Thieme

# *O*-Methyl Phytocannabinoids: Semi-synthesis, Analysis in Cannabis Flowerheads, and Biological Activity*

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Key words
*Cannabis sativa*, Cannabaceae, meroterpenoids, phytocannabinoids, *O*-methylation, PPAR

**received** February 1, 2019
**revised** March 18, 2019
**accepted** March 20, 2019

Bibliography
**DOI** https://doi.org/10.1055/a-0883-5383
Published online | Planta Med © Georg Thieme Verlag KG Stuttgart · New York | ISSN 0032-0943

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## ABSTRACT

A general protocol for the selective mono-*O*-methylation of resorcinyl phytocannabinoids was developed. The availability of semisynthetic monomethyl analogues of cannabigerol, cannabidiol, and cannabidivarin (**1a**–**3a**, respectively) made it possible to quantify these minor phytocannabinoids in about 40 different chemotypes of fiber hemp. No chemotype significantly accumulated mono-*O*-methyl cannabidiol (**2b**) or its lower homologue (**3b**), while at least three chemotypes containing consistent amounts (≥ 400 mg/kg) of *O*-methylcannabigerol (**1b**) were identified. *O*-Methylation of alkyl phytocannabinoids (**1b**–**3b**) does not significantly change the activity on peroxisome proliferator-activated receptors in contrast to what was reported for phenethyl analogues.



## Introduction

Phytocannabinoids, the hallmark secondary metabolites of Cannabis (*Cannabis sativa* L., Cannabaceae), are meroterpenoids resulting from the convergence of mevalonate and polyketide pathways [1]. Their structure is based on a resorcinyl core *para*-substituted with a monoterpenyl and pentyl groups, although compounds with a different prenyl moiety (e.g., sesquiterpenyl [1, 2]) or with a shortened alkyl group (methyl, propyl, or, more rarely, ethyl and butyl) are also present in the plant [1], and a recent review has listed almost 150 Cannabis phytocannabinoids [1]. This impressive chemodiversity is mostly the result of changes (cyclizations, rearrangements, oxidations, additions, etc.) in the terpenyl moiety, but a certain degree of diversity is also present in the resorcinyl core. Thus, in addition to the acidic precursors [1] of neutral phytocannabinoids and their terpenyl esters [3], cannabinoquinoids [4] and *O*-methyl phytocannabinoids have also been reported [5, 6].

The mono-*O*-methyl derivatives of CBG (1a) (▶ **Fig. 1**) and CBD (2a) (▶ **Fig. 1**) were first isolated as Beam test negative constituents of a Japanese chemotype of *C. sativa* L. (Minamishihara number 1) [5, 6], and were next also identified as trace constituents of

* Dedicated to Professor Dr. Cosimo Pizza 70th birthday in recognition of his outstanding contribution to natural product research.

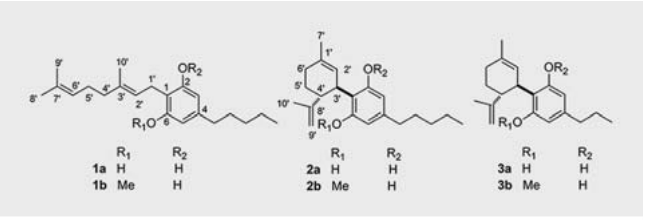
**ABBREVIATIONS**

| | |
|---|---|
| $CB_1$-$CB_2$ | cannabinoid receptors 1 and 2 |
| CBD | cannabidiol |
| CBDV | cannabidivarin |
| CBG | cannabigerol |
| GCC | gravity column chromatography |
| LOQ | limit of quantitation |
| PE | petroleum ether |
| PPARs | peroxisome proliferator-activated receptors |
| TBAF | tetrabutylammonium fluoride |
| *t*BDMS-Cl | *t*-butyldimethylsilyl chloride |
| $TMSCHN_2$ | trimethylsilyldiazomethane |
| TRP | transient receptor potential |

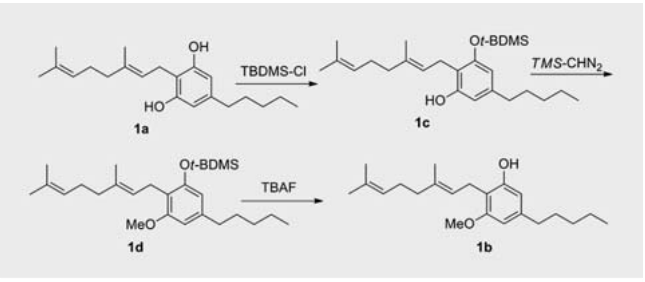
some Western chemotypes of Cannabis [1]. Just like their resorcinyl analogues, *O*-methyl CBG (1b) and *O*-methyl CBD (2b) are not narcotic [5,6], but little is known about their biological profile, especially their activity on other targets of the biological space of phytocannabinoids, which includes not only metabotropic receptors ($CB_1$ and $CB_2$), but also ionotropic receptors (thermo-TRPs), transcription factors (PPAR-$\alpha$ and -$\gamma$), and various enzymes involved in the metabolism of eicosanoids and endocannabinoids [7]. Our interest for this class of minor phytocannabinoids was fostered by the observation that mono-*O*-methylation of phenethyl phytocannabinoids (amorfrutinoids) promotes PPAR-$\gamma$ activity [8], and a similar increase of potency was observed for the inhibition of 15-lipooxygenase (15-LO) when the monomethyl ether of CBD (2b) was compared to its parent resorcinol (CBD, 2a) [1]. There is growing evidence that the phytocannabinoid structural motif is a privileged structure for bioactivity [1], and we have therefore developed a semi-synthesis of mono-*O*-methyl phytocannabinoids to gain further information about their biological profile and to quantify their occurrence in various chemotypes of fiber hemp.

## Results and Discussion

Although Cannabis produces almost 150 phytocannabinoids [1], many of them are only available in minute amounts by isolation, therefore, synthesis or semi-synthesis are the only option to try to advance their medicinal potential. Over the years, we have isolated the monomethyl ethers of CBG (**1a**), CBD (**2a**), and CBDV (**3a**) from some European chemotypes of fiber hemp in yields unattractive to sustain a medicinal chemistry effort (maximum 40 mg/kg). To increase the availability of these compounds, we decided to investigate the selective monomethylation of their corresponding, and much more easily available, resorcinol analogues [CBG (**1a**), CBD (**2a**), and CBDV (**3a**)] (▶ **Fig. 1**). The methylation of phytocannabinoids has already been investigated previously [5,9], but a general and chemoselective protocol for the monomethylation is still lacking. Indeed, treatment with diazomethane or other common methylating agents (methyl iodide, dimethylsulfate) and bases affords reaction mixtures containing major, or exclusive, amounts of the dimethylated products. It has been observed that the formation of these compounds could not be avoided even with substoichiometric amounts of methylating reagents [5,9]. This observation can be rationalized considering that the electron-donating properties of the *O*-alkyl methyl seemingly increase the reactivity of the other resorcinyl hydroxyl, making the monoalkylated product more reactive than the starting diphenol. The selective mono-*O*-methylation observed for the alkyl esters of acidic phytocannabinoids can be related to the presence of a strong intramolecular hydrogen bonding between the ester carbonyl and the *ortho*-hydroxyl group, with a resulting decrease of reactivity compared to the non-hydrogen-bonded hydroxyl group [9].




▶ **Fig. 1** Chemical structures of CBG (**1a**), CBD (**2a**), and CBDV (**3a**) and their corresponding *O*-methyl analogues (**1b**–**3b**).




▶ **Fig. 2** Chemical conversion of CBG (**1a**) into the corresponding *O*-methyl analogue (**1b**). A parallel procedure was also applied to CBD (**2a**) and CBDV (**3a**).

In sharp contrast with the methylation, silylation with the bulky reagent *t*BDMS-Cl was highly chemoselective, exclusively providing the mono-protected resorcinols **1c**–**3c** from **1a**–**3a**, respectively (▶ **Fig. 2**). "Slenderer" silylating agents like triethylsilylchloride and trimethylsilyl chloride were less selective, providing, together with the *O*-monosilylated derivatives, the major reaction products, and also significant amounts of the *O*-disilylated analogues. Methylation with $TMSCHN_2$ [10] of **1c**–**3c** cleanly afforded **1d**–**3d** (▶ **Fig. 2**), and subsequent deprotection (TBAF) was uneventful, providing **1b**–**3b** in an excellent overall yield (▶ **Fig. 2**).

The NMR spectra of the mono-methylated phytocannabinoids **1b**–**3b** were fully assigned by using 2D experiments and are listed in the Materials and Methods section. The most evident effect of *O*-methylation on NMR resonances was the expected downfield shift experienced by the *O*-methylated carbons compared to the OH- linking ones. Moreover, *O*-methylation could increase the steric hindrance to the rotation of the phenyl-cyclohexenyl bond in **2b**/**3b** compared to **2a**/**3a**. Thus, in addition to the obvious electronic effects, this could explain the slight splitting in the proton resonances of H-3 and H-5 ($\delta$ 6.22 and 6.20, in $CD_3OD$) in **2b**/**3b**

compared to their coincidence in the non-methylated **2a**/**3a** ($\delta$ 6.07, in $CD_3OD$) [11].

The availability of reference standards of *O*-methyl cannabinoids made it possible to quantify their presence in a wide selection of chemotypes of fiber hemp registered in the EU or under development in breeding centers. Because of this, plant samples (flowerheads) were extracted with dichloromethane, and the crude extracts were directly analyzed by GC-MS using tribenzylamine as the internal standard. This procedure was designed to exclude any extraction/chromatographic artifact formation of the *O*-methyl derivatives of the phytocannabinoids. Cannabis samples contain mixtures of native phytocannabinoids (acidic phytocannabinoids), and their decarboxylated version (neutral phytocannabinoids) formed on storage, but, under GC conditions, acidic cannabinoids (= pre-cannabinoids) are decarboxylated, thus simplifying the analysis. CBG (**1a**) and CBD (**2a**) are typical constituents of fiber chemotypes of Cannabis (hemp), and we comparatively analyzed the concentration of these compounds and their *O*-methyl derivatives in a series of chemotypes registered in the EU (Eletta Campana, Fibranova, Tiborszallasi, Bialobrzeskie, Beniko) or under development in breeding centers (▶ **Table 1**). All these plants accumulated significant amounts of CBD (samples 1–8), CBDV (samples 9–23), or CBG (samples 24–29), but none of them accumulated monomethyl phytocannabinoids as the major constituent. Nevertheless, some chemotypes contained significant (> 100 mg/kg) amounts of *O*-methyl phytocannabinoids, with the highest concentration (490 mg/kg) observed in the chemotype GBC-1.

*O*-Methylation of phytocannabinoids presumably occurs via an *S*-adenosylmethionine transfer reaction and is more common in phenethyl phytocannabinoids compared to alkyl phytocannabinoids. Nothing is known on the enzyme(s) responsible for this activity, but CBG seems to be a better substrate than CBD, since higher amounts of the *O*-methyl derivative occur with the former compared to the latter, while phytocannabinoids devoid of two free resorcinyl hydroxyls, like $\Delta^9$-THC and CBC, do not seem to be substrates for this enzymatic activity, since *O*-methyl derivatives of these compounds have never been reported as natural products.

Very little is known about the biological profile of *O*-methyl phytocannabinoids from *C. sativa*. Their parent compounds [CBG (**1a**), CBD (**2a**) and CBDV (**3a**)] do not significantly modulate the activity of cannabinoid receptors, but they can, nevertheless, modulate the endocannabinoid system by allosteric modulation of the receptors (CBD) [7] or by interaction with the enzymes involved in the synthesis and degradation of endocannabinoids [7]. In addition, CBG is also the electron-donating properties powerful antagonist of the menthol receptor TRPM8 [12], while both compounds can also modestly interact with the transcription factor PPAR-γ. Since we had enough *O*-monomethyl derivative phytocannabinoids (**1b**–**3b**) on hand, we tested them on this endpoint, revealing that the activity remained modest and not significantly different from the parent compounds **1a**–**3a**. Similar results were obtained for the interaction with PPAR-α. These observations are in contrast with data reported for phytocannabinoid analogues showing a phenethyl group in place of the alkyl one, for which *O*-methylation increased the potency toward PPAR-γ [8]. Since **1a**–**3a** show a symmetry plane bisecting the resorcinyl hydroxyls, it is tempting to assume that a change in one hydroxyl can be compensated, at least for the interaction with PPARs, by the other one. On the other hand, in phenethyl phytocannabinoids, the presence of an aromatic ring in the alkyl substituent could bias the conformation of the resorcinyl ring, desymmetrizing, in terms of bioactivity, the two phenolic hydroxyls.

In conclusion, *O*-methylation is the most common modification of the resorcinyl core of phytocannabinoids, and seems more common in compounds, like CBG (**1a**), having a non-cyclized isoprenyl residue compared with compounds like CBD (**2a**) where this moiety is cyclized. A systematic survey of about 40 cultivars identified that a couple of them produce considerable amounts of **1b**. Overall, the concentration of *O*-methyl phytocannabinoids in Cannabis flowerheads is highly variable and of chemotaxonomic relevance for a better characterization of Cannabis cultivars. From a biological standpoint, *O*-methylation of alkyl phytocannabinoids does not significantly alter the activity on PPARs, in contrast to what has been reported for phenethyl analogues.

## Materials and Methods

### General experimental procedures

IR spectra were registered on an Avatar 370 FT-IR Techno-Nicolet apparatus. $^1$H (500 MHz) and $^{13}$C (125 MHz) NMR spectra were measured on Varian INOVA 500 MHz NMR spectrometers. Chemical shifts were referenced to the residual solvent signal ($CD_3OD$: $\delta_H$ = 3.34, $\delta_C$ = 49.2). Homonuclear $^1$H connectivities were determined by the COSY experiment. One-bond heteronuclear $^1$H-$^{13}$C connectivities were determined with the HSQC experiment. Two- and three-bond $^1$H-$^{13}$C connectivities were determined by gradient 2D HMBC experiments optimized for a $^{2,3}J$ = 9 Hz. Low- and high-resolution ESIMS were obtained on an LTQ OrbitrapXL (Thermo Scientific) mass spectrometer.

Reactions were monitored by TLC on Merck 60 $F_{254}$ (0.25 mm) plates, visualized by staining with 5% $H_2SO_4$ in ethanol and heating. Organic phases were dried with $Na_2SO_4$ before evaporation. Chemical reagents and solvents were purchased form Sigma-Aldrich and were used without any further purification unless stated otherwise. PE with a boiling point between 40–60 °C was used. Silica gel 60 (70–230 mesh) used for GCC was purchased from Macherey-Nagel.

### *O*-Silyl cannabinoids: synthesis of *O*-*tert*-butyldimethylsilyl cannabigerol (1c) as exemplificative

To a stirred solution of CBG (**1a**, 1.19 g, 3.8 mmol) in dry $CH_2Cl_2$ (10 mL), imidazole (1.02 g, 15.0 mmol, 4 mol. equivalents) and *t*BDMS-Cl (1 *M* in $CH_2Cl_2$, 4.5 mL, 4.5 mmol, 1.2 mol. equivalents) were sequentially added. The solution was stirred at room temperature overnight, then quenched by the addition of 2 *M* $H_2SO_4$ (20 mL) and $CH_2Cl_2$ (10 mL). The organic layer was washed with brine (3 × 10 mL) then dried, filtered, and evaporated. The residue was purified by GCC on silica gel (PE/$CH_2Cl_2$ 9 : 1 as the eluent) to afford **1c** as a brown oil (667 mg, 52%). Under the same conditions, the yield was 54% for *O*-*t*BDMS-cannabidiol (**2c**) and 66% for *O*-*t*BDMS-cannabivarin (**3c**).

▶ **Table 1** Concentration of mono-*O*-methyl phytocannabinoids in different chemotypes of fiber hemp.

| N° | Samples | Chemotype | 1b µg/g ± SD | 2b µg/g ± SD | 3b µg/g ± SD |
|---|---|---|---|---|---|
| 1 | Kompolti | CBD | 125.35 ± 10.21 | 15.55 ± 1.32 | nd |
| 2 | Ferimon | CBD | 5.42 ± 0.43 | 3.54 ± 0.16 | nd |
| 3 | Fedora17 | CBD | 9.52 ± 0.76 | 2.46 ± 0.17 | nd |
| 4 | Felina 32 | CBD | 6.38 ± 0.35 | < LOQ | nd |
| 5 | Epsilon 68 | CBD | < LOQ | nd | nd |
| 6 | Futura 75 | CBD | nd | nd | nd |
| 7 | Charmaeleon | CBD | 6.71 ± 0.44 | nd | nd |
| 8 | Markant | CBD | < LOQ | < LOQ | nd |
| 9 | Ivor | CBD | < LOQ | nd | nd |
| 10 | Delta Ilosa | CBD | 12.29 ± 0.20 | < LOQ | nd |
| 11 | Carma monoica | CBD | 15.92 ± 1.11 | 2.29 ± 0.15 | nd |
| 12 | Carmagnola | CBD | 20.14 ± 1.00 | 2.38 ± 0.14 | nd |
| 13 | Carmaleonte | CBD | nd | nd | nd |
| 14 | Denise | CBD | nd | < LOQ | nd |
| 15 | Uso 31 | CBD | nd | < LOQ | nd |
| 16 | Eletta Campana | CBD | 293.31 ± 15.59 | 50.32 ± 0.28 | nd |
| 17 | Fibranova | CBD | 157.93 ± 0.52 | 475.25 ± 5.42 | nd |
| 18 | Fibrol | CBD | 23.43 ± 1.31 | 5.84 ± 0.22 | nd |
| 19 | Tiborszallasi | CBD | 40.67 ± 3.43 | 19.73 ± 1.31 | nd |
| 20 | KC Dora | CBD | 62.89 ± 4.89 | 22.81 ± 2.02 | nd |
| 21 | Monoica | CBD | 195.31 ± 9.44 | 84.16 ± 0.16 | nd |
| 22 | Bialorbrzeskie | CBD | < LOQ | < LOQ | nd |
| 23 | Beniko | CBD | < LOQ | 2.28 ± 0.11 | nd |
| 24 | GBC-1 | CBDV | 491.91 ± 1.78 | 81.81 ± 1.51 | 26.98 ± 0.92 |
| 25 | GBC-2 | CBDV | 68.11 ± 2.41 | 30.14 ± 1.69 | 7.20 ± 0.27 |
| 26 | GBC-3 | CBDV | 333.61 ± 2.60 | 84.22 ± 4.21 | 48.71 ± 0.29 |
| 27 | GBC-4 | CBDV | 57.37 ± 2.83 | 14.69 ± 0.58 | 4.46 ± 0.18 |
| 28 | GBC-5 | CBDV | 339.70 ± 6.90 | 101.88 ± 4.11 | 19.66 ± 0.24 |
| 29 | GBC-6 | CBDV | 16.20 ± 0.22 | 175.23 ± 2.59 | 4.62 ± 0.10 |
| 30 | GBC-7 | CBDV | 20.22 ± 0.73 | 12.75 ± 0.61 | 4.87 ± 0.14 |
| 31 | GBC-8 | CBD | 209.97 ± 5.33 | 57.28 ± 1.17 | 7.53 ± 0.08 |
| 32 | GBC-9 | CBDV | 469.00 ± 27.8 | 69.35 ± 1.66 | 18.28 ± 0.40 |
| 33 | GBC-10 | CBDV | 177.37 ± 6.03 | 64.69 ± 0.19 | 9.71 ± 0.14 |
| 34 | GBC-11 | CBDV | 83.27 ± 1.41 | 24.68 ± 0.22 | 9.13 ± 0.10 |
| 35 | GBC-14 | CBDV | 400.15 ± 6.68 | 84.96 ± 2.31 | 18.01 ± 0.20 |
| 36 | GBC-16 | CBDV | 197.98 ± 3.35 | 100.72 ± 2.51 | 19.01 ± 0.28 |
| 37 | GBC-18 | CBG | 31.21 ± 0.52 | 2.00 ± 0.05 | < LOQ |

nd = not detected

## *O*-Methyl-*O*-silyl cannabinoids: synthesis of *O*-*tert*-butyldimethylsilyl-*O*-methylcannabigerol (1d) as exemplificative

To a stirred solution of **1c** (500 mg, 1.2 mmol) in methanol (5 mL), trimethylsilyl diazomethane (2 *M* in ether, 11.6 mL, 23.2 mmol, 20 mol. equivalents) was added. The solution was stirred at room temperature overnight, and then quenched with sat. $NaHCO_3$ (20 mL) and $CH_2Cl_2$ (20 mL). The organic layer was washed with brine (3 × 10 mL) dried, filtered, and evaporated. The residue was purified by GCC on silica gel (PE as the eluent) to afford **1d** as a colorless oil (589 mg, 98%). Under the same conditions, the yield was also > 90% for 2-*O*-*t*BDMS-6-OMe-cannabidiol (**2d**) and 2-*O*-*t*BDMS-6-OMe-cannabivarin (**3d**).

## Final desylyation. Synthesis of *O*-methylcannabigerol (1b) as exemplificative

To a stirred solution of **1d** (500 mg, 1.1 mmol) in tetrahydrofuran (THF) (5 mL), acetic acid (65 µL, 1.1 mmol, 1 mol. equivalent) and TBAF were (1 *M* in THF, 1.1 mL, 1.1 mmol, 1 mol. equivalent) sequentially added. The solution was stirred at room for 10 min, and then quenched with EtOAc (20 mL) and brine (20 mL). The organic layer was washed with brine (2 × 10 mL), dried, and evaporated. The residue was purified by GCC on silica gel (PE : EtOAc 95 : 5 as the eluent) to afford **1b** as a pale yellow oil (352 mg, 94 %). Under the same conditions, the yield was also > 90 % for **2b** and **3b**.

*O-Methylcannabigerol (**1b**)*: Pale yellow oil. $[\alpha]_D = 0$ ($c = 0.2$ in $CH_3OH$). IR (KBr) $cm^{-1}$ 3429, 1616, 1588, 1451, 1424, 1212, 1163, 1099; $^1H$ NMR ($CD_3OD$, 400 MHz) $\delta$ 6.26 (2H, s, H-3–5), 5.17 (1H, t, 7.2 Hz H-2′), 5.05 (1H, t, 6.8 Hz H-6′), 3.75 (3H, s, OMe), 3.25 (2H, d, 7.2 Hz, H-1′), 2.48 (2H, t, 7.5 Hz H-1′′), 2.04 (2H, q, 7.7 Hz, H-5′), 1.93 (2H, t, 7.7 Hz, H-4′), 1.74 (3H, s, H-10′), 1.60 (3H, s, H-8′), 1.60 (3H, s, H-9′), 1.59 (2H, overlapped, H-2′′), 1.34 (4H, m, H-3′′-4′′), 0.91 (3H, t, 6.9 Hz H-5′′). $^{13}C$ NMR ($CD_3OD$, 100 MHz): $\delta$ 159.6 (C-6), 151.5 (C-2), 142.6 (C-4), 134.4 (C-3′), 131.9 (C-7′), 125.5 (C-6′), 124.9 (C-2′), 115.0 (C-1), 109.2 (C-5), 103.7 (C-3), 56.0 (OMe-6), 40.9 (C-4′), 37.1 (C-1′′), 32.7 (C-3′′), 32.4 (C-2′′), 27.7 (C-5′), 25.9 (C-9′), 23.6 (C-1′), 22.9 (C-4′′), 17.7 (C-8′), 16.2 (C-10′), 14.4 (C-5′′). HR-ESIMS: *m/z* $[M–H]^-$ 329.2469 (calcd. for $C_{22}H_{33}O_2$, 329.2475).

*O-Methylcannabidiol (**2b**)*: Pale yellow oil. $[\alpha]_D^{25} = +110$ ($c = 0.2$ in $CH_3OH$). $^1H$ NMR ($CD_3OD$, 500 MHz) $\delta$ 6.22 (1H, s, H-5), 6.20 (1H, s, H-3), 5.21 (1H, s, H-2′), 4.40 (2H, s, H-9′), 3.93 (1H, d, 9.8 Hz, H-3′), 3.68 (3H, s, -$OCH_3$), $\delta$ 2.89 (1H, td, 10.7 Hz, 4.7 Hz, H-4′), 2.45 (2H, t, 7.7 Hz, H-1′′), 2.19 (1H, m, H-6′a), 1.99 (1H, bd, 16.5 Hz, H-6′b), 1.73 (2H, m, H-5′), 1.66 (3H, s, H-10′), 1.60 (3H, s, H-7′), 1.57 (2H, m, H-2′′), 1.33 (4H, m, H-3′′-4′′), 0.90 (3H, t, 6.7 Hz, H-5′′); $^{13}C$ NMR ($CD_3OD$, 125 MHz) $\delta$ 159.6 (C-6), 156.5 (C-2), 150.4 (C-8′), 142.9 (C-4), 127.4 (C-2′), 112.0 (C-1), 110.4 (C-9′), 109.9 (C-5), 105.4 (C-3), 56.1 (OMe), 46.5 (C-4′), 37.4 (C-3′), 37.0 (C-1′′), 32.6 (C-3′′), 32.1 (C-2′′), 31.7 (C-5′), 30.8 (C-6′), 23.7 (C-7′), 23.6 (C-4′′), 19.4 (C-10′), 14.4 (C-5′′). HRESIMS *m/z* $[M–H]^-$ 327.2321 (calcd. for $C_{22}H_{31}O_2$, 327.2324).

*O-Methylcannabivarin (**3b**)*: Pale yellow oil. $[\alpha]_D^{25} = +102$ ($c = 0.2$ in $CH_3OH$). IR (KBr) $cm^{-1}$ 3433, 1615, 1589, 1456, 1429, 1211, 1168, 1101 $^1H$ NMR ($CD_3OD$, 500 MHz) $\delta$ 6.22 (1H, s, H-5), 6.20 (1H, s, H-3), 5.21 (1H, s, H-2′), 4.40 (2H, s, H-9′), 3.93 (1H, d, 9.8 Hz, H-3′), 3.68 (3H, s, -$OCH_3$), $\delta$ 2.89 (1H, td, 10.7 Hz, 4.7 Hz, H-4′), 2.44 (2H, t, 7.7 Hz, H-1′′), 2.18 (1H, m, H-6′a), 1.99 (1H, bd, 16.5 Hz, H-6′b), 1.74 (2H, m, H-5′), 1.66 (3H, s, H-10′), 1.60 (3H, s, H-7′), 1.60 (2H, overlapped, H-2′′), 0.92 (3H, t, 7.7 Hz, H-3′′); $^{13}C$ NMR ($CD_3OD$, 125 MHz) $\delta$ 159.6 (C-6), 156.5 (C-2), 150.4 (C-8′), 142.7 (C-4), 127.4 (C-2′), 112.0 (C-1), 110.4 (C-9′), 109.9 (C-5), 105.4 (C-3), 56.1 (OMe), 46.5 (C-4′), 39.1 (C-1′′), 37.4 (C-3′), 31.7 (C-5′), 30.8 (C-6′), 25.5 (C-2′′), 23.7 (C-7′), 19.4 (C-10′), 14.1 (C-3′′). HRESIMS *m/z* $[M–H]^-$ 299.2007 (calcd. for $C_{20}H_{27}O_2$, 299.2011).

## Analysis of *O*-methylcannabinoids in Cannabis flowerheads

Powdered plant material (1.0 g) was extracted with $CH_2Cl_2$ (2 × 10 mL). After evaporation to constant weight, a sample of the extract (5.0 mg) was diluted with $CH_2Cl_2$ (500 µL) and a solution of tribenzylamine (100 mg/mL) was added as the internal standard. GC analysis was carried out on a Trace GC apparatus coupled to a Polaris Q ion trap mass spectrometer (Thermo Finnigan). The gas chromatograph was operated in split mode using a 1-µL injection with the injector set and was maintained at 270 °C. Helium was used as the carrier gas at a flow rate of 1.0 mL/min. The separation was performed on a TG-5MS capillary column (30 m, 0.25 mm I.D., 0.25 mm thickness) (Thermo Fisher Scientific). The oven column temperature was programmed as follow: the initial temperature of 150 °C was maintained for 2 min and was next increased from 150 to 270 °C at a rate of 5 °C/min, eventually staying at 270 °C for 15 min. Electron ionization was operated at 70 eV. The transfer line and ion source were kept at 270 °C and 250 °C respectively. The MS was used in full scan (33–350 *m/z*) and tandem MS/MS. The selected parent/daughter ions transitions were: *m/z* 287 → 210 for **1b,** *m/z* 245 → 188 and *m/z* 245 → 174 for **2b**, and *m/z* 287 → 196 for the internal standard. Calibration curves were 2–2000 ppm for **1b** and 0.1–100 ppm for **2b**. The LOQ was 1 ppm for **1b** and 0.1 ppm for **2b.** The quantity (µg) of analyte per gram of each sample was calculated based on the concentration of analytes in the extracts, derived by interpolation with their respective calibration curves.

## Peroxisome proliferator-activated receptor activity assays

Human embryonic kidney epithelial cells 293 T cells were obtained from the American Type Culture Collection (CRL-3216) and cultured in DMEM supplemented with 10 % fetal calf serum and antibiotics. To analyze PPAR transcriptional activities, HEK-293 T cells were cultured in 24-well plates (2 × 104 cells/well) and transiently co-transfected with either GAL4-PPARγ (50 ng) or GAL4-PPARα (50 ng) vectors together with the luciferase reporter vectors GAL4-luc (Firefly luciferase) (50 ng) and pRL-CMV (Renilla luciferase) (100 ng) using Roti-Fect (Carl Roth). Twenty hours after transfection, the cells were stimulated with increasing concentrations of the compounds (1, 5, 10, 25, and 50 µM) for 6 h and luciferase activities were quantified using a Dual-Luciferase Assay (Promega). Rosiglitazone (1 µM) (Cayman Chemical) was used as a positive control for PPARγ activation (50-fold induction over basal activity) and WY14643 (5 µM) (Tocris Bioscience) was used as a positive control for PPARα activation (60-fold induction over basal activity). Test compounds and control stocks were prepared in DMSO, and the final concentration of the solvent was always less than 0.5 % vol/vol. The plasmids GAL4-PPARγ and GAL4-PPARγ were obtained from Prof. Christopher Sinal (Dalhousie University, Canada).

## Acknowledgements

We are grateful to MIUR for financial support to the groups in Novara and Naples (PRIN2017, Project 2017WN73PL, Bioactivity-directed exploration of the phytocannabinoid chemical space).

## Conflict of Interest

The authors declare no conflict of interest.

## References


[1] Hanuš LO, Meyer SM, Muñoz E, Taglialatela-Scafati O, Appendino G. Phytocannabinoids: a unified critical inventory. Nat Prod Rep 2016; 33: 1357–1392

[2] Pollastro F, Taglialatela-Scafati O, Allarà O, Muñoz E, Di Marzo V, De Petrocellis L, Appendino G. Bioactive prenylogous cannabinoid from fiber hemp (*Cannabis sativa*). J Nat Prod 2011; 74: 2019–2022

[3] Ahmed SA, Ross SA, Slade D, Radwan MM, Zulfiqar F, Matsumoto RR, Xu YT, Viard E, Speth RC, Karamyan VT, ElSohly MA. Cannabinoid ester constituents from high-potency *Cannabis sativa*. J Nat Prod 2008; 71: 536–542

[4] Mechoulam R, Ben-Zvi Z, Gaoni Y. Hashish – XIII: On the nature of the beam test. Tetrahedron 1968; 24: 5615–5624

[5] Yamauchi T, Shoyama Y, Matsuo Y, Nishioka K. Cannabigerol monomethyl ether, a new component of hemp. Chem Pharm Bull 1968; 16: 1164–1165

[6] Shoyama Y, Kuboe K, Nishioka I, Yamaguchi T. Cannabidiol monomethyl ether. A new neutral cannabinoid. Chem Pharm Bull 1972; 20: 2072

[7] Izzo AA, Borrelli F, Capasso R, Di Marzo V, Mechoulam R. Non-psychotropic plant cannabinoids: new therapeutic opportunities from an ancient herb. Trends Pharmacol Sci 2009; 10: 515–527

[8] Fuhr L, Rousseau M, Plauth A, Schroeder FC, Sauer S. Amorfrutins are Natural PPARγ Agonists with Potent Anti-inflammatory Properties. J Nat Prod 2015; 78: 1160–1164

[9] Takeda S, Noriyuki U, Yamamoto I, Watanabe K. Cannabidiol-2′,6′-dimethylether, a cannabidiol derivative, is a highly potent and selective 15-lipoxygenase inhibitor. Drug Metab Dispos 2009; 37: 1733–1737

[10] Presser A, Hufner A. Trimethylsilyldiazomethane – a mild and efficient reagent for the methylation of carboxylic acids and alcohols in natural products. Monatsh Chem 2004; 135: 1015–1022

[11] Choi YH, Hazekamp A, Peltenberg-Looman AMG, Frederich M, Erkelens C, Lefeber AWM, Verpoorte R. NMR assignment of the major cannabinoids and cannabiflavonoids isolated from flowers of *Cannabis sativa*. Phytochem Anal 2004; 15: 345–354

[12] Borrelli F, Pagano E, Romano B, Panzera S, Maiello F, Coppola D, De Petrocellis L, Buono L, Orlando P, Izzo AA. Colon carcinogenesis is inhibited by the TRPM8 antagonist cannabigerol, a Cannabis-derived non-psychotropic cannabinoid. Carcinogenesis 2014; 35: 2787–2797