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Isolation and Characterization of New Cannabis Constituents from a High Potency Variety

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Abstract

Phytochemical investigation of a high potency variety of *Cannabis sativa* L. resulted in the isolation of six new metabolites, (±)-6,7-*trans*-epoxycannabigerolic acid (**2**), (±)-6,7-*cis*-epoxycannabigerolic acid (**3**), (±)-6,7-*cis*-epoxycannabigerol (**4**), (±)-6,7-*trans*-epoxycannabigerol (**5**), 5′-methyl-4-pentylbiphenyl-2,2′,6-triol (**7**), and 7-methoxycannabispirone (**8**), along with seven known compounds namely, cannabigerolic acid (**1**), 5′-methoxycannabigerolic acid (**6**), cannabispirone (**9**), *β*-cannabispiranol (**10**), dehydrocannabifuran (**11**), cannflavin B (**12**) and cannabigerol (**13**). The antimicrobial as well as the antileishmanial activities were investigated.

Keywords

Cannabis sativa; Cannabaceae; epoxy cannabigerolic acid; cannabispirone; antimicrobial activity; antileishmanial activity

Introduction

Cannabis sativa L. (Cannabaceae), one of the oldest plants known in medicine, is the most widely used illicit drug in the world today. A total of almost 500 natural constituents have been isolated and/or identified from Cannabis [1], with Δ^9-THC as the main biologically active component [2]. The availability of high potency marijuana on the illicit market with unprecedented Δ^9-THC concentrations (> 20% by dry weight) [3] has renewed our interest in the discovery of new constituents from cannabis. We herein report the isolation and structure elucidation of six new metabolites (**2**, **3**, **4**, **5**, **7** and **8**) and seven known compounds (**1**, **6** and **9–13**). This is the first report of the full NMR data for **1**, **6** and **11**. The antimicrobial and antileishmanial activities of the isolates are also reported.

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Materials and Methods

General experimental procedures

^{1}H-NMR (400 MHz), ^{13}C-NMR (100 MHz) and 2D-NMR spectra were recorded using the residual solvent signal as an internal standard on a Varian AS 400. IR spectra were measured on a Bruker Tensor 27. UV spectra were obtained on a Varian Cary 50 Bio UV-Visible spectrophotometer. Optical rotation was measured on an Autoplot IV automatic polarimeter. High resolution mass spectra were measured using a Bruker BioApex. HPLC was performed on a Waters Delta Prep 4000 Preparative Chromatography System connected to a Waters 486 Tunable Absorbance detector (206 nm) using a Phenomenex Luna C18 column (250×21.2 mm, 5 μm, 100 Å). Flash silica gel (J.T. Baker, 40–63 μm, 60 Å), C18 silica gel (Fluka, 40–63 μm, 60 Å) and Sephadex LH 20 (Fluka) were used for column chromatography. GC-MS analyses were 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 ion trap detector. The injector temperature was 250 °C and 1 μL injections were performed in the 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 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 350μA. Data was recorded using an IBM Netfinity 3000 workstation with Microsoft Windows NT 4.0 operating system and Xcalibur (Version 1.2) data acquisition and analysis software.

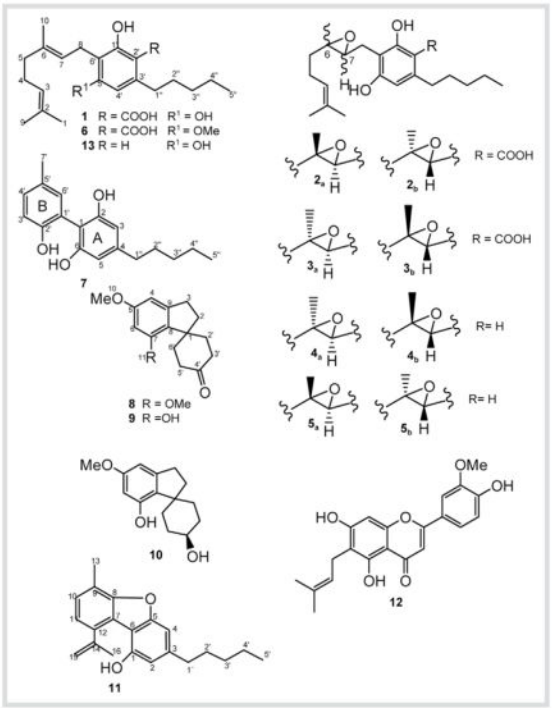
Plant material

C. sativa plants were grown from high potency Mexican seeds (variety code CHPF-01). The seeds and plants were authenticated by Dr. Suman Chandra, The University of Mississippi, and the specimen (S1310V1) was deposited at the Coy Waller Complex, The University of Mississippi. Whole buds of mature female plants were harvested, air-dried, packed in barrels (# 1196) and stored at low temperature (−24 °C). The THC, CBG and CBD contents in the plant material determined by GC/FID analysis were 9.89 %, 0.42% and 0.25 %, respectively.

Extraction and isolation

The plant material (9.0 kg) was sequentially extracted with hexanes (2×60 L), CH_2Cl_2 (2×24 L), EtOAc (2×20 L), EtOH (2×20 L), EtOH/H_2O (36 L, 1: 1) and H_2O (40 L) at room temperature. The extracts were evaporated under reduced pressure at 40 °C to afford hexanes (1.48 kg), CH_2Cl_2 (0.15 kg), EtOAc (0.13 kg), EtOH (0.09 kg), EtOH/H_2O (0.77 kg) and H_2O (0.54 kg) extracts for a total extract of 3.16 kg (35.1%, w/w). A portion of the hexanes extract (40 g) was chromatographed on flash silica gel (1.2 kg, 10×50 cm) eluting with *n*-hexane. Fractions with *Rf* close to that of Δ^9-THC according to silica gel TLC (*n*-hexane/EtOAc, 9: 1) were combined and purified by flash silica chromatography and Sephadex LH-20 (*n*-hexane as eluent), followed by final purification by preparative C18 HPLC (MeCN, 25 mL/min) to afford **4** (3.3 mg, rt = 9.5 min), **5** (9.0 mg, rt = 9.0 min), **11** (2.6 mg,

rt = 5.3 min.) and **13** (45 mg, rt = 2.5 min). Portions of the CH_2Cl_2, EtOAc and EtOH extracts were combined (232.0 g) since they showed similar TLC profiles (EtOAc/*n*-hexane, 4: 6), and the resulting extract was subjected to VLC over silica gel (6 kg, 15×90 cm) eluting with EtOAc/*n*-hexane [0:100, 10: 90, 20: 80, 30: 70, 40: 60, 50: 50, 75: 25, 100:0 (2 L of each mixture)] followed by EtOH (4 L), yielding 9 fractions (I – IX). Fraction III (1.70 g) was chromatographed on silica gel (50 g, 2.0×50 cm, *n*-hexane/EtOAc, 100:0 to 85:15), yielding 36 fractions ($III_{1–36,}$ 100 mL each). Fraction $III_{10–14}$ (35 mg) was purified by C18 HPLC (MeCN/H_2O, 95:5, 25 mL/min) to afford **6** (3.0 mg, rt = 7.4 min) and **8** (7.4 mg, rt = 8.8 min). Fraction $III_{15–30}$ (526 mg) afforded **1** (382 mg) upon precipitation from *n*-hexane/EtOAc. Fraction $III_{32–36}$ (130 mg) was purified by C18 HPLC (MeOH/H_2O, 65: 35, 25 mL/min) to give **2** (13.4 mg, rt = 9.4), **3** (4.0 mg, rt = 10.8 min) and **7** (2.4 mg, rt = 5.1 min). Fraction VII (6.70 g) was subjected to silica gel chromatography (400 g, 7.0×80 cm, *n*-hexane/EtOAc, 80:20) yielding 13 fractions ($VII_{1–13}$, 250 mL each). Fraction $VII_{9–10}$ (41 mg) was purified by C18 HPLC (MeOH/H_2O, 75: 25) to afford **9** (5.3 mg, rt = 15.3 min) and **10** (5.0 mg, rt = 4.0 min). Fraction VII_{11} (2.78 g) was purified by Sephadex LH-20 (50 g, 2.0 × 50 cm, MeOH) followed by C18 flash chromatography (MeOH/H_2O, 8: 2) to afford **12** (264.1 mg).



Isolates

Cannabigerolic acid (**1**): White amorphous powder; UV (EtOH): λ_{max} = 255, 299 nm; IR (neat): ν_{max} = 3390, 1650 cm^{-1}; ^1H- and ^{13}C-NMR, see Table 1 and Table 2; HR-ESI-MS (negative ion mode): *m/z* = 359.2214 $[M - H]^-$ (calcd. for $C_{22}H_{31}O_4$:359.2222). (±)-*6,7-trans-Epoxycannabigerolic acid* (**2**): Yellow oil; UV (MeOH): λ_{max} = 215, 260, 300 (sh) nm; $[\alpha]_D^{25}$: 0 (*c* 1.2, MeOH); IR (neat): ν_{max} = 3402, 1650 cm^{-1}; ^1H- and ^{13}C-NMR, see Table 1

and Table 2; HR-ESI-MS (positive ion mode): *m/z* = 399.2156 $[M + Na]^+$ (calcd. for $C_{22}H_{32}O_5Na$: 399.2147).

(±)-*6,7-cis-Epoxycannabigerolic acid* (**3**): Yellow oil; UV (MeOH): λ_{max} = 215, 260, 300 (sh) nm; $[\alpha]_D^{25}$: 0 (*c* 1.2, MeOH); IR (neat): ν_{max} = 3402, 1650 cm^{-1}; ^1H- and ^{13}C-NMR, see Table 1 and Table 2; HR-ESI-MS (positive ion mode): *m/z* = 399.2194 $[M + Na]^+$ (calcd. for $C_{22}H_{32}O_5Na$: 399.2147) and HR-ESI-MS (negative ion mode): *m/z* = 375.2116 $[M - H]^-$ (calcd. for $C_{22}H_{31}O_5$: 375.2172).

(±)-*6,7-cis-Epoxycannabigerol* (**4**): Yellow oil; UV (MeOH): λ_{max} = 215, 260, 300 (sh) nm; $[\alpha]_D^{25}$: 0 (*c* 1.2, MeOH); IR (neat): ν_{max} = 3402, 1610 cm^{-1}; ^1H- and ^{13}C-NMR, see Table 1 and Table 2; HR-ESI-MS (positive ion mode): *m/z* = 333.2493 $[M + H]^+$ (calcd. for $C_{21}H_{33}O_3$:333.2431).

(±)-*6,7-trans-Epoxycannabigerol* (**5**): Yellow oil; UV (MeOH): λ_{max} = 215, 260, 300 (sh) nm; $[\alpha]_D^{25}$: 0 (*c* 1.2, MeOH); IR (neat): ν_{max} = 3402, 1610 cm^{-1}; ^1H- and ^{13}C-NMR, see Table 1 and Table 2; HR-ESI-MS (positive ion mode): *m/z* = 333.2486 $[M + H]^+$ (calcd. for $C_{21}H_{33}O_3$:333.2431).

5′-Methoxycannabigerolic acid **(6):** Yellow oil; UV (EtOH): λ_{max} = 221, 262, 300 nm; IR (neat): ν_{max} = 3400, 1650 cm^{-1}; ^1H-and ^{13}C-NMR, see Table 1 and Table 2; HR-ESI-MS (positive ion mode): *m/z* = 375.2540 $[M + H]^+$ (calcd. for $C_{23}H_{35}O_4$: 375.2535).

5′-Methyl-4-pentylbiphenyl-2,2′,6-triol (**7**): Yellow oil; UV (EtOH): λ_{max} = 210, 282 nm; IR (neat): ν_{max} = 3390, 2910, 1615 cm^{-1}; ^1H- and ^{13}C-NMR, see Table 1 and Table 2 HR-ESI-MS (positive ion mode): *m/z* = 287.1612 $[M+H]^+$ (calcd. for $C_{18}H_{23}O_3$:287.1647). *7-Methoxycannabispirone* (**8**): White needles; m.p.123–124 °C; UV (EtOH): λ_{max} = 224, 275 nm; IR (neat): ν_{max}=3370, 1710, 1600 cm^{-1}; ^1H- and ^{13}C-NMR, see Table 3; HR-ESI-MS (positive ion mode): *m/z*=261.1434 $[M+H]^+$ (calcd. for $C_{16}H_{21}O_3$:261.1491).

Biological activity

The isolated compounds were tested *in vitro* against a culture of *Leishmania donovani* (L. Rivas, Centro de Investigaciones Biologocas CSIC. Madrid, Spain) promastigotes, using pentamidine (Sigma) and amphotericin B (Sigma) as positive controls (IC_{50}=0.15 and 0.9 ng/mL, resectively) [4]. Their antimicrobial activity against *Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Cryptococcus neoformans*, *Mycobacterium intracellulare* and *Aspergillus fumigates* (all from ATCC) [5] as well as the cytotoxicity [6] against Vero cells (African green monkey kidney fibroblast; ATCC) were also tested.

GC-MS trimethylsilyl derivatization

Dried samples (ca. 100 μg) were mixed with pyridine (5 μL, silylation grade, Pierce) and BSTFA [N,O-bis(trimethylsilyl)trifluoroacetamide] (100 μL, 98+%, Acros Organics), followed by heating at 75 °C for 1 h. After cooling to room temperature, CH_2Cl_2 (0.9 mL) was added to the reaction mixture and the solution analyzed by GC-MS.

Results and Discussion

Compound **1** was isolated as a white amorphous powder. Its HR-ESI-MS displayed a pseudomolecular ion at *m/z* = 359.2214 $[M - H]^-$, indicating the molecular formula $C_{22}H_{32}O_4$. The IR spectrum of **1** showed the presence of hydroxy groups (3390 cm^{-1}) and a chelated carboxyl group (1650 cm^{-1}). The ^{1}H-NMR spectrum of **1** in DMSO-d_6 (Table 1) showed three methyl singlets at δ_H = 1.59 (H-1), 1.67 (H-9) and 1.82 (H-10), three methylenes at δ_H = 2.12 (m, H-4), 2.22 (m, H-5) and 3.43 (d, H-8) and two olefinic protons at δ_H = 5.06 (t, H-3) and 5.28 (t, H-7), attributed to a geranyl substituent. It also displayed an aromatic proton at δ_H = 6.28 (s, H-4′) corresponding to δ_C = 111.5 (C-4′) in the HMQC spectrum. The ^{13}C-NMR, DEPT-135 and HMQC spectra showed 22 resonances, including 4 methyl, 7 methylene, 3 methine and 8 quaternary carbons. Two of these resonances were assigned to aromatic carbons bearing hydroxy groups at δ_C = 160.7 (C-5′) and 163.9 (C-1′), while a carboxyl group at δ_C = 176.6 (COOH) was attached to C-2′ (δ_C = 103.4). The structure was further confirmed by GC-MS: **1** spontaneously decarboxylated on injection to give cannabigerol (**13**) ($[M]^+$ = 316). The trimethylsilyl derivative of **1** ($[M]^+$ = 576) confirmed the HR-ESI-MS result and the presence of two phenolic and one carboxyl groups. The ^{1}H-NMR and IR data of **1** were similar to those previously reported for cannabigerolic acid [7], however, this is the first report of the ^{13}C-NMR, DEPT, 2D-NMR and HR-ESI-MS data for **1**.

Compound **2** was isolated as a yellow, optically inactive oil. Its molecular formula, $C_{22}H_{32}O_5$, was derived from HR-ESI-MS (*m/z* = 399.2156 $[M + Na]^+$, 775.4364 $[2M+ Na]^+$). The IR spectrum of **2** diplayed hydroxy groups at 3402 cm^{-1} and a chelated carboxyl group at 1650 cm^{-1}. The ^{1}H- and ^{13}C-NMR, DEPT and HMQC data of **2** in DMSO-d_6 (Table 1 and Table 2) were similar to those of **1** except for the presence of a 6,7-epoxy group [δ_H = 3.62 (t, *J* = 5.2 Hz, H-7); δ_C = 66.9 (C-7), 78.9 (C-6)] instead of the 6,7-double bond [δ_H = 5.28 (t, *J* = 6.8 Hz, H-7); δ_C = 121.7 (C-7), 138.7 (C-6)]. The 6,7-epoxy position was determined by the HMBC correlations of H-7 with C-5, C-6, C-10, C-8 and C-6′, and H_3–10 with C-5, C-6 and C-7 (Fig. 1). The structure was further confirmed by GC-MS: the trimethylsilylderivative of **2** ($[M]^+$=592) confirmed the HR-ESI-MS result and the presence of two phenolic and one carboxyl groups. The lack of a NOESY correlation between H-7 and H_3–10 indicated a 6,7-*trans*-configuration, while lack of any optical rotation points to a racemic mixture of enantiomers (**2a** and **2b**). Thus, **2** is (±)-6,7-*trans*-epoxycannabigerolic acid. Compound **3** was isolated as an optically inactive yellow oil. The HR-ESI-MS of **3** afforded an $[M + Na]^+$ ion at *m/z* = 399.2194 implying a molecular formula $C_{22}H_{32}O_5$. The ^{1}H-, ^{13}C- and 2D-NMR data of **3** (Table 1 and Table 2) are similar to those of **2** except for the downfield shift of C-6 (+ 3 ppm) [8] and the ROESY correlation between H-7 and H_3–10 (Fig. 1), these findings indicated the 6,7-*cis* configuration (**3a** and **3b**). Therefore, **3** is (±)- 6,7-*cis*-epoxycannabigerolic acid.

Compound **4** was obtained as an optically inactive oil, with a molecular formula $C_{21}H_{32}O_3$ based on HR-ESI-MS (*m/z* = 333.2493 $[M + H]^+$). GC-MS analysis (rt = 38.84 min) displayed a base peak at *m/z* = 193. The IR, ^{1}H- and ^{13}C-NMR data of **4** in $CDCl_3$ were similar to those of cannabigerol (**13**) [9] (Table 1 and Table 2) except for the presence of a 6,7-epoxy group [δ_H = 3.88 (t, *J* = 5.2 Hz, H-7); δ_C = 67.3 (C-7), 81.4 (C-6)], while the

ROESY correlation between H-7 and H_3–10, established **4** as (±)-6,7-*cis*-epoxycannabigerol.

Compound **5** was also isolated as an optically inactive oil and its molecular formula was determined as $C_{21}H_{32}O_3$ by HR-ESI-MS and ^{13}C-NMR spectroscopy. GC-MS analysis of **5** (rt = 38.68 min) revealed a base peak at *m/z* = 193, while the NMR data were almost identical to those of **4** except for an upfield shift of C-6 (Table 2) and the absence of ROESY correlation between H-7 and H_3–10. Based on the above, **5** was elucidated as (±)-6,7-*trans*-epoxycannabigerol.

Compound **6** was isolated as a yellow oil. On the basis of its HR-ESI-MS at *m/z* = 375.2540 $[M + H]^+$ and ^{13}C-NMR spectroscopic data, the molecular formula was established as $C_{23}H_{34}O_4$. The structure was determined by comparing its ^{1}H- and ^{13}C-NMR data in $CDCl_3$ (Table 1 and Table 2)with **1**. Compound **6** contained an additional methoxy group [δ_H = 3.86 (s, O*Me*); δ_C = 55.7 (O*Me*)] instead of a hydroxy group. The location of the methoxy group was determined to be at C-5′ from HMBC correlations (O*Me*/C-5′; O*Me*/C-6′; H-4′/O*Me*), establishing **6** as 5′-methoxycannabigerolic acid. Although **6** is a known cannabis constituent [10], this is the first report of its full NMR assignments.

Compound **7** was isolated as a yellow oil. It gave a molecular formula of $C_{18}H_{22}O_3$ based on HR-ESI-MS (*m/z*= 287.1612 $[M+H]^+$, 285.1581 $[M - H]^-$), GC-MS ($[M]^+$ 286) and ^{13}C-NMR data. The IR absorption bands at 3390, 1615, 1242 and 1035 cm^{-1} indicated the presence of hydroxy and benzene ring functionalities. The ^{1}H-NMR (Table 1) and COSY spectra of **7** indicated two aromatic ring systems, 1,2,4,6-tetrasubstituted ring A with two magnetically equivalent protons [δ_H = 6.45 (2H, s, H-3 and H-5)] and an ABX spin system for ring B [δ_H = 6.99 (1H, d, *J* = 8.2 Hz, H-3′), 7.15 (1H, dd, *J* = 2.0, 8.2 Hz, H-4′), 7.03 (1H, d, *J* = 2.0 Hz, H-6′)]. The ^{1}H-NMR also showed the presence of one aromatic methyl singlet at δ_H = 2.30 (H-7′) and an *n*-pentyl moiety. The ^{13}C-NMR (Table 2), DEPT-135 and HMQC spectra revealed the presence of 18 carbon resonances, including 2 methyl, 4 methylene, 5 sp^2 methine, 3 oxyaryl and 4 quaternary carbons. The ^{1}H- and ^{13}C-NMR data of ring A are suggestive of a phenyl substituted olivetol (biphenyl) [11], and together with HMBC correlations (Fig. 2) [H_3 –7′/C-4′, C-6′; H-3′/C-2′, C-1′] indicates that **7** is 5′-methyl-4-pentylbiphenyl-2,2′,6-triol.

Compound **8** was obtained as white needles. HR-ESI-MS at *m/z* = 261.1434 $[M + H]^+$ and ^{13}C-NMR established the molecular formula as $C_{16}H_{20}O_3$. GC-MS analysis of **8** showed a base peak at *m/z* = 203 and two other characteristic ions at *m/z* = 189 and 175, indicating that **8** is a spiroindane derivative [12]. ^{1}H- and ^{13}C-NMR data of **8** were similar to those reported for cannabispirone **9** (Table 2) [13], [14], except for a methoxy instead of hydroxy group at C-7, establishing **8** as 7-methoxycannabispirone. The structure was confirmed by DEPT-135, COSY, HMQC and HMBC analysis. Although **8** is a known synthetic product [14], [15], this is the first report of its isolation from a natural source.

Compounds **9–13** were identified as cannabispirone [12], *β*-cannabispiranol [15], dehydrocannabifuran [16], [17], cannflavin B [9] and cannabigerol [9] by comparing their

spectroscopic data with reported values. However, this is the first report for the ^{13}C-NMR assignments for dehydrocannabifuran (**11**) (Table 3).

Compound **13** exhibited selective antimicrobial activity against *Mycobacterium intracellulare* with an IC_{50} value of 15.0 μg/mL. Compounds **1** and **12** displayed moderate antileishmanial activity with IC_{50} values of 12.0 and 5.0 μg/mL, respectively. All isolates lacked cytotoxicity against Vero cells (African green monkey kidney fibroblast).

Acknowledgments

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Abbreviations

CBG	cannabigerol
CBD	cannabidiol
FID	flame ionization detector
Δ^9-THC	Δ^9-tetrahydrocannabinol
VLC	vacuum liquid chromatography

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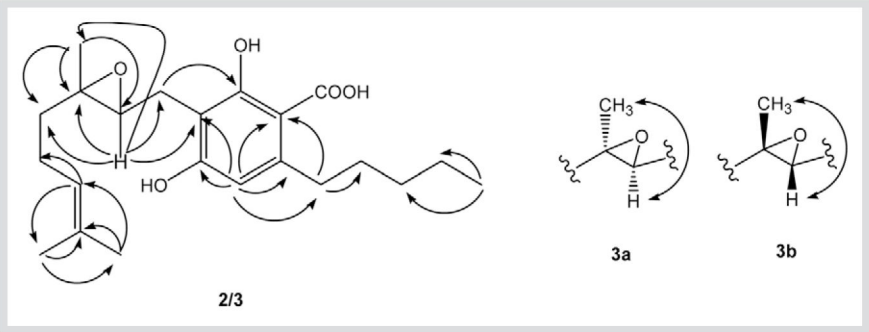

Fig. 1.
HMBC correlations of **2** and **3** (→) and key ROESY correlations of **3** (↔).

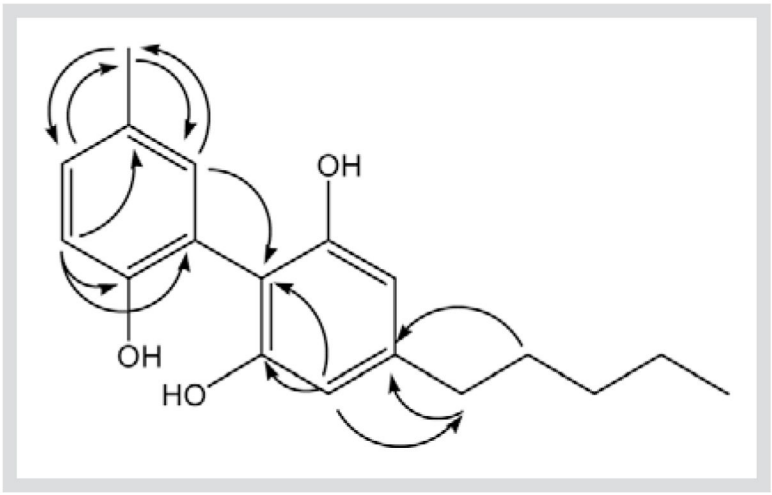

Fig. 2.
Key HMBC correlations of **6**.

Table 1

^{1}H-NMR data for **1–7** and **13** (400 MHz, δ in ppm, J in Hz)

Position	1[a]	2[a]	1[b]	3[b]	4[b]	5[b]	6[b]	13[b]	7[b]
1	1.59 s	1.53 s	1.52 s	1.57 s	1.57 s	1.57 s	1.55 s	1.60 s	–
3	5.06 t (6.4)	5.06 t (6.4)	5.03 t (6.4)	5.08 t (6.4)	5.08 t (6.4)	5.07 t (6.4)	5.06 (m)	5.07 m	6.45 s
4	2.12 m	2.10 m	1.91 t (6.8)	2.13 m	2.13 m	2.11 m	2.03 m	2.09 m	
5	2.22 m	1.49 m	2.10 t (7.2)	1.53 m	1.53 m	1.53 m	1.94 m	2.09 m	6.45 s
6	–	–	–	–	–	–	–	–	–
7	5.28 t (6.8)	3.62 t (5.2)	5.17 t (6.8)	3.88 t (5.2)	3.88 t (5.2)	3.87 t (5.2)	5.18 t (6.6)	5.29 m	–
8	3.43 d (6.8)	2.68 dd (5.2, 16.4) 2.33 dd (8.4, 16.8)	3.19 d (6.8)	2.45 m	2.45 m	2.43 m	3.32 d (7.6)	3.41 d (7.0)	–
9	1.67 s	1.61 s	1.59 s	1.66 s	1.66 s	1.65 s	1.63 s	1.69 s	–
10	1.82 s	1.04 s	1.71 s	1.32 s	1.32 s	1.32 s	1.76 s	1.82 s	–
2′	–	–	–	–	6.20 s	6.20 s	–	6.26 s	–
3′	–	–	–	–	–	–	–	–	6.99 d (8.2)
4′	6.28 s	6.18 s	6.25 s	6.30 s	6.30 s	6.28 s	6.31 s	6.26 s	7.15 dd (2.0, 8.2)
6′	–	–	–	–	–	–	–	–	7.03 d (2.0)
7′	–	–	–	–	–	–	–	–	2.30 s
1″	2.88 t (7.2)	2.86 t (7.2)	2.80 t (7.2)	2.90 t (7.2)	2.90 t (7.2)	2.84 t (7.2)	2.88 t (7.6)	2.45 t (7.5)	2.52 t (5.6)
2″	1.60 m	1.45 m	1.60 m	1.58 m	1.58 m	1.57 m	1.58 m	1.56 q(7.8)	1.59 m
3″	1.35 m	1.23 m	1.28 m	1.26 m	1.26 m	1.26 m	1.35 m	1.33 m	1.33 m
4″	1.35 m	1.23 m	1.28 m	1.26 m	1.26 m	1.26 m	1.35 m	1.33 m	1.33 m
5″	0.91 t (7.2)	0.83 t (6.4)	0.85 t (6.8)	0.88 t (6.4)	0.88 t (6.4)	0.88 t (6.4)	0.89 t (6.4)	0.90 t (6.9)	0.89 t (6.4)
OCH_3	–	–	–	–	–	–	3.86 s	–	–

[*] Assignments confirmed by DEPT-135, gHMQC, gCOSY, and gHMBC experiments.

[a] In DMSO-*d6*.

[b] In $CDCl_3$.

Table 2

^{13}C-NMR data for **1–7** and **13** (100 MHz, δ in ppm)

Position	1[a]	2[a]	1[b]	3[b]	4[b]	5[b]	6[b]	13[b]	7[b]
1	17.9	18.1	17.9	17.7	17.8	17.8	17.9	17.6	117.3
2	132.1	131.4	131.0	132.8	132.8	132.2	131.3	132.0	154.1
3	124.1	125.2	124.6	123.4	123.4	124.2	124.7	123.8	108.4
4	26.8	21.6	26.7	21.9	21.9	21.9	26.1	26.4	146.6
5	40.0	38.8	40.0	37.1	36.9	36.9	40.0	39.7	108.4
6	138.7	78.9	133.9	81.9	81.4	78.4	135.3	138.0	154.1
7	121.7	66.9	123.1	67.2	67.3	68.3	122.4	121.8	–
8	22.3	26.7	22.4	26.0	26.0	26.0	22.1	22.5	–
9	25.9	26.2	25.9	25.8	25.8	25.9	25.9	25.6	–
10	16.4	18.1	16.3	17.7	17.8	17.8	16.3	16.1	–
1′	163.9	151.4	163.3	153.0	152.8	154.5	163.0	154.8	152.5
2′	103.4	106.1	103.6	105.0	110.6	109.6	104.0	108.4	117.3
3′	147.7	138.8	145.1	145.1	143.3	143.3	147.5	142.7	132.0
4′	111.5	107.1	110.3	108.4	108.4	107.5	106.2	108.4	131.6
5′	160.7	156.0	160.0	153.8	152.8	153.6	162.3	154.8	132.1
6′	112.1	116.1	112.6	110.9	110.6	110.6	115.2	110.7	116.4
7′	–	–	–	–	–	–	–	–	20.7
1″	36.8	36.4	36.2	35.2	36.8	36.9	37.4	35.5	36.1
2″	31.7	31.1	31.6	31.2	31.3	31.0	31.9	30.8	30.8
3″	32.2	31.8	32.0	32.1	31.8	31.7	32.3	31.5	31.7
4″	22.8	22.6	22.0	22.7	22.7	22.7	22.7	22.2	22.8
5″	14.3	14.5	14.3	14.2	14.3	14.2	14.3	14.0	14.2
COOH	176.6	170.0	174.3	176.4	–	–	176.6	–	–
OMe	–	–	–	–	–	–	55.7	–	–

[*] Assignments confirmed by DEPT-135, gHMQC, gCOSY and gHMBC experiments.

[a] In DMSO-d_6.

[b] In $CDCl_3$.

Table 3

^{1}H-NMR and ^{13}C-NMR data for **8**, **9** and **11** ($CDCl_3$, δ in ppm, J in Hz)

Position	8		9		11	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	–	48.2	–	47.8	–	154.3
2	2.22 t (7.2)	35.5	2.21 t (7.2)	35.6	7.06 bs	110.5
3	2.93 t (7.2)	31.2	2.93 t (7.2)	31.2	–	134.8
4	6.37 bs	101.0	6.35 bs	102.0	6.66 bs	103.5
5	–	160.8	–	160.5	–	145.0
6	6.28 bs	97.4	6.15 bs	101.0	–	114.5
7	–	157.5	–	153.6	–	138.7
8	–	125.8	–	126.9	–	155.6
9	–	145.7	–	146.5	–	117.1
10	3.75 s	55.6	3.72 s	55.6	7.04 d (8.0)	127.0
11	3.78 s	55.1	–	–	7.13 d (8.0)	121.2
12	–	–	–	–	–	128.3
13	–	–	–	–	2.59 s	15.2
14	–	–	–	–	–	150.2
15	–	–	–	–	5.23 bs 5.64 bs	110.7
16	–	–	–	–	2.31 s	22.7
1′	–	–	–	–	2.73 (7.2)	36.3
2′	2.40 m	39.2	2.42 m	39.2	1.55 m	29.9

Position	8		9		11	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
	2.53 dt (6.4, 13.2)		2.53 dt (6.4, 13.2)			
3′	1.82 dd (2.8, 6.4) 2.63 dt (4.6, 13.2)	34.5	1.82 dd (2.8, 6.4) 2.67 dt (4.6, 13.2)	34.5	1.25 m	31.5
4′	–	213.3	–	214.5	1.25 m	22.7
5′	1.81 dd (2.8, 6.4) 2.63 dt (4.6, 13.2)	34.5	1.82 dd (2.8, 6.4) 2.67 dt (4.6, 13.2)	34.5	0.88 t (6.8)	14.2
6′	2.40 m 2.53 dt (6.4, 13.2)	39.2	2.42 m 2.53 dt (6.4, 13.2)	39.2	–	–

* Assignments confirmed by DEPT-135, gHMQC, gCOSY and gHMBC experiments.