HHS Public Access

Author manuscript

Tetrahedron Lett. Author manuscript; available in PMC 2016 September 30.

Published in final edited form as:

Tetrahedron Lett. 2012 July 11; 53(28): 3560–3562. doi:10.1016/j.tetlet.2012.04.139.

Cannabisol, a novel Δ⁹-THC dimer possessing a unique methylene bridge, isolated from *Cannabis sativa*

Fazila Zulfiqar[a], **Samir A. Ross**[a,b,*], **Desmond Slade**[a,†], **Safwat A. Ahmed**[a,‡], **Mohamed M. Radwan**[a], **Zulfiqar Ali**[a], **Ikhlas A. Khan**[a,b], and **Mahmoud A. ElSohly**[a,c,*]

[a]National Center for Natural Products Research, School of Pharmacy, The University of Mississippi, University MS 38677, USA

[b]Department of Pharmacognosy, School of Pharmacy, The University of Mississippi, University MS 38677, USA

[c]Department of Pharmaceutics, School of Pharmacy, The University of Mississippi, University MS 38677, USA

Abstract

Cannabisol (**1**), a unique dimer of Δ^9-tetrahydrocannabinol (Δ^9-THC) with a methylene bridge, was isolated from *Cannabis sativa*. This is the first example of a C-bridged dimeric cannabinoid. The structure of **1** was unambiguously deduced by HRESIMS, GCMS, and NMR spectroscopy. A plausible biogenesis of **1** is described.

Keywords

Cannabis sativa; Cannabisol; C-bridged dimer

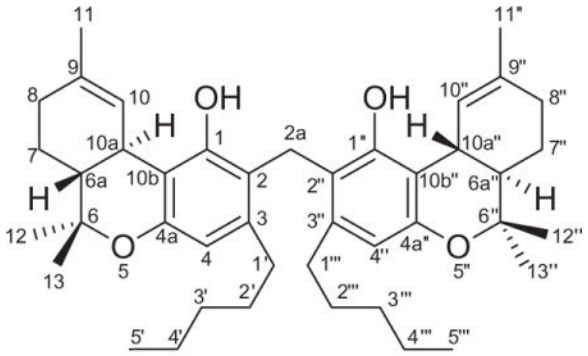
Cannabis, the only genus in the plant family Cannabaceae, consists of only one highly variable species: *Cannabis sativa* L. Other previously reported species, for example *C. indica* Lam. and *C. ruderalis* Janisch, are now recognized as varieties of *C. sativa* L. *C. sativa* L. is not only one of the oldest medicinal plants but also the most widely used illicit drug in the world. More than 535 constituents have been isolated and/or identified from *C. sativa* L.,[1] with Δ^9-THC being recognized as the main biologically active component.[2] The chemotypes of *C. sativa* L. can be divided into drug type (marijuana), intermediate type (the source for hashish), and fiber type (hemp), with the Δ^9-THC content ranging from 1 to 20%, 0.3 to 1.0%, and <0.3%, respectively.[3,4] The availability of high potency marijuana on the illicit market with unprecedented Δ^9-THC concentrations (>20% by dry weight)[5] prompted our phytochemical investigation of this high potency variety. Herein we describe the isolation and structure elucidation of a dimeric cannabinoid, named cannabisol (**1**) from a high potency variety of *C. sativa* L., seized in the USA, as well as its possible biogenetic origin.

*Corresponding authors. sross@olemiss.edu (S.A. Ross), melsohly@olemiss.edu (M.A. ElSohly).
†Present address: MRIGlobal, 425 Volker Boulevard, Kansas City, MO 64110, USA.
‡Present address: Department of Pharmacognosy, Faculty of Pharmacy, Ismailia, Egypt.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2012.04. 139.

Thirty six potency monitoring plant samples with high CBG content (1.4–3.8%) were combined (630 g) and sequentially extracted with hexanes, ethyl acetate, and methanol at room temperature. The extracts were evaporated under reduced pressure to afford hexanes (100.7 g), ethyl acetate (5.4 g), and methanol (16.0 g) extracts. The hexanes extract (100.7 g) was subjected to vacuum liquid chromatography (VLC) on flash silica gel eluted with hexanes, EtOAc, and MeOH gradients. Fractions eluted with hexanes were combined according to their TLC profiles to afford two fractions: F1 (33 g) and F2 (44 g). The extract obtained after fractionation of F1 over flash silica gel (hexanes) and pooling of fractions with R_f-values higher than that of Δ^9-THC according to TLC (hexanes/EtOAc, 9:1), was purified by flash silica gel column chromatography (hexanes/$CHCl_3$, 1:1), yielding **1** (10 mg).



Cannabisol (1)

Cannabisol (**1**),[6] was obtained as a brown solid and displayed a protonated molecular ion $[M+H]^+$ at *m/z* 641.4528 (calcd 641.4570 for $C_{43}H_{60}O_4$ + H) in the positive mode HRESIMS, corresponding to a molecular formula of $C_{43}H_{60}O_4$. The ^{13}C NMR spectrum, however, displayed only 22 resonances, suggesting the possibility of a dimeric structure (Table 1). A relatively low intensity sp^3 methylene resonance at δ_C 24.2 (C-2a) was observed in the DEPT-135 and ^{13}C NMR spectra, and showed HMQC correlation with a proton singlet at δ_H 3.95 (2H, H-2a) in the ^1H NMR spectrum. Rest of the NMR spectroscopic data were close to those of Δ^9-THC[7] except for the resonance of one of the aromatic methine in Δ^9-THC that was replaced with a quaternary carbon in **1**. Due to symmetric nature of the molecule, the NMR data of only one unit are discussed.

The DEPT NMR spectrum resolved the 22 carbon resonances as four methyl, seven methylene, four methine, and seven quaternary carbons. The ^{13}C and ^1H NMR spectra of **1** displayed resonances for three tertiary methyls [δ_C 19.4/δ_H 1.05 (s), C-12; δ_C 27.6/δ_H 1.39 (s), C-13; δ_C 23.5/δ_H 1.60 (s), C-11)], one primary methyl [δ_C 14.2/δ_H 0.88 (t, J = 6.6 Hz), C-5′], one aromatic methine [δ_C 111.6/δ_H 6.35 (s), C-4], and one olefinic methine [δ_C 124.3/δ_H 6.11 (s), C-10]. Furthermore, the resonances for one hydroxyl group [δ_H 5.61 (s)], two aliphatic methines [δ_C 46.1/δ_H 1.66 (m), C-6a; δ_C 34.0/δ_H 3.11 (d, J = 10.8 Hz), C-10a], and seven aliphatic methylenes [δ_C 22.7/δ_H 1.34 (m), C-4′; δ_C 32.0/δ_H 1.34 (m), C-3′; δ_C 30.5/δ_H 1.60 (m), C-2′; δ_C 34.2/δ_H 2.64 (m), C-1′; δ_C 25.2/δ_H 1.89 (m), C-7; δ_C 31.3/δ_H 2.12 (m), C-8; δ_C 24.2/δ_H 3.95 (s), C-2a] were observed. The ^{13}C NMR spectrum, in association with the DEPT-135 spectrum, displayed six aromatic and/or olefinic

quaternary carbons (δ_C 111.1, 112.4, 134.1, 141.0, 154.0, 155.2) and one oxyquaternary aliphatic carbon (δ_C 77.4). Analysis of the ^{1}H–^{1}H COSY, HMQC, and HMBC spectra led to partial structures A–F (Fig. 1).

The following HMBC correlations (Fig. 2) located partial structure A at C-3 [δ_H/C 2.64 (H-1′)/112.4, 141.0, 111.6 (C-2, 3, 4); δ_H/C 1.60 (H-2′)/141.0 (C-3)], B and C at C-6 [δ_H/C 1.66 (H-6a)/77.4, 19.4, 27.6 (C-6, 12, 13); δ_H/C 1.39, 1.05 (H-12, 13)/77.4, 46.1 (C-6, 6a)], B at C-10b [δ_H/C 6.11 (H-10)/111.1 (C-10b)], and D at C-9 [δ_H/C 1.60 (H-11)/31.3, 134.1, 124.3 (C-8, 9, 10]. Further correlations revealed the position of the C-9/C-10 double bond [δ_H/C 1.60 (H-11)/134.5, 124.3 (C-9, 10); δ_H/C 6.11 (H-10)/31.3, 124.3, 23.5 (C-8, 9, 11)], hydroxyl at C-1 [δ_H/C 5.61(OH)/155.2, 112.4, 111.1 (C-1, 2, 10b)], and an aromatic methine at C-4 [δ_H/C 6.35 (H-4)/112.4, 154.0, 111.1, 34.2 (C-2, 4a, 10b, 1′)]. The vicinal methine resonances at δ_H 3.11 (d, J = 10.8 Hz, H-10a) and 1.66 (m, H-6a) displayed a coupling constant of $J_{10a–6a}$ = 10.8 Hz, indicating a *trans*-configuration, and reflecting Δ^9-THC units with a quaternary carbon at C-2 (δ_C 112.4).

The absence of correlations in the ^{1}H–^{1}H COSY spectrum for CH2-2a indicated an isolated moiety (partial structure E in Fig. 1), while the HMBC correlations (Fig. 2) of 2H–2a (δ_H 3.95) with C-1/C-1″ (δ_C 155.2), C-2/C-2″ (δ_C 112.4), and C-3/C-3″ (δ_C 141.0) revealed the position of C-2a bridging two Δ^9-THC units at C-2/2″. The associations of δ_H 3.95 (2H-2a) in the ROESY spectrum (Fig. 3) with δ_H 5.61 (OH/OH″) and δ_H 2.64 (2H-1′/2H-1‴) further confirmed the sandwiched position of methylene C-2a. GC–MS analysis[8] of **1** displayed two chromatographic peaks with m/z 314 and 328 molecular ions $[M]^+$, indicating Δ^9-THC and 2-methyl-Δ^9-THC, respectively.

A plausible biogenetic pathway for the formation of 1 includes initial decarboxylation of acid functionality of Δ^9-THCA followed by nucleophilic attack of resulting anion on the electrophilic carbonyl carbon of the coenzyme A ester of Δ^9-THCA to form 1a, which enzymatically reduced to the methylene bridge between the two Δ^9-THC units (Fig. 4).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The project described was supported in part by Grant No. 5P20RR021929 from the National Center for Research Resources. The project has been funded in part with Federal funds from the National Institute on Drug Abuse, National Institutes of Health, Department of Health and Human Services, under Contract No. N01DA-10-7773. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Center for Research Resources or the National Institutes of Health. We are grateful to Dr. Bharathi Avula for conducting the mass data.

References and notes

1. (a) ElSohly MA, Slade D. Life Sci. 2005; 78:539–548. [PubMed: 16199061] (b) Radwan M, ElSohly MA, Slade D, Ahmed SA, Khan IA, Ross SA. J Nat Prod. 2009; 72(5):906–911. [PubMed: 19344127] (c) Pollastro F, Orazio T, Allara M, Munoz E, Di Marzo V, De Petrocellis L, Appendino G. J Nat Prod. 2011; 74(9):2019–2022. [PubMed: 21902175]
2. Williamson EM, Evans FJ. Drugs. 2000; 60:1303–1314. [PubMed: 11152013]

3. Clarke, RC.; Watson, DP. In Cannabis and Cannabinoids: Pharmacology, Toxicology, and Therapeutic Potential. Grotenhermen, F.; Russo, E., editors. The Haworth Press, Inc.; Binghamton, New York: 2002. p. 3-14.Chapter 1-Botany of Natural Cannabis Medicines

4. Clarke, RC.; Watson, DP. In Marijuana and the Cannabinoids. ElSohly, MA., editor. Humana Press Inc; Totowa, New Jersey: 2007. p. 1-16.Chapter 1-Cannabis and Natural Cannabis Medicines

5. ElSohly MA, Ross SA, Mehmedic Z, Arafat R, Yi B, Banahan BF. J Forensic Sci. 2000; 45:24–30. [PubMed: 10641915]

6. Cannabisol (1). Brown powder; $[\alpha]_D^{27} - 51.5$ (c 4.4, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 284 (3.90); NMR: see Table 1; ESIMS *m/z* 641 $[M+H]^+$, 663 $[M+Na]^+$, 679 $[M+K]^+$; HRESIMS *m/z* 641.4528 $[M+H]^+$ (calcd 641.4570), 663.4371 $[M+Na]^+$ (calcd 663.4389), 679.4009 $[M+K]^+$ (calcd 679.4129).

7. Ahmed SA, Ross SA, Slade D, Radwan MM, Zulfiqar F, Matsumoto RR, Xu Y-T, Viard E, Speth RC, Karamyan VT, ElSohly MA. J Nat Prod. 2008; 71:1119. Erratum to article in J. Nat. Prod., 2008, 71, 536–542.

8. GC–MS analysis was carried out on a ThermoQuest Trace 2000 GC, equipped with a single split/splitless capillary injector, a ThermoQuest AS2000 autosampler, and a Phenomenex ZB-5 column (30 m × 0.25 mm × 0.25 μm), interfaced to a ThermoQuest–Finnigan Trace MS quadrupole detector. The injector temperature was 250 °C, and 1 μL injection was performed in splitless mode, with the splitless time set at 60 s, the split flow set at 50 mL/min, and the septum purge valve set to close for 60 s after the injection occurred. The oven temperature was raised from 70 to 270 °C (hold 20 min) at a rate of 5 °C/min, for a total run time of 60 min; the transfer line temperature was 250 °C. Helium was used as the carrier gas at a constant pressure of 20 psi. The mass spectrometer was operated in the electron impact mode (EI^+) and scanned from 40 to 800 amu at 1 scan/s, with an ionizing voltage of 70 eV and an emission current of 350 μA. Data were recorded using an IBM Netfinity 3000 workstation with Microsoft Windows NT 4.0 operating system (Build 1381, Service pack 6) and Xcalibur data acquisition and analysis software (Version 1.2).

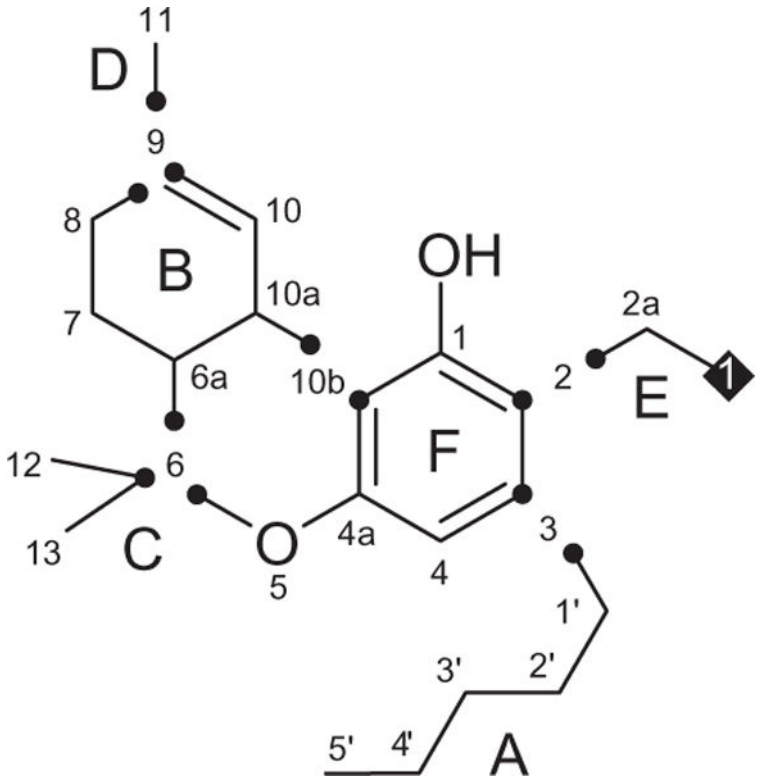


Figure 1.
Partial structures of **1**.

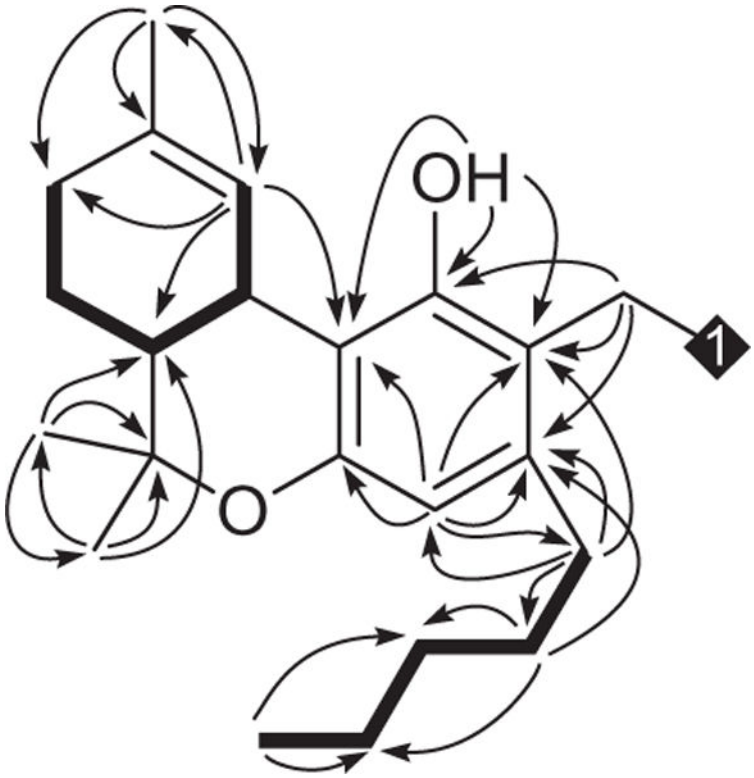


Figure 2.
HMBC (→) and COSY (–) correlations of **1**.

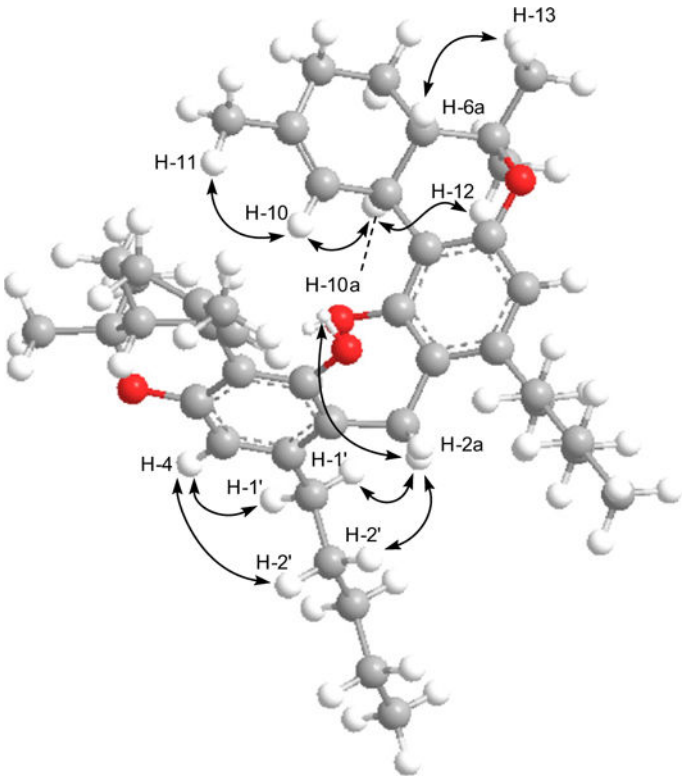


Figure 3.
Computer generated lowest energy three-dimensional diagram and selected ROESY correlations of **1**.

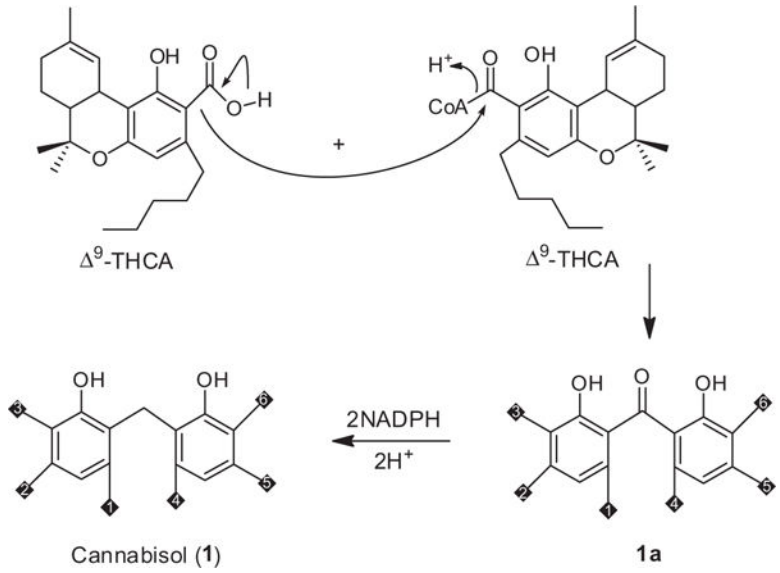


Figure 4.
Proposed biogenetic pathway for **1**.

Table 1

^{1}H and ^{13}C NMR spectroscopic data for cannabisol (**1**) (400 MHz, δ in ppm, J in Hz, $CDCl_3$)

Carbon	δ_C	δ_H (mult, J)
1/1″	155.2	–
2/2″	112.4	–
3/3″	141.0	–
4/4″	111.6	6.35 (s)
4a/4a″	154.0	–
6/6″	77.4	–
6a/6a″	46.1	1.66 (m)
7/7″	25.2	1.89 (m)
8/8″	31.3	2.12 (m)
9/9″	134.1	–
10/10″	124.3	6.11 (s)
10a/10a″	34.0	3.11 (d, 10.8)
10b/10b″	111.1	–
11/11″	23.5	1.60 (s)
12/12″	19.4	1.05 (s)
13/13″	27.6	1.39 (s)
1′/1″	34.2	2.64 (m)
2′/2″	30.5	1.66 (m)
3′/3″	32.0	1.34 (m)
4′/4″	22.7	1.34 (m)
5′/5″	14.2	0.88 (t, 6.6)
2a	24.2	3.95 (s)
OH	–	5.61 (s)