients suffered from lipoid pneumonia,[5] airway-centered chemical pneumonitis,[6] a combination of both, or something else altogether. However, on November 8, 2019, CDC reported that vitamin E acetate (VEA) was detected in bronchoalveolar lavage (BAL) fluid samples received from 10 states in 29 out of 29 patients with EVALI. From this study they concluded that "these findings provide direct evidence of vitamin E acetate at the primary site of injury within the lungs."[1,2] In a follow-up study, CDC reported that VEA was detected in BAL fluid of 48 out of 51 patients (94%) and 47 out of 50 patients (94%) either had detectable levels of THC or had reported vaping within 90 days of the onset of illness.[7]

VEA has been detected in approximately 50% of the THC-containing vaping liquids analyzed by the FDA's Forensic Chemistry Center (FCC), which is currently examining EVALI-related products for presence of diluents, pesticides, poisons, heavy metals, and toxins.[8,9] A subset of these samples has been found to contain VEA concentrations between 23% and 88%. Although the presence of VEA in THC-containing e-liquids was reported early in the investigation,[10] this study demonstrates that THC and VEA in the vaping mixtures form a hydrogen bonded complex.

EXPERIMENTAL SECTION

Samples. All samples were submitted to the FCC from various sources (hospitals, FDA investigators, state laboratories, etc.), and mixtures were prepared in house. Infrared spectra of THC samples and controls examined in this study were consistent with Δ^9-tetrahydrocannabinol. All mixtures prepared for vaping were heated to 90 °C for at least 30 min to decrease viscosity of the THC and vortexed prior to dispensing approximately 0.9 g of each mixture into empty 510 threaded vaping cartridges (ATMOS ACCESSORIES C5 Ceramic Black Tip 1 mL, locally purchased) using a 3 mL Luer-Lok syringe with a 16G needle (BD Precision Glide Needle, Becton, Dickinson and Company, Franklin Lakes, NJ). A portion of the unvaped contents of the cartridges and in-house mixtures were used as controls.

Vaping Apparatus for Aerosol Production and Collection. A direct method for aerosol collection was adapted from Olmedo et al.[11] Due to the increased viscosity of THC products compared to nicotine-based products, minor modifications were required. The aerosol was generated using a peristaltic pump (drive no. 07522-20 and head no. 77200-62, Cole-Parmer, Vernon Hills, IL), which pulls the aerosol from the 510 threaded vaping device (ATMOS MICRO PAL battery, locally purchased) using a 16 cm piece of Tygon tubing (0.19 in. internal diameter (i.d.), Masterflex L/S 15 E-3606, Cole-Parmer, Vernon Hills, IL). The 16 cm tube was connected to a series of four 1 mL pipet tips (one cut to fit inside the 0.19 in. i.d. tubing and the rest uncut to fit over the

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1/8 in. i.d. tubing), three 6 cm sections of Tygon tubing (R-3603 Tygon, 1/8 in. i.d. tubing, Cole-Parmer, Vernon Hills, IL), and one 2 cm section of 1/8 in. i.d. Tygon tubing placed inside of a 20 mL glass screw top vial (DWK Life Sciences, Millville, NJ). The stopper portion of the screw top cap (PTFL/Butyl Headspace Cap (DWK Life Sciences, Millville, NJ) was removed, and the last pipet tip was inserted in to the vial to allow for ventilation around the edges of the pipet tip.

As with the aerosol collection system described by Olmedo et al.,[11] the puffed aerosol condensed as it flowed through the system of pipets and tubing. However, the thick condensate produced by the THC-based products could not flow through the system to the glass collection vial. Therefore, the material was collected by centrifuging (Eppendorf 5810 centrifuge, Eppendorf, Hauppauge, NY) each portion of the tubing system containing condensed material at 4000 rpm for 2 min into a glass vial. For direct analysis of the aerosol, the system of pipet tips and additional tubing was removed and one piece of the 0.19 in. i.d. tubing was used to carry the aerosol from the vaping device to the instrument. For all vaping experiments, the vaping device was operated at the highest power setting of 3.7 V and the peristaltic pump was programmed to operate at a flow rate of 1.0 L/min and a puff topography of 4 s puffs every 30 s. This topography is similar to that of an average experienced e-cigarette slow user as described by Talih et al.[12]

FT-IR. Infrared spectra were collected on a Nicolet 8700 FT-IR spectrometer (Thermo Scientific, Waltham, MA). For the analysis of condensed-phase samples, the spectrometer was outfitted with a micromATR vision (Czitek, Danbury, CT) attenuated total reflection (ATR) accessory with a diamond internal reflection element (IRE). A microdroplet of each sample was placed on the surface of the IRE, and a spectrum was collected. Spectra were the result of 64 coadditions at 4 cm^{-1} resolution. For the analysis of aerosolized vapors, the infrared spectrometer was outfitted with a 4.5 cm long and 2 cm radius cylindrical Pyrex cell with two 5 mm radius cylindrical transfer lines. Each transfer line was outfitted with a 2 mm straight bore stopcock with a polytetrafluoroethylene stopper plug (Corning, Corning, NY) to regulate the vapor flow. The cell was capped at either end with 4 mm thick and 2.5 cm radius BaF_2 windows; the cell/window was secured together using a Janos Technology Inc. (Keene, NH) gas cell assembly device. This device was then inserted into the spectrometer at which point one transfer line of the cell was connected to the vaping machine and the other transfer line of the cell was connected to the vacuum nozzle inside a fume hood, both using a rubber hose. Prior to analysis, each stopcock was opened and the aerosolized vapor was pumped into the cell. Once the gas cell was full, a spectrum was collected. Due to the rapid condensation rate of the aerosolized vapor, each spectrum only consisted of 1 scan at 4 cm^{-1} resolution.

NMR. Approximately 9 mg/mL solutions were prepared for each sample by mixing with $CDCl_3$ plus 0.1% TMS (CIL, 99.8% D) followed by vortexing. 700 uL of each solution was transferred to a new nuclear magnetic resonance (NMR) tube (Wilmad Economy, Vineland, NJ, USA, 5 mm, 7″, 600 MHz). Samples were analyzed using ^{13}C NMR on a Bruker AVANCE III 500 (500 MHz proton frequency) equipped with a 5 mm probe (Bruker, Billerica, MA, USA). TopSpin version 3.5 pl7 was used for data collection and analysis. All chemical shifts were referenced to TMS.

DART-HRMS. Solutions of samples and standards at concentrations of approximately 500 μg/mL in HPLC-grade CH_3CN (Fisher Scientific, Fair Lawn, NJ) were analyzed by direct analysis in real time ionization coupled to a high-resolution mass spectrometer (DART-HRMS). DIP-it tips (IonSense, Saugus, MA) were dipped into the solutions, allowed to dry, and then analyzed. Mass spectra were acquired on a Thermo Scientific Q Exactive mass spectrometer (Bremen, Germany). This instrument was equipped with a DART SVP (standardized voltage and pressure) ionization source, VAPUR interface, and linear rail, on which was mounted a module capable of holding 12 DIP-it tips (all from IonSense). Spectra were acquired with the hardware operating in positive polarity mode. The DART SVP source was operated with helium gas at a temperature of 250 °C, grid voltage of +300 V, and positioned directly in line with the VAPUR interface inlet at a distance of approximately 8.0 mm, or approximately 4.5 mm from the DIP-it tip during analysis. The mass spectrometer was operated with a nominal resolving power (fwhm at m/z 200) of 140 000 at a nominal rate of 1 Hz, over the range m/z 100−1000, with an automatic gain control (AGC) value of 10^6. MS/MS spectra were acquired using the same parameters with an isolation width of ±0.6 Da and higher energy collisional dissociation (HCD) energy of 30 eV. Analysis of the DIP-it tips was performed by moving the rail at 0.5 mm/s.

Real-time analysis of the aerosolized sample was performed on the same instrument, using appropriate environmental controls and personal protective equipment to minimize inhalation risk, by removing the linear rail and directing the output of the vaping apparatus between the DART source and the VAPUR interface to the HRMS. All parameters were the same as described above, except the nominal resolving power was set to 17 500 at a nominal rate of 10 Hz to ensure acquisition of an adequate number of spectra over the duration of the vaping apparatus puff.

RESULTS

The infrared spectrum of a representative suspect THC/VEA-containing unvaped e-liquid sample is shown in Figure 1 along with those of an unvaped THC/VEA control mixture (~50:50 w/w) prepared in house, a THC control, and a VEA control. As shown in Figure 1a,b, the unvaped suspect THC/VEA and in-house prepared mixture are consistent with each other but exhibit sufficient differences compared to the individual THC and VEA controls, Figure 1c,d, respectively (band assignments for each spectrum are provided in Table S1). Significant differences are noted with dashed lines in Figure 1 and include an additional C═O stretching absorption at 1736 cm^{-1}, an additional C−O stretching absorption at 1228 cm^{-1}, and a shifted OH stretching absorption toward higher wavenumber values at 3449 cm^{-1}. Similarly, Moskala et al. discovered that when polyvinyl acetate (PVA) and polyvinylphenol (PVPh) are mixed together, the OH stretching absorption characteristic of PVPh shifts toward higher wavenumber values and the ester carbonyl absorption characteristic of PVA splits from one peak to two peaks; the amount of OH peak shifting increases with PVA concentration and the intensity of the additional carbonyl absorption increases with PVPh concentration.[13] The authors attributed these observations to intermolecular hydrogen bonding between the PVA carbonyl group and PVPh alcohol group. Based on this information from the literature and the IR data presented here, it is proposed that THC and VEA-

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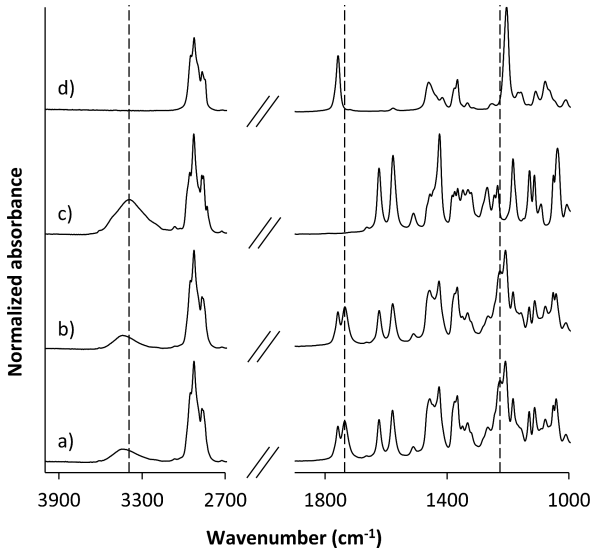

Figure 1. Representative infrared spectra of (a) an unvaped suspect THC and VEA-containing e-liquid, (b) a mixture containing ∼50:50 w/w THC and VEA control prepared in house, (c) a THC control, and (d) a VEA control.

containing suspect e-liquid samples actually contain the hydrogen bonded THC/VEA complex shown in Figure 2. Such hydrogen bonding has been predicted for THC and fatty acid binding proteins in the brain using computational methods[14] and observed directly between the THC hemisuccinate ester carbonyl and a random methylated beta-cyclodextrin hydroxyl group.[15]

While observation of the hydrogen bonding between THC and VEA in the unvaped material is useful for characterizing the suspect products, the examination of the aerosol and condensate generated from vaping this mixture is necessary to understand the potential impact inhalation of such mixtures could have on the end user. IR results demonstrated that the hydrogen bonded complex stayed intact and did not dissociate during or after the vaping process. For example, the same shifted OH stretching absorptions, split ester carbonyl stretching absorptions, and additional C−O stretching absorptions observed with the unvaped liquid (Figure 3a) were also observed with the aerosolized vapor (Figure 3b) and postvape condensate (Figure 3c).

The proposed structure shown in Figure 2 is supported by comparison of the ^{13}C NMR spectra obtained for a THC control, VEA control, THC/VEA mixture prior to being vaped and THC/VEA postvape condensate as shown in Figure 4a−d, respectively. As expected, no carbonyl peak is observed in the THC control spectrum. While the VEA control spectrum exhibited a carbonyl peak at 169.760 ppm, the THC/VEA prevape mixture and THC/VEA postvape condensate spectra

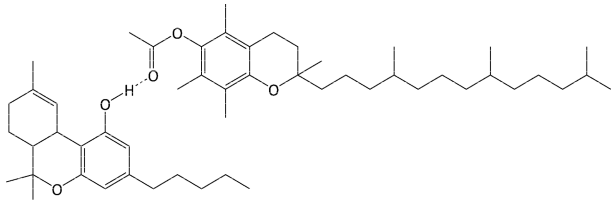

Figure 2. Proposed hydrogen bonded THC/VEA complex found in suspect e-liquids.

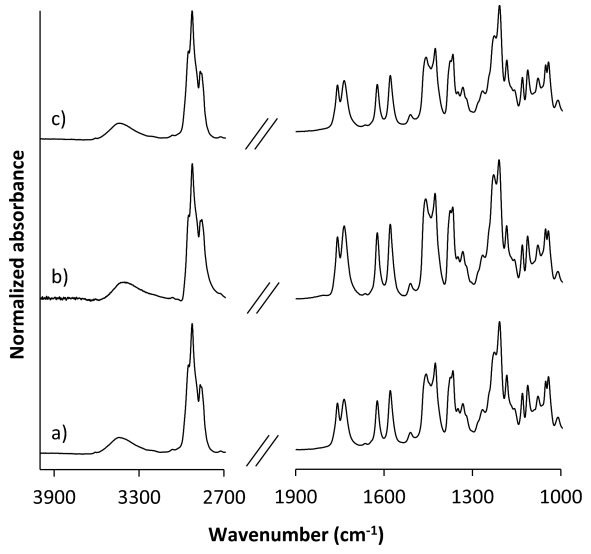

Figure 3. Infrared spectra of (a) an unvaped THC/VEA mixture, (b) an aerosolized THC/VEA mixture following vaping, and (c) a condensate following vaporization of a THC/VEA mixture.

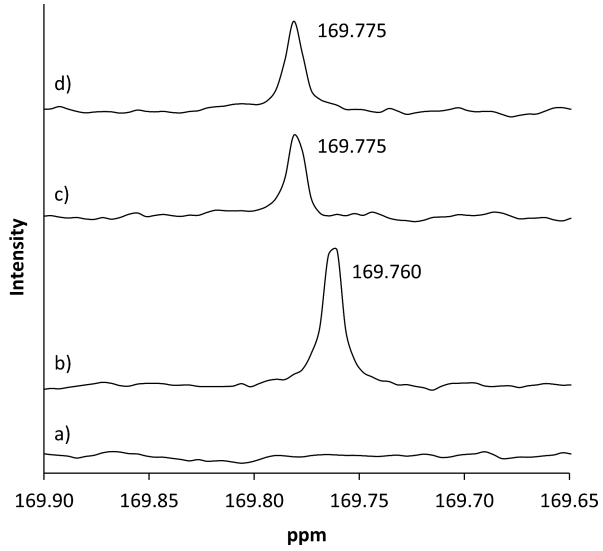

Figure 4. NMR spectra in the ^{13}C carbonyl region for (a) THC control, (b) VEA control, (c) THC/VEA mixture prior to being vaped, and (d) THC/VEA postvape condensate.

both exhibited a carbonyl peak downfield at 169.775 ppm. This shift is the result the carbonyl carbon becoming deshielded from having electron density pulled away. Such a downfield shift has been previously attributed to hydrogen bonding between the PVPh hydroxyl group and PVA ester carbonyl.[16,17]

The structure shown in Figure 2 is also supported by results generated using DART-HRMS. For example, the mass spectrum of a representative suspect THC and VEA-containing e-liquid sample diluted in acetonitrile and sampled via DIP-it tip is shown in Figure 5a, along with those of similarly prepared THC and VEA controls in Figures 5b,c, respectively. The controls exhibit features that may be reasonably expected: a large relative abundance $[THC + H]^+$ peak (Figure 5b) and large relative abundance $[VEA + H]^+$, $[VEA + NH_4]^+$, and $[2VEA + NH_4]^+$ peaks (Figure 5c). NH_4^+ adducts are frequently observed for carbonyl-containing molecules, such

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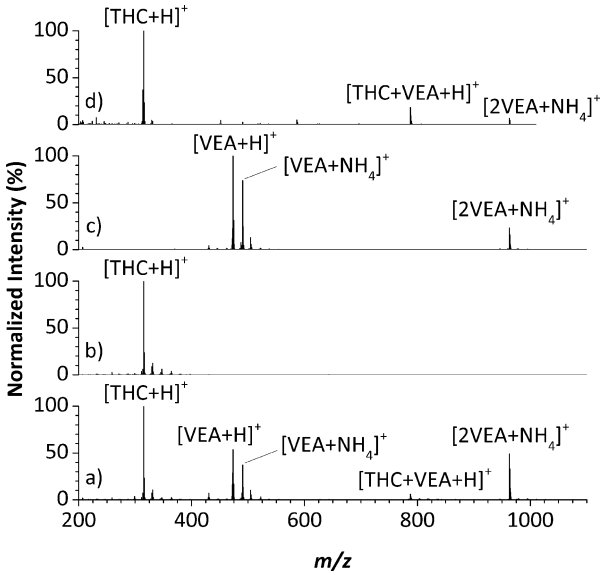

Figure 5. Representative DART mass spectra of (a) a suspect THC and VEA-containing e-liquid, (b) a THC control, (c) a VEA control, and (d) a THC/VEA vapor.

as esters, ionized via DART.[17] The mass spectrum shown in Figure 5a for the sample containing THC and VEA exhibits all of these peaks, plus an additional peak at m/z 787.627 that corresponds to $[THC + VEA + H]^+$. This assignment is based on accurate-mass measurement ($\Delta = -3.8$ ppm) and MS/MS spectra (see Figures S1−S10), which exhibit peaks that correspond to THC and VEA monomers. The presence of this mixed dimer peak with H^+ adduct is noteworthy, particularly when considering that dimers of THC were not observed in considerable abundance while dimers of VEA with an NH_4^+ adduct were. It is expected that the NH_4^+ adduct binds to the carbonyl oxygen atoms of the ester functionality of VEA and VEA dimer.[18] The prevalence of the H^+ adduct of the mixed dimer indicates that the carbonyl ester oxygen of VEA is not accessible for NH_4^+ binding, which would be the case for the proposed hydrogen bonded complex and supports the IR and NMR findings.

DART-HRMS analysis of the aerosolized THC/VEA mixture further demonstrated that the hydrogen bonded complex stayed intact and did not dissociate during the vaping process. Specifically, DART-HRMS spectra of the aerosolized vapor also exhibited a peak for the H^+ adduct of the mixed dimer. Interestingly, the mass spectrum of the aerosolized vapor (Figure 5d) exhibits a significant abundance of the mixed dimer, greater than either the VEA dimer or monomer, the latter of which is not even visible in the spectrum, indicating that a significant amount of the hydrogen bonded complex exists in the aerosolized vapor.

CONCLUSIONS

CDC recently concluded that VEA is associated with EVALI since this compound was detected in 29 out of 29 patients[1,2] and later detected in 48 out of 51 patients[7] diagnosed with the lung illness. The results of these studies have already led to additional research focused on determining adverse health effects associated with vaping VEA.[19,20] These preliminary studies mainly focused on the potential toxins produced by the thermal decomposition of VEA under various conditions. However, as Blount et al. indicated, it is possible that other compounds could act synergistically with VEA to increase the risk of EVALI.[2] While analysis of the effects of vaporizing VEA is beneficial, understanding the interactions between VEA and other constituents commonly found in the vape cartridges in various phases could provide additional information needed to better understand the cause of EVALI. The work presented here demonstrates that unvaped, aerosolized, and condensed aerosol from mixtures of VEA and THC, which have been associated with EVALI, result in the formation of a hydrogen bonded complex between THC and VEA that has not yet been reported. While this work does not directly link the hydrogen bonded complex to EVALI, it demonstrates that the complex is present in the aerosol produced under the vaping conditions used here and would likely be delivered to the lungs in this form. If so, that would put the THC/VEA complex at the primary site of the lung injury. Additional work should therefore be considered, including determining how the complex may impact the formation of toxic pyrolysis byproducts, to determine how the lungs are affected by the complex.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.analchem.9b05536.

Table of infrared band assignments and additional mass spectra (PDF)

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Notes

The contents of this manuscript are the authors' opinions and should not be considered as opinions or policy of the U.S. FDA. The mention of trade names and manufacturers is for technical accuracy and should not be considered as an endorsement of a specific product or manufacturer.
The authors declare no competing financial interest.

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