Synthesis and Spectroscopic Characterization of Cannabinolic Acid

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Abstract

Cannabinoids, the main constituents of the cannabis plant, are being increasingly studied for their medicinal properties. Cannabinolic acid (CBNA; **1**) was synthesized from tetrahydrocannabinolic acid (THCA; **2**), a major constituent of the cannabis plant, by aromatization using selenium dioxide mixed with trimethylsilyl polyphosphate as catalyst in chloroform. Purification was achieved by centrifugal partition chromatography, and the final product had a purity of over 96% by GC analysis. Spectroscopic data on CBNA such as ^{1}H-NMR and IR, and molar extinction coefficients, as well as chromatographic data are presented as useful references for further research on CBNA. The developed method allows production of CBNA on a preparative scale, making it available for further studies on its biological activities as well as use as a reference standard for analytical procedures.

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The cannabis plant (*Cannabis sativa* L., Cannabaceae) is under intense study for its medicinal properties in a variety of illnesses such as multiple sclerosis, Gilles de la Tourette syndrome, chronic pain and wasting syndrome associated with AIDS/HIV and anorexia [1]. Although so far at least 489 compounds have been identified in cannabis [2], most studies focus on the effects of the cannabinoids. As a contribution to the study of the lesser known cannabinoids, we recently published the standardized spectroscopic and chromatographic data of a variety of natural cannabinoids [3]. In that study, data on cannabinolic acid (CBNA; **1**) was incomplete, due to unavailability of a calibrated standard. Compound **1** is formed during storage and aging of plant samples by degradation of tetrahydrocannabinolic acid (THCA; **2**), a major component of cannabis resin [4], [5]. The biological activities of **1** have not been studied in detail, and analytical study is complicated by the fact that the published spectroscopic data are incomplete. Although a full synthesis of the closely related cannabinol (CBN; **3**) has been described [6], the synthesis and/or preparative isolation of CBNA (**1**) has not been reported.

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In this letter, we describe the production of CBNA by dehydrogenation of THCA using selenium dioxide mixed with trimethylsilyl polyphosphate (PPSE) as the catalyst in carbon tetrachloride (see Fig. **1**) [10]. As a minor modification, we found that carbon tetrachloride could be replaced by the less toxic chloroform without effects on the final transformation yield. Finally, the significant amount of 26 mg of purified CBNA was obtained in a single experiment. The final product was highly pure (96% by GC analysis), therefore rendering a quantified CBNA solution suitable for use as reference standard for analytical or biological studies. Under the selected conditions for LC-MS analysis, isolated CBNA was mildly fragmented. Nevertheless, the highest intensity peak was the decarboxylated [M – CO_2] product, indicating the relative instability of the carboxylic group.

Unfortunately, the conversion rate under the applied conditions was only 10%. Reaction products other than the starting material **2** or the desired product **1** were not further identified. However, the described method for dehydrogenation is relatively simple and well described [10], and THCA is easy to obtain in large amounts from cannabis plant materials [7]. Purification can be achieved by centrifugal partition chromatography (CPC), a technique which permits easy up-scaling. Therefore, the described procedure allows the production of analytical grade CBNA on a preparative scale.

Full spectroscopic data for compound **1** (UV, IR, ^{1}H-NMR, MS) are presented as Supporting Information, facilitating studies on the role of CBNA as a component of cannabis products. The spectroscopic and chromatographic data were published in a systematic manner, complementing the data that were earlier obtained on 17 natural cannabinoids [3].

Materials and Methods

Chemicals and solvents: Selenium dioxide (SeO_2, purity > 98%, reagent grade), hexamethyldisiloxane (HMDSO, purity > 98%) and phosphorus pentoxide (P_2O_5, purity > 97%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Organic solvents (analytical or HPLC reagent grade) were purchased from J.T. Baker (Deventer, The Netherlands). Cannabinoid standards for THCA and CBN (purity ≥ 98%) were produced and quantified as previously reported [7], [8].

Synthesis: PPSE was prepared from P_2O_5 and HMDSO [9]. Thus, HMDSO in chloroform (12% v/v) was refluxed for 30 minutes under nitrogen gas, followed by addition of P_2O_5 (50 mg/mL) and additional refluxing for 2 hours. The clear chloroform phase, containing PPSE, was separated from residual solid P_2O_5 and transferred to a reaction vessel. SeO_2 (30 mg/mL final concentration) and THCA (dissolved in chloroform, 50 mg/mL final concentration) were added, giving a molar ratio between SeO_2 and substrate of circa 2 : 1 [10]. The resultant mixture was mildly refluxed for 6 – 8 hours to allow dehydrogenation of THCA. Subsequently, the liquid phase containing the cannabinoids was separated from the solid SeO_2. The liquid phase was evaporated under vacuum and reconstituted in hexane, resulting in precipitation of PPSE. The hexane fraction contained crude CBNA.

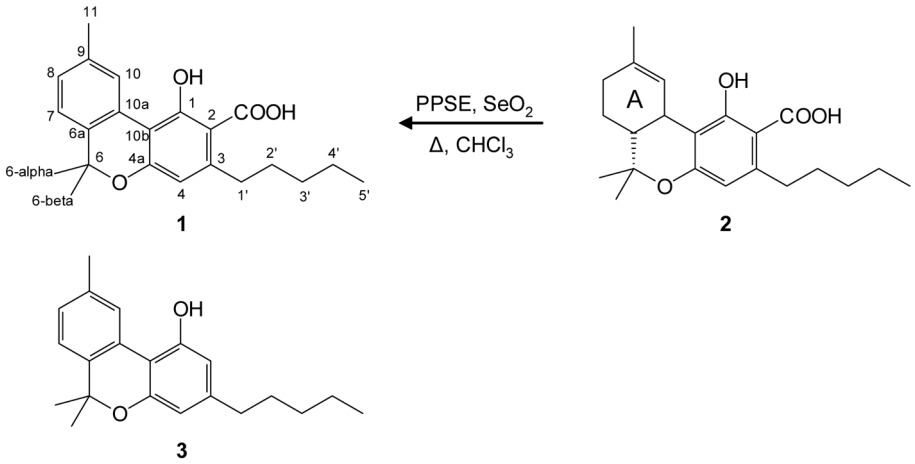

Fig. **1** Chemical structures of cannabinolic acid (**1**), tetrahydrocannabinolic acid (**2**) and cannabinol (**3**). The formation of **1** by dehydrogenation of ring A of **2** is indicated.

Isolation and characterization: Purified CBNA (**1**) (26 mg) was obtained by fractionation of the crude synthesis sample by centrifugal partition chromatography, using hexane/methanol/water, 5 : 3 : 2 (v/v/v) with 0.1% formic acid [7]. The eluent was monitored at the maximal absorption wavelength for CBNA of 261 nm. Fractions containing CBNA were detected by LC-DAD-MS. The purified compound was positively identified by comparing retention times in HPLC and GC [3] and spectroscopic data (HPLC-DAD-MS) to literature data [3], [11], [12] (Figs. **1S** – **3S**, Supporting Information). A quantitative ^{1}H-NMR method was used to prepare a quantified ethanolic solution of CBNA [8]. The purity of isolated **1** was determined by GC analysis at a concentration of 1 mg/mL (5 μL injected). The quantified solution was used to measure the molar extinction coefficients of CBNA in the range of 200 – 400 nm and to measure the infrared (IR) spectrum in FT-IR (Fig. **4 S**, Supporting Information) [3].

LC-DAD-MS analysis: LC-DAD-MS data were obtained with an Agilent 1100 series HPLC system consisting of an auto sampler, low-pressure mixing pump, column oven and DAD detector, connected to an Agilent single-quadrupole mass spectrometer equipped with an Agilent APCI ion probe.

HPLC conditions: Vydac (Hesperia, CA, USA) RP18 column (type 218MS54, 4.6 × 250 mm, 5 μm); Waters Bondapak RP18 (2 × 20 mm, 50 μm) guard column. Solvent system: A = H_2O, 0.1% formic acid, B = MeOH, 0.1% formic acid; 65 – 100% B over 25 min, 100% B for 3 min; flow rate: 1.5 mL/min; injection volume: 10 μL. DAD conditions: 228, 261 nm, and spectra 210 – 400 nm.

APCI-MS conditions: Positive ion mode; scan range: 200 – 400 amu; fragmentor voltage: 100 and 240 V; gas temperature: 350 °C; vaporizer temperature: 400 °C; drying gas (N_2) flow rate: 4 L/min; nebulizer pressure: 45 psig (lb/in^2); capillary voltage: 4000 V; corona current: 4.0 μA.

Nuclear magnetic resonance spectroscopy (^{1}H-NMR): Spectra were recorded in $CDCl_3$ using a Bruker DPX 300 MHz spectrometer. 64 scans were recorded with the following parameters: 32 K datapoints for zero filling, pulse width of 4.0 μs and relaxation delay of 1 second. FID's were Fourier transformed with LB of 0.5 Hz. Peak assignment was done by comparison to the NMR-spectrum of CBN (**3**) [13] (Table **1 S**, Supporting Information). Quantification of isolated CBNA in ethanol solution was performed by quantitative ^{1}H-NMR [8].

Cannabinolic acid (**1**)*:* greenish oil; R_f = 0.25, silica gel 60 F_{254}, MeOH/H_2O/acetic acid (19 : 1 : 0.05); R_f = 0.54, RP-18 F_{254}, $CHCl_3$/MeOH (19 : 1); UV (EtOH): λ_{max} (log ε) = 261 (4.70), 298 (4.30), 324 (4.11); IR (KBr): ν_{max} = 2925, 1620, 1260 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz): δ = 8.40 (1H, s, H-10), 7.11 (2H, dd, J = 12.31, 8.58 Hz, H-7, H-8), 6.40 (1H, s, H-4), 2.96 (2H, t, J = 7.78 Hz, H-1′), 2.38 (3H, s, H-11), 2.15 (2H, m, H-2′), 1.60 (6H, s, H-6α, H-6β), 1.32 (4H, m, H-3′, H-4′), 0.83 (3H, t, J = 6.91 Hz, H-5′); APCI-MS: m/z = 355.2 [M + H$^+$], 337.2 [M – H_2O], 311.2 [M – CO_2].

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