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Structural revision of a Wnt/β-catenin modulator and confirmation of cannabielsoin constitution and configuration†

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In this report, we revise the structure for a previously reported synthetic product proposed to be the 1*R*,2*S*-cannabidiol epoxide and reassign it as cannabielsoin using anisotropic NMR and synthetic chemistry methods. These results provide a direct link to the first known biological target and function of cannabielsoin.

Cannabinoids, phytochemicals isolated primarily from the herbaceous plant *Cannabis sativa*, have recently come to the forefront of many biological and pharmacological investigations due to their ability to interact with different receptors in the body producing a wide range of effects that can impact pain, sleep, mood, memory and other physiological factors.[1] Within this class of more than 112 known secondary metabolites, Δ^9-tetrahydrocannabinol (THC, **1**) and cannabidiol (CBD, **2**), along with their congeners, have attracted the most attention (Fig. 1).[2] Notably, CBD (**2**) has been reported to afford therapeutic effects without the psychoactive properties of THC (**1**).[2] While most of the structures of this class of phytochemicals have been proposed or elucidated since the mid-1970's, surprisingly little is known about the specific biochemical receptors and biological function of many cannabinoid analogs and their biosynthetic intermediates in humans.[1,3] Furthermore, few studies have been conducted to confirm historically proposed chemical structures using modern spectroscopic and synthetic chemistry techniques. In one prominent contemporary example, a report describing the structure of a newly identified CBD analog, anhydrocannabimovone (**3**) was subsequently revised to structure **4** after a total synthesis of cannabimovone required correction of the assigned relative configuration of **3**.[4]

In our efforts to explore the structural characteristics required for modulating the biological activities of various cannabinoids, we noted that Dar, Ali and coworkers had recently communicated the synthesis of 1*R*,2*S*-CBD epoxide (**5**) from CBD (**2**) utilizing Oxone® as an oxidizing reagent (Scheme 1).[5] These researchers also described the potent binding affinity of their oxidized CBD derivative for the Wnt/β-catenin receptor in the context of developing a treatment for neuropathic pain.[5]

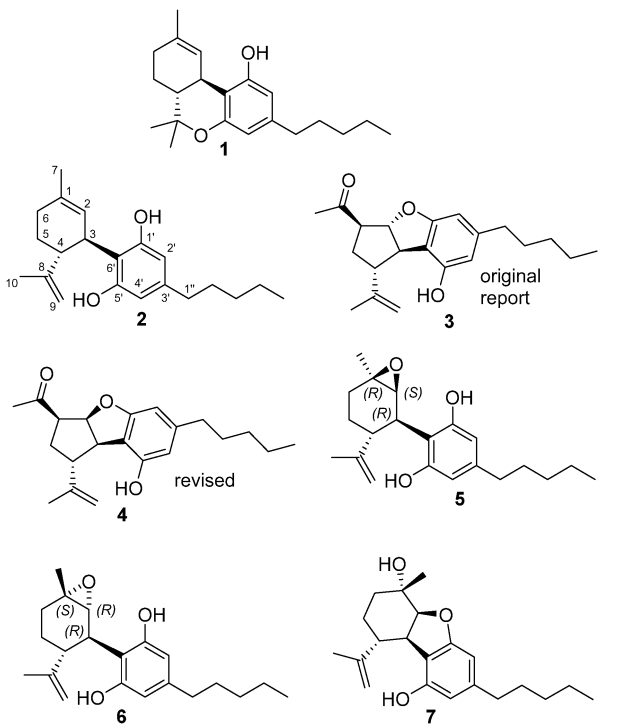

Fig. 1 Structures of THC (**1**), CBD (**2**), original structure reported for anhydrocannabimovone (**3**), the revised structure of anhydrocannabimovone (**4**), and the structures of 1*R*,2*S*-CBD epoxide (**5**), 1*S*,2*R*-CBD epoxide (**6**), and CBE (**7**).

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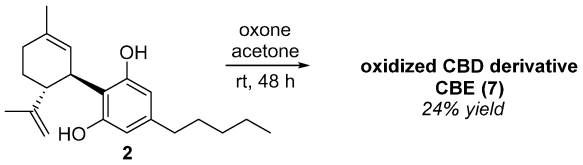


Scheme 1 Attempted synthesis of compound **5**.

Interestingly, when we attempted to reproduce this intriguing discovery, we noted inconsistencies between the method for generating the proposed synthetic product and its spectroscopic data (ESI†). In our hands, the major product matching published spectra was produced in only 24% yield, accompanied by intractable polar material with incomplete conversion after 48 hours (Scheme 1). The original report also noted a "mismatch" of NMR data previously reported for **5** but attributed these discrepancies to prior structural misassignment and formation of the alternate diastereomer (**6**).[6] Most notable in our initial NMR analysis for this CBD derivative was the C2 ^{13}C signal resonating far downfield at 82.3 ppm, an NMR chemical shift totally inconsistent with an epoxide functionality.

Analysis of 1D and 2D NMR data including 1D ^{1}H and ^{13}C, COSY-45, HSQC, HMBC and ROESY experiments suggested that this product was actually the tricyclic structure cannabielsoin (CBE, **7**). Despite no corroborating $^{n}J_{CH}$ HMBC correlations across the cyclic ether ring juncture, comparison with ^{1}H NMR chemical shifts reported independently by Shani and Uliss substantiated this hypothesis.[7] Further assessment of this seminal report indicated that the chirality at C2 and C3 had been conclusively proven by the authors through comparison to a common product formed from both CBE and olivetoyl pinene (ESI†).[7a,b] However, the chirality at C1 was lost through a dehydration step leaving no structural feature to corroborate their assignment of relative configuration at that stereocenter. The C1 assignment was originally made by reference to the deshielding influence of an axial hydroxyl group on an adjacent axial hydrogen.[8] This phenomenon used to assign the chirality at the C1 stereocenter of cannabielsoic acid and by extension, iso-cannabielsoic acid (ESI†).[7b] However, in this case, the chemical shifts and coupling pattern of the key H3 proton were quite similar for the pair of diastereomers (3.38 ppm 5.2, 9.0 Hz) vs (3.30 ppm, 5.2, 10.0 Hz), respectively.[7] Uliss' work supported the proposed structure revisions but cyclization with the alternate epoxide diastereomer (**5**) was not evaluated and the resulting cyclized product (**7**) was not fully characterized by NMR or other spectral techniques.[7c] Additional ambiguity was introduced by the report from Dar and Ali, which proposed stereochemical inversion at C1.[5] Furthermore, their reported ^{1}H NMR chemical shift for H3 was 3.32 ppm with coupling constants of 5.9 and 10.9 Hz, which is closer to those of iso-cannabielsoic acid.[5]

CBE (**7**) has been reported as a plant and mammalian metabolite of CBD and, at the time of this report, has been the topic of 42 peer-reviewed manuscripts and 115 patents with 45 of these issued in 2020 alone (ESI†).[9] As a result of the heightened interest in this captivating cannabinoid, our group

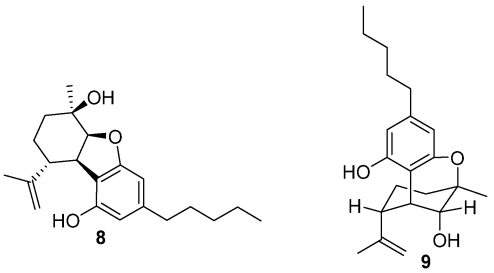


Fig. 2 Structures of CBE with an alternate configuration at C1 (**8**) and a 6-membered cyclic ether (**9**).

initiated an in-depth study to unequivocally define the molecular constitution and configuration of naturally occurring CBE by comparison with its synthetically-derived equivalent. As the first step of this effort, conformational searches were performed using the OPLS3e forcefield as implemented in the Schrödinger MacroModel® software package for the proposed epoxide product (**5**), CBE (**7**), CBE with an alternate configuration at C1 (**8**), and a 6-membered cyclic ether (**9**, Fig. 2).[10] Density functional theory (DFT) methods were then used to generate geometry-optimized conformations and ^{13}C chemical shift calculations were performed with the Gaussian 16 software package at the mPW1PW91/6-311+G(2d,p)//M06-2X-D3/6-31G(d,p) level for all structures identified within 5 kcal mol^{-1} of the global minimum.[10,11] After Boltzmann distribution weighting, these results clearly pointed toward the CBE structure as the best fit with a mean average error (MAE) of 2.2 ppm for the originally proposed CBE configuration and MAE of 2.7 ppm for CBE with an inverted C1 stereocenter (Fig. 3). These results also revealed that the configuration at C1 for these two possible structures (**7**, **8**) could not be differentiated with data from conventional ROESY or NOESY experiments (ESI†).

Calculation of spin–spin coupling constants using the "mixed" selection in the Gaussian software package showed that the expected $^{3}J_{CH}$ coupling constant for H2 to C1′, with an

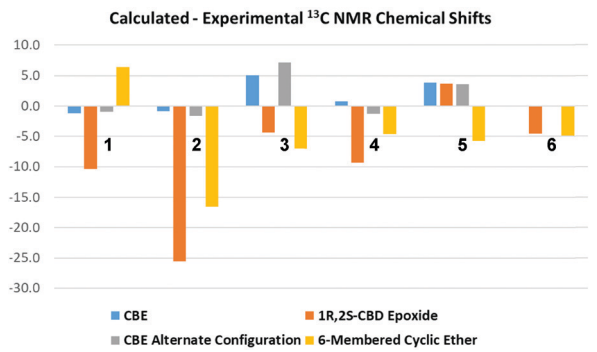


Fig. 3 Differences in calculated *vs.* experimental ^{13}C NMR chemical shifts, for the terpenoid ring carbons in ppm proposed 1*R*,2*S*-CBD epoxide product (**5**), CBE (**7**), CBE with an alternate configuration at C1 (**8**), and the 6-membered cyclic ether analog (**9**). DFT ^{13}C NMR chemical shift calculations were performed at the mPW1PW91/6-311+G(2d,p)//M06-2X-D3/6-31G(d,p) level.

HCOC bond angle of −83°, was −0.2 Hz and the $^4J_{CH}$ coupling constant from H2′ to C2 was calculated to be −0.1 Hz, thus reconciling why neither of these responses were observed in HMBC experiments optimized for 3 Hz or 8 Hz.[10]

Orthogonal spectroscopic confirmation of the originally proposed structure for CBE was accomplished using the recently reported "one-shot" method for the measurement of residual chemical shift anisotropy (RCSA) in poly-γ-benzyl-L-glutamate (PBLG).[12] Since every organic molecule contains carbon, the NMR chemical shift of each carbon can provide information on the overall molecular structure being studied; the ^{13}C RCSA values measured in an aligned vs an isotropic state provide orientational relationships between the chemical shielding tensors of different carbon atoms.[13]

The single or tandem application of anisotropic (RDC, RCSA, and/or residual quadrupolar coupling (RQC)) NMR parameters can be used to provide an unequivocal, investigator bias-independent evaluation of the correctness of molecular constitution and/or relative configuration.[14,15] Besides only requiring a single 1D ^{13}C NMR data set for the anisotropic NMR analysis, these "one-shot" data do not require an external or internal ^{13}C NMR chemical shift reference and thus eliminate systematic errors due to solvent evaporation and/or weighing errors. To perform this experiment, 4.0 mg of CBE was combined with 66.1 mg of PBLG in 600 μL $CDCl_3$ (ESI†). The mixture was homogenized as described by Liu, *et al.* and the resulting 1D ^{13}C data from this analysis are shown in Fig. 4.[16]

These RCSA data were used to perform a singular value decomposition (SVD) analysis for each structure with iterative optimization of Boltzmann populations in the MSPIN software package.[17] The results for the conformational ensembles are shown in Table 1 and details can be found in the ESI.† It is significant to note that when the RCSA-optimized Boltzmann population was used for calculated ^{13}C chemical shift weighting, the overall MAE for CBE (7) improved from 2.2 ppm to 1.8 ppm providing another example demonstrating that anisotropic NMR data can be used to refine Boltzmann populations determined from DFT methods.[12]

Table 1 SVD results for the various conformational ensembles with corresponding *Q*-factor for the proposed 1*R*,2*S*-CBD epoxide product (**5**), CBE (**7**), CBE with an alternate configuration at C1 (**8**), and the 6-membered cyclic ether analog (**9**)

Structure	5	7	8	9
SVD Q_{factor}	0.149	0.068	0.110	0.256

Structural confirmation of CBE encouraged attempts to improve the reaction yield in the Oxone® oxidation. Despite the ambiguity around reaction concentration and solvent quality in the original publication, varying the concentration from 0.1–1.0 M and adding water did not improve the yield of 7. Alternate epoxidation conditions were considered where the Payne epoxidation[6,19] of CBD generated 1*R*,2*S*-CBD epoxide (**5**) in 43% yield (Scheme 2). In order to bring closure to the structural assignment of CBD epoxide compounds, we reproduced the reported epoxidation of **10** with *m*CPBA.[7,20] Epoxide **11** could be isolated in 42% yield from a mixture of products under slightly modified literature conditions. Epoxides **5** and **11** were subjected a battery of 1D and 2D NMR experiments

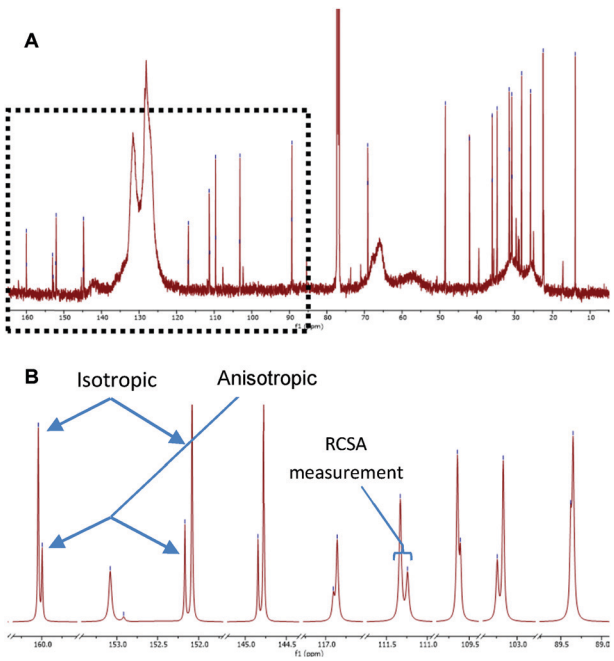
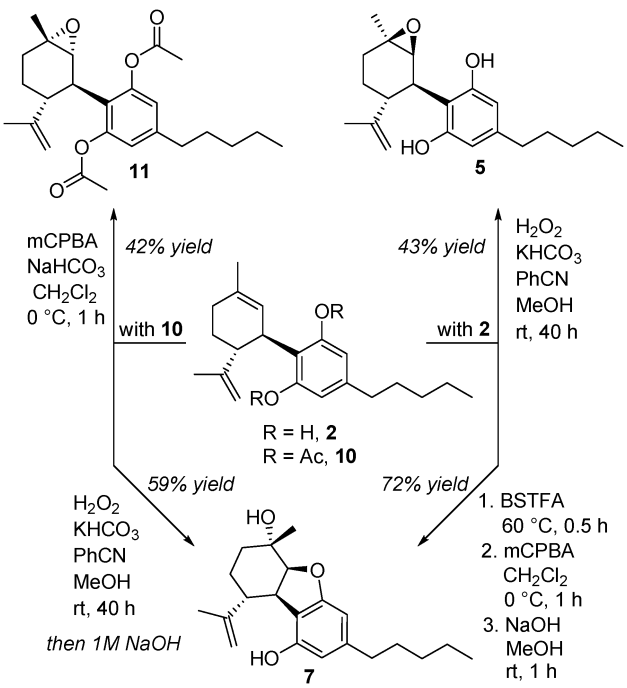


Fig. 4 (A) Native 1D ^{13}C NMR spectrum data (150.9 MHz) for CBE in $CDCl_3$/PBLG. (B) Expansion (boxed region) of 1D ^{13}C NMR data processed with Global Spectral Deconvolution (GSD).[18]



Scheme 2 Synthesis of epoxides **5** and **11** leading to improved synthesis of CBE (**7**).

confirming their structures and adding additional support to the assignment of Shani and Uliss (ESI†).[7] Acetate protection of the CBD alcohols appears to invert the major facial selectivity of the epoxidation, analogous to what is observed for the dimethoxy CBD derivative.[21] Efforts to convert epoxide **5** to a CBE stereoisomer by cyclization under a variety of basic conditions failed. Resistance to cyclization is consistent with a higher energy barrier for equatorial attack of nucleophiles on cyclohexane-derived epoxides.[22]

The Payne epoxidation of CBD diacetate (**10**) leads to a 59% yield of CBE following acetate deprotection. The epoxidation method was reported to generate 1*R*,2*S*-CBD epoxide (**5**);[6] however, in our hands, a mixture of products was formed by TLC prior to complete deacylation. Relying on the data we had collected, a one-pot synthesis of CBE from CBD was attempted with temporary phenol protection. CBD was fully silylated, followed by epoxidation, and final deprotection to furnish CBE in 72% yield.

In summary, we have confirmed the structure of the naturally occurring CBE (**7**) and developed improved conditions for its synthesis. Through alternate epoxidation conditions, 1, 2-CBD epoxide derivatives **5** and **11** were synthesized and fully characterized. This work finally clarifies conflicting structural proposals presented in previous key reports involving CBE and 1,2-CBD epoxide.[5,6,20]

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Conflicts of interest

There are no conflicts to declare.

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