DOI: 10.1002/ejoc.200901464

Cannabimovone, a Cannabinoid with a Rearranged Terpenoid Skeleton from Hemp

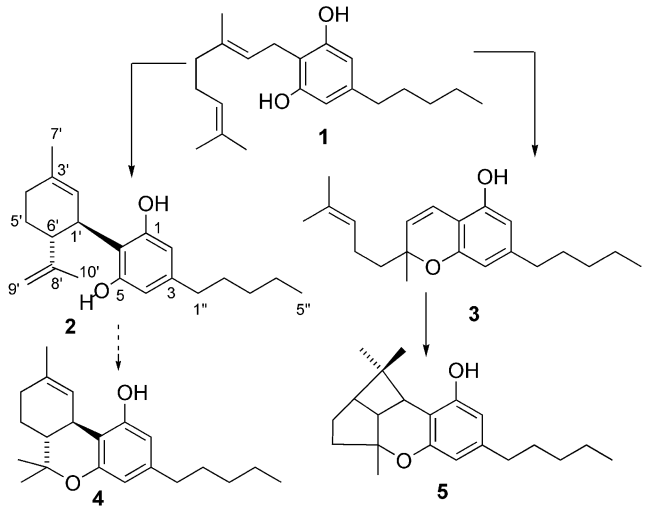
Orazio Taglialatela-Scafati,*[a] Alberto Pagani,[b] Fernando Scala,[a] Luciano De Petrocellis,[c] Vincenzo Di Marzo,[d] Gianpaolo Grassi,[e] and Giovanni Appendino*[b]

Keywords: Hemp / Phytocannabinoids / Aldol reactions / Biomimetic synthesis / Cannabinoid receptors / Terpenoids

An investigation of the polar fractions from a nonpsychotropic variety of hemp (*Cannabis sativa* L.) afforded cannabimovone (**6**), a polar cannabinoid with a rearranged 2(3→4) *abeo*-terpenoid skeleton, biogenetically originating from the intramolecular aldolization of a 2′,3′-*seco*-menthanyl precursor. The structure of cannabimovone was elucidated by spectroscopic analysis, whereas attempts to mimic its biogenetic derivation from cannabidiol (**2**) gave only anhydrocannabimovone (**12**), the intramolecular oxy-Michael adduct of the crotonized version (**11b**) of the elusive natural products. Biological evaluation of cannabimovone (**6**) against metabotropic (CB_1, CB_2) and ionotropic (TRPs) cannabinoid receptors showed a significant activity only for ionotropic receptors, especially TRPV1, whereas anhydrocannabimovone (**12**) exhibited strong activity at both ionotropic and metabotropic cannabinoid receptors. Overall, the biological profile of anhydrocannabimovone (**12**) was somewhat similar to that of THC (**4**), suggesting a remarkable tolerance to constitutional and configurational changes.

Introduction

With an inventory of over 500 secondary metabolites identified so far, *Cannabis sativa* L. is one of the phytochemically best characterized plant species.[1] The biomedical and agricultural relevance of hemp undoubtedly underlies the wealth of data on its constituents and their biological activities,[2] and cannabinoids, a class of unique meroterpenoids derived from the alkylation of olivetol with a monoterpene unit, are the most typical constituents of cannabis.[3] Over 100 different cannabinoids have been described,[1] basically derived from the geranyl derivative cannabigerol (CBG, **1**, Scheme 1) either by oxidative prototropic cyclization [cannabidiol (CBD, **2**)] or by oxidative pericyclic cyclization [cannabichromene (CBC, **3**)]. Further elaboration involves formal intramolecular cyclization either in a prototropic [tetrahydrocannabinol (THC, **4**)] or in a pericyclic way (cannabicyclol, **5**).[4]

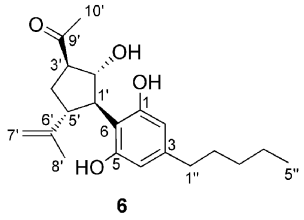


Scheme 1. Biogenetic relationships of the major phytocannabinoids.

Most phytocannabinoids are derived from the dehydrogenation or oxidative modification of these five primary terpenoid skeletons, so structural variation within these compounds is limited.[1]

[a] Dipartimento di Chimica delle Sostanze Naturali, Università di Napoli Federico II,
Via Montesano 49, 80131 Napoli, Italy
Fax: +39-081-678552
E-mail: scatagli@unina.it
[b] Dipartimento di Scienze Chimiche, Alimentari, Farmaceutiche e Farmacologiche, Università del Piemonte Orientale,
Viale Ferrucci 33, 28100 Novara, Italy
Fax: +39-0321-375621
E-mail: appendino@pharm.unipmn.it
[c] Endocannabinoid Research Group, Institute of Cybernetics, Consiglio Nazionale delle Ricerche,
Pozzuoli (NA), Italy
[d] Endocannabinoid Research Group, Institute of Biomolecular Chemistry, Consiglio Nazionale delle Ricerche,
Pozzuoli (NA), Italy
[e] CRA-CIN,
Viale G. Amendola 82, 45100 Rovigo, Italy
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejoc.200901464.

In past centuries, the unmatched excellence of some Italian varieties of fiber hemp was recognized worldwide.[5] Remarkably, there is only limited phytochemical information available for these plants, which have so far been investigated mostly for the presence of THC and some of the major cannabinoids.[5] While investigating the phenolic fraction from an industrial Italian cultivar of hemp rich in CBD (**2**), we isolated a cannabinoid with an unprecedented *abeo*-menthane terpenoid structure.[6] We named this compound cannabimovone (**6**, Figure 1), and here we present details on its structure elucidation, its attempted biomimetic preparation from CBD (**2**), and its biological profile against a series of metabotropic and ionotropic phytocannabinoid targets.[7]



6

Figure 1. Structure of cannabimovone (**6**).

Results and Discussion

Cannabimovone (**6**, Figure 1) was isolated as an optically active, light yellow, amorphous solid from a polar fraction of an acetone extract of a CBD-rich nonpsychotropic variety of *C. sativa* derived from the ancient Italian variety "Carmagnola".[5] Its ESI (positive ions) mass spectrum exhibited pseudomolecular ions at *m/z* 347 ($[M + H]^+$) and 369 ($[M + Na]^+$). These data, along with 1D NMR spectroscopic data, pointed to the molecular formula $C_{21}H_{30}O_4$, indicative of seven degrees of unsaturation, as confirmed by HREIMS. The ^1H NMR spectrum of **6** (500 MHz, $CDCl_3$, Table 1) displayed two relatively downfield-shifted methyl singlets (δ_H = 1.70 and 2.27 ppm) and a methyl triplet at δ_H = 0.87 ppm. In addition to these signals, the proton spectrum was completed by a 2H sharp singlet at δ_H = 6.18 ppm and two 1H broad singlets at δ_H = 4.71 and 4.63 ppm, four multiplets between δ_H = 3.00 and 4.80 ppm, and a series of signals in the δ_H = 1.20–2.50 ppm region. The ^{13}C NMR spectrum of **6** (125 MHz, $CDCl_3$, Table 1) showed 19 distinct resonances, including a ketone carbonyl at δ_C = 211.1 ppm and six signals in the sp^2 region. Both the presence of a 2H singlet at δ_H = 6.18 ppm in the ^1H NMR spectrum and the overlap of two sets of carbon resonances in the ^{13}C NMR spectrum could be explained by the presence of a symmetrically tetrasubstituted phenyl ring.

The combined inspection of 2D NMR COSY and HSQC spectra indicated two spin systems (highlighted in bold in Figure 2): the first one was a typical *Cannabis* phenyl-linked pentyl moiety, whereas the second was attributed to a tetrasubstituted cyclopentyl moiety bearing an oxymethine carbon (δ_H = 4.73 ppm, δ_C = 77.9 ppm). Moreover, the HSQC spectrum associated the two singlets at δ_H = 4.71 and 4.63 ppm with the same carbon at δ_C = 110.4 ppm, implying the presence of an olefinic methylene moiety.

Table 1. ^{13}C (125 MHz) and ^1H (500 MHz) NMR data for cannabimovone (**6**) in $CDCl_3$.

Position	δ_H (mult., *J* in [Hz])	δ_C
1/5		155.4 (C)
2/4	6.18 (s)	109.0 (CH)
3		144.1 (C)
6		110.0 (C)
1′	3.52 (dd, 11.3, 8.9)	48.4 (CH)
2′	4.73 (dd, 8.9, 6.8)	77.9 (CH)
3′	3.03 (ddd, 11.2, 6.8, 6.8)	58.0 (CH)
4′a	2.13 (m)	31.2 (CH_2)
4′b	2.04 (m)	
5′	3.35 (ddd, 11.3, 11.3, 9.2)	45.3 (CH)
6′		146.3 (C)
7′a	4.71 (br. s)	110.4 (CH_2)
7′b	4.63 (br. s)	
8′	1.70 (s)	20.0 (CH_3)
9′		211.1 (C)
10′	2.27 (s)	29.5 (CH_3)
1′′	2.41 (t, 7.6)	35.5 (CH_2)
2′′	1.55 (m)	30.7 (CH_2)
3′′	1.30 (m)	31.5 (CH_2)
4′′	1.27 (m)	22.7 (CH_2)
5′′	0.87 (t, 7.0)	14.3 (CH_3)

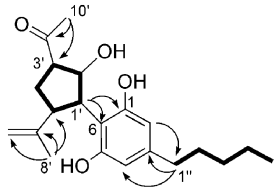
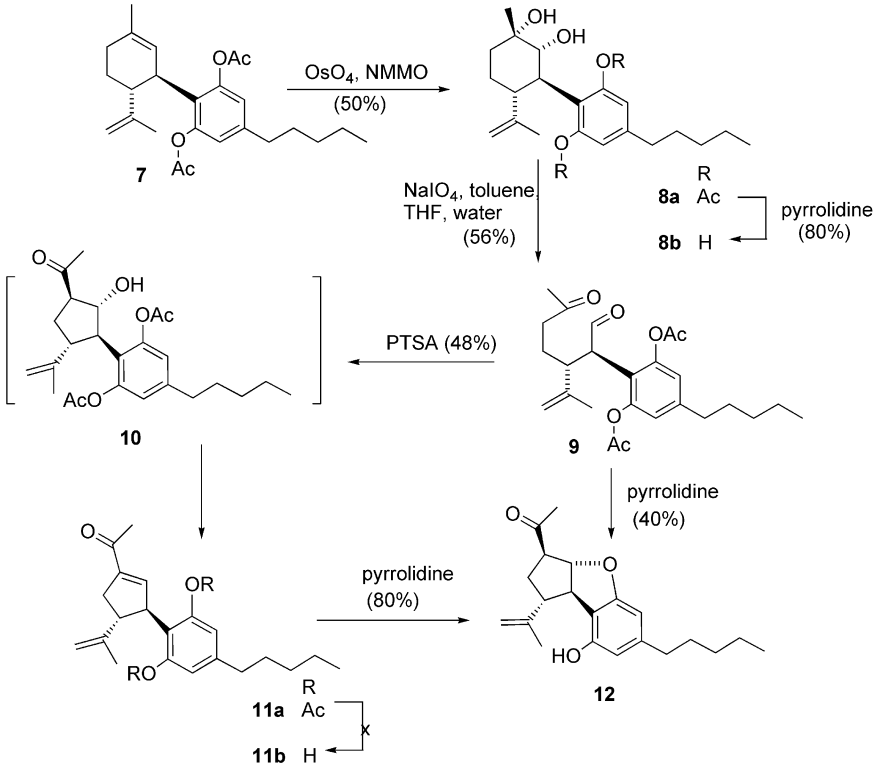


Figure 2. COSY (in bold) and key H→C HMBC correlations observed in cannabimovone (**6**).

The 2D NMR HMBC spectrum (see Figure 2) provided critical information with which the structure of cannabimovone (**6**) could be pieced together. In particular, H_3-10′ showed $^{2,3}J_{H,C}$ cross-peaks with the ketone carbonyl and with C-3′, showing the presence of an acetyl group on the cyclopentane ring. An isopropenyl group was also linked to the five-membered ring on the basis of the HMBC cross-peaks of H_3-8′ with C-5′, C-6′, and C-7′. Finally, cross-peaks of H-1′ with C-6 and with the oxygen-bearing carbon atoms C-1/C-5 (δ_C = 155.4 ppm) and of H-1′′ with C-3 and with the protonated C-2/C-4 (δ_C = 109.0 ppm) aromatic pair completely defined the planar structure of cannabimovone (**6**).

The five-membered ring of this compound features four stereogenic centers, the relative orientation of which was assessed on the basis of the 2D NMR ROESY spectrum. The ROESY cross-peaks of H-3′/H-1′ and H-2′/H-5′ indicated the *cis* orientation of these protons. The spatial proximity of H-3′ with H-7′ indicated their *trans* relative orientation.

A plausible biogenetic derivation of cannabimovone begins with CBD (**2**), the major constituent of the extract, and involves stereoselective dihydroxylation of the endocyclic double bond, followed by oxidative cleavage of the



Scheme 2. Attempted biomimetic synthesis of cannabimovone.

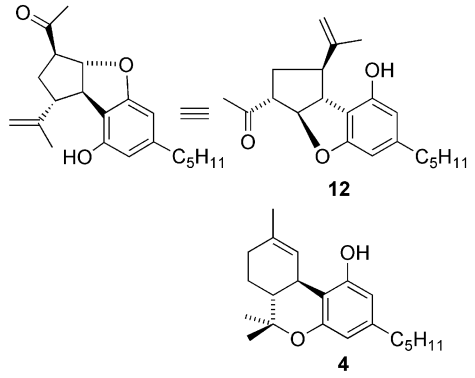
glycol system and stereoselective aldolization of the resulting dicarbonyl intermediate (Scheme 2). In view of the plausibility of this scheme, it was assumed that the absolute configuration of **6** should be the same as that of its precursor.

Only minute amounts of cannabimovone (**6**) were obtained by isolation, and so it was attempted to synthesize cannabimovone from its easily available putative precursor. CBD was degraded during dihydroxylation, due to the high sensitivity of its resorcinyl moiety to oxidation,[8] but protection of the two phenolic hydroxy groups made the reaction manageable. Excellent chemoselectivity has been reported in the epoxidation of CBD,[9] and we were therefore confident that the dihydroxylation of its acetate (**7**) could proceed with preferential attack of the endocyclic double bond. Considerable efforts were in fact required to steer dihydroxylation chemoselectively to the endocyclic double bond, and the reaction course was strongly dependent on the protocol employed.

The best results in terms of chemoselectivity were observed with the Upjohn protocol (cat. OsO_4/NMMO), whereas the asymmetric dihydroxylation (AD-mix-α or AD-mix-β) was poorly selective, affording almost equimolar mixtures of the endocyclic and the exocyclic diols. Conversely, excellent stereoselectivity was observed in the attack at the endocyclic double bond, with exclusive attack *trans* to the olivetyl moiety.[10] The diol **8a** was surprisingly stable with respect to acyl migration, an event that would have required remodulation of the synthesis, with a change from ester to ether protection for the phenolic hydroxy groups. Periodate cleavage of **8a** afforded the keto-aldehyde **9**, which proved rather resistant to aldolization, presumably because of steric strain in its aldolization product **10**, in which the cyclopentane ring features four adjacent substituents. Despite considerable experimentation, only the crotonized analogue of the natural product (**11a**) could be obtained in acidic medium, whereas under basic conditions (pyrrolidine) crotonization was accompanied by loss of the phenolic acetates and eventual formation of the intramolecular oxy-Michael adduct **12** (anhydrocannabinovone) as the only reaction product. The Michael addition was completely stereoselective, affording exclusively the isomer with a *trans* relationship between the oxygen bridge and the acetyl group. The fast Michael trapping of **11b** was further attested to by the impossibility of deacetylation of the enone **11a** without formation of the tricyclic compound **12**. Eventually, under both acidic and basic conditions, isolation of the natural product from the aldolization mixture remained elusive. While not disproving the biogenetic proposal for the formation of cannabimovone (**6**), these results point to the existence of considerable steric tension in the natural product, consistently with the surprising stability of the keto-aldehyde **9** toward aldolization. Compound **12** could not be isolated from the extract that yielded **6**, testifying to the mildness of the isolation scheme employed during the fractionation step.

Phytocannabinoids can interact with a host of metabotropic (CB_1 and CB_2) and ionotropic (TRPV1, TRPM8, and TRPA1) targets[7] and in view of the structural novelty of cannabimovone (**6**) it was interesting to assess its profile

of activity toward these end-points. The cyclized analogue (anhydrocannabimovone, **12**) and the oxidized *p*-menthane precursor (dihydroxy-CBD, **8b**) of the natural product were also investigated. Cannabimovone (**6**) and dihydroxy-CBD (**8b**) were devoid of significant affinity for either CB1 or CB2 (K_i > 10 μM) receptors, but anhydrocannabimovone (**12**) showed high affinity for both receptors (K_i ca. 100 nM). On the other hand, cannabimovone (**6**) was a full, albeit weak, TRPV1 agonist (EC_{50} = 6.4 μM), more potent than **8b** (EC_{50} = 17.1 μM) and more efficacious than **12**. It also activated TRPA1 (EC_{50} = 6.3 μM). In comparison, **12** was more potent (EC_{50} = 1.7 μM) and efficacious toward TRPA1, whereas **8b** exhibited weaker potency (EC_{50} = 15.4 μM) and efficacy than the natural product. Finally, of the three compounds investigated, only **12**, like several plant cannabinoids,[7] exhibited significant functional antagonist activity at TRPM8 channels (IC_{50} = 1.83 μM). Taken together, these observations suggest that cannabimovone (**6**) has a biological profile similar to that of its biogenetic precursor CBD, with modest affinity for metabotropic cannabinoid (CB1, CB2) receptors and relatively good activity at TRPV1. On the other hand, anhydrocannabimovone (**12**) exhibited potent cannabinoid activity at both CB1 and CB2 receptors, as well as at TRPA1 channels and TRPM8, mimicking to some extent the biological profile of THC (**4**).[7] Indeed, as highlighted in Scheme 3, the tricyclic cannabinoid skeleton of anhydrocannabimovone (**12**) shows a certain structural similarity with THC (**4**), suggesting a surprising preservation of the biological profile through changes of constitution and configuration.



Scheme 3. Structural similarity between anhydrocannabimovone (**12**) and THC (**4**).

Conclusions

Cannabis is one of the most thoroughly investigated plants in terms of secondary metabolites,[1] and the isolation of a cannabinoid of a novel skeletal type highlights the potential of modern techniques to expand the current inventory of natural products substantially, affording, even from well known "old" plants, new chemotypes useful to explore uncharted areas of the druggable space.

Experimental Section

General: Optical rotations ($CHCl_3$) were measured at 589 nm with a Perkin–Elmer 192 polarimeter with a sodium lamp (λ = 589 nm) and a 10 cm microcell. ^1H (500 MHz) and ^{13}C (125 MHz) NMR spectra were measured with a Varian INOVA spectrometer. Chemical shifts were referenced to the residual solvent signal ($CDCl_3$: δ_H = 7.26 ppm, δ_C = 77.0 ppm). Homonuclear ^1H connectivities were determined by COSY experimentation. One-bond heteronuclear ^1H–^{13}C connectivities were determined by HSQC experiments. Through-space ^1H connectivities were determined by use of a ROESY experiment with a mixing time of 500 ms. Two- and three-bond ^1H–^{13}C connectivities were determined by means of gradient 2D HMBC experiments optimized for $^{2,3}J$ = 9 Hz. Low- and high-resolution EIMS spectra (70 eV) were performed with a VG Prospec (Fisons) mass spectrometer. ESIMS spectra were performed with a LCQ Finnigan MAT mass spectrometer. Silica gel 60 (70–230 mesh) was used for gravity column chromatography. Reactions were monitored by TLC on Merck plates (60 F_{254}, 0.25 mm), which were visualized by UV inspection and/or staining with H_2SO_4 in ethanol (5%) and heating. Organic phases were dried with Na_2SO_4 before evaporation.

Plant Material: *C. sativa*, derived from a greenhouse cultivation at CRA-CIN, Rovigo (Italy), where a voucher specimen is kept, was collected in November 2008. The isolation and manipulation of all cannabinoids was done in accordance with their legal status (Authorization SP/101 of the Ministero della Salute, Rome, Italy).

Extraction and Isolation of Cannabimovone (6): Dried flowerheads of *C. sativa* (500 g) were heated at 120 °C in a ventilated oven for 2.5 h to decarboxylate pre-cannabinoids. After cooling to room temp., the plant material was extracted with acetone (2 × 10 L). Removal of the solvent left a gummy residue that was partitioned between 1:1 aqueous methanol (1 L) and petroleum ether (1 L). The defatted polar phase was concentrated and extracted with CH_2Cl_2. The organic phase was dried (Na_2SO_4) and evaporated to afford a black gum (10 g), which was purified by flash chromatography on RP-18 silica gel (Biotage equipment, 250 mL column, linear gradient, from methanol water 55:45 to 90:10). Overall, five fractions were collected. The more polar one was further fractionated by gravity column chromatography on silica gel, with use of acidified (0.5% HOAc) petroleum ether/EtOAc mixtures. After four chromatographic steps, crude cannabimovone (10 mg) was obtained from a fraction directly eluted after the stilbenoid cannabiprene. The crude fraction was further purified by HPLC (eluent *n*-hexane/EtOAc 7:3) to provide pure cannabimovone (**6**, 7.0 mg, 14 ppm based on dried plant material).

Cannabimovone (6): Colorless, amorphous solid. $[\alpha]_D^{22}$ = –10 (*c* = 0.07, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): see Table 1. ^{13}C NMR (125 MHz, $CDCl_3$): see Table 1. ESI-MS: *m*/*z* = 347 $[M + H]^+$, 369 $[M + Na]^+$. HREIMS calcd. for $C_{21}H_{30}O_4$ $[M]^+$ 346.2144; found 346.2148.

Diacetylcannabidiol (7): Acetic anhydride (188.0 μL, 6 equiv.) and DMAP (19.5 mg, 0.5 equiv.) were added to a solution of cannabidiol (**2**, 100 mg, 0.32 mmol) in pyridine (300 μL). After having been stirred at room temperature for 16 h, the reaction mixture was worked up with methanol (200 μL) to destroy excess acetic anhydride, and washed five times with H_2SO_4 (2 N) and with brine. The organic phase was dried with Na_2SO_4 and filtered, and the solvents were evaporated. The residue was purified by column chromatography on silica gel (hexane/EtOAc 98:2) to afford 1,5-diacetylcannabidiol (**7**, 54 mg, 0.14 mmol, 44% yield) as a viscous, colorless oil. ^1H NMR (500 MHz, $CDCl_3$): δ_H = 6.86, 6.82 (2 × s, 2 H, 2-H,

4-H), 5.56 (br. s, 1 H, 2′-H), 4.67 (br. s, 1 H, 9′a-H), 4.61 (br. s, 1 H, 9′b-H), 3.92 (m, 1 H, 1′-H), 3.20 (m, 1 H, 6′-H), 2.51 (m, 2 H, 1′′-H), 2.32 (s, 6 H, 2×OAc), 2.21 (m, 2 H, 4′-H), 1.84 (m, 2 H, 5′-H), 1.80 (s, 3 H, 7′-H), 1.68 (s, 3 H, 10′-H), 1.56 (q, J = 7.6 Hz, 2 H, 2′′-H), 1.30 (overlapped signals, 4 H, 3′′-H, 4′′-H), 0.88 (t, J = 7.0 Hz, 3 H, 5′′-H) ppm. ESI-MS: m/z = 421 $[M + Na]^+$.

1,5-Diacetyl-2′,3′-dihydroxycannabidiol (8a): OsO_4 (cat., 25.4 μL of a 5.0% toluene solution) and *N*-methylmorpholine oxide (NMMO, 117 mg, 4 mol. equiv.) were added to a solution of 1,5-diacetylcannabidiol (**7**, 100 mg, 0.25 mmol) in acetone/water (4:1, 800 μL). After the system had been stirred at room temperature for 6 h, further *N*-methylmorpholine oxide (117 mg, 4 equiv.) was added, and after 6 h the reaction mixture was worked up by dilution with EtOAc and sequential washing with H_2SO_4 (2 N), satd. $NaHCO_3$, and brine. After drying (Na_2SO_4), the organic phase was filtered and concentrated, and the residue was purified by gravity column chromatography on silica gel (hexane/EtOAc 9:1→8:2 as eluent) to afford 1,5-diacetyl-2′,3′-dihydroxycannabidiol (**8a**, 54 mg, 0.12 mmol, 50% yield) as an amorphous, white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ_H = 6.87, 6.83 (2×s, 2 H, 2-H, 4-H), 4.68 (br. s, 1 H, 9′a-H), 4.62 (br. s, 1 H, 9′b-H), 3.67 (br. d, J = 8.5 Hz, 1 H, 2′-H), 3.20 (t, J = 8.5 Hz, 1 H, 1′-H), 2.60 (ddd, J = 8.5, 8.5, 2.1 Hz, 1 H, 6′-H), 2.55 (t, J = 7.0 Hz, 2 H, 1′′-H), 2.40 (s, 3 H, OAc), 2.36 (s, 3 H, OAc), 1.84 (m, 1 H, 5′a-H), 1.68 (s, 3 H, 10′-H), 1.65 (m, 1 H, 5′b-H), 1.50 (m, 2 H, 2′′-H), 1.40 (m, 1 H, 4′a-H), 1.36 (overlapped signals, 1 H, 4′b-H), 1.32 (m, 4 H, 3′′-H, 4′′-H), 1.29 (s, 3 H, 7′-H), 0.88 (t, J = 7.3 Hz, 3 H, 5′′-H) ppm. ESI-MS: m/z = 453 $[M + Na]^+$.

Compound **8a** was deacetylated by treatment with 10 equimolecular of pyrrolidine in CH_2Cl_2, to afford **8b** (80% yield) as an amorphous solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ_H = 6.28 (br. s, 2 H, 2-H, 4-H), 4.66 (br. s, 1 H, 9′a-H), 4.61 (br. s, 1 H, 9′b-H), 3.69 (br. d, J = 8.5 Hz, 1 H, 2′-H), 3.16 (t, J = 8.5 Hz, 1 H, 1′-H), 2.61 (ddd, J = 8.5, 8.5, 2.1 Hz, 1 H, 6′-H), 2.47 (t, J = 7.0 Hz, 2 H, 1′′-H), 1.87 (m, 1 H, 5′a-H), 1.71 (s, 3 H, 10′-H), 1.62 (m, 1 H, 5′b-H), 1.51 (m, 2 H, 2′′-H), 1.41 (m, 1 H, 4′a-H), ca. 1.35 (m, 1 H, 4′b-H), 1.32 (m, 4 H, 3′′-H, 4′′-H), 1.26 (s, 3 H, 7′-H), 0.89 (t, J = 7.3 Hz, 3 H, 5′′-H) ppm. ESI-MS: m/z = 369 $[M + Na]^+$.

Periodate Cleavage of 1,5-Diacetyl-2′,3′-dihydroxycannabidiol (8a): Sodium periodate (214 mg, 5.0 equiv.) was added to a solution of 1,5-diacetyl-2′,3′-dihydroxycannabidiol (**8a**, 100 mg, 0.23 mmol) in toluene/THF/H_2O (1:1:1, 1.0 mL). After having been stirred at room temperature for 16 h the reaction mixture was worked up by dilution with EtOAc and washing with satd. $NaHCO_3$ and brine. After drying (Na_2SO_4), filtration, and concentration, the residue was purified by gravity column chromatography on silica gel (hexane/EtOAc 9:1) to afford compound **9** (56 mg, 56%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ_H = 9.72 (s, 1 H, 2′-H), 6.18 (s, 2 H, 2-H, 4-H), 4.68 (br. s, 1 H, 9′a-H), 4.52 (br. s, 1 H, 9′b-H), 3.80 (m, 1 H, 1′-H), 3.52 (m, 1 H, 6′-H), 2.77 (m, 1 H, 4′a-H), 2.56 (t, J = 7.6 Hz, 2 H, 1′′-H), 2.41 (m, 1 H, 4′b-H), 2.16 (s, 3 H, 7′-H), 2.07 (s, 6 H, 2×OAc), 1.78 (m, 2 H, 5′-H), 1.72 (s, 3 H, 10′-H), 1.58 (q, J = 7.6 Hz, 2 H, 2′′-H), 1.30 (overlapped signal, 2 H, 3′′-H), 1.28 (overlapped signal, 2 H, 4′′-H), 0.89 (t, J = 7.0 Hz, 3 H, 5′′-H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ_C = 209.2 (s, 2′-C), 198.9 (s, 3′-C), 169.4 (s, OAc), 150.5 (s, 1-C), 150.5 (s, 5-C), 145.2 (s, 8′-C), 143.8 (s, 3-C), 121.4 (t, 9′-C), 117.9 (s, 6-C), 115.6 (d, 2-C), 115.6 (d, 4-C), 54.7 (d, 1′-C), 45.0 (d, 6′-C), 42.3 (t, 4′-C), 36.1 (t, 1′′-C), 32.1 (t, 3′′-C), 30.9 (t, 2′′-C), 29.8 (q, 7′-C), 25.6 (t, 5′-C), 22.2 (t, 4′′-C), 21.0 (q, OAc), 17.8 (q, 10′-C), 14.3 (q, 5′′-C) ppm. ESI-MS: m/z = 451 $[M + Na]^+$.

Crotonization of the *seco*-Cannabinoid 9: *p*-Toluenesulfonic acid (PTSA, 394 mg, 1.0 equiv.) was added to a solution of **9** (100 mg, 0.23 mmol) in THF (1.0 mL). After having been stirred at room temperature for 12 h, the reaction mixture was worked up by dilution with CH_2Cl_2 and sequential washing with satd. $NaHCO_3$ and brine. The organic phase was dried with Na_2SO_4, filtered, and concentrated, and the residue was purified by gravity column chromatography on silica gel (hexane/EtOAc 9:1 as eluent) to afford **11a** (43 mg, 0.11 mmol, 48% yield) as a yellowish, amorphous solid. $[\alpha]_D^{22}$ = –52 (c = 0.10, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ_H = 6.75 (s, 2 H, 2-H, 4-H), 6.55 (d, J = 1.5 Hz, 1 H, 2′-H), 4.74 (s, 1 H, 7′a-H), 4.73 (s, 1 H, 7′b-H), 4.16 (dd, J = 7.5, 1.5 Hz, 1 H, 1′-H), 3.18 (ddd, J = 1.5, 7.5, 7.3, 2.5 Hz, 1 H, 5′-H), 2.91 (dd, J = 12.1, 7.3 Hz, 1 H, 4′a-H), 2.56 (overlapped signal, 1 H, 4′b-H), 2.55 (overlapped signal, 2 H, 1′′-H), 2.30 (s, 3 H, 10′-H), 2.17 (s, 6 H, 2×OAc), 1.70 (s, 3 H, 8′-H), 1.60 (m, 2 H, 2′′-H), 1.32 (m, 4 H, 3′′-H, 4′′-H), 0.88 (t, J = 7.3 Hz, 3 H, 5′′-H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ_C = 196.9 (s, 9′-C), 169.2 (s, 2×OAc), 150.1 (s, 1-C, 5-C), 146.1 (s, 6′-C), 144.2 (s, 3-C), 143.7 (s, 3′-C), 123.9 (d, 2′-C), 123.7 (d, 2-C, 4-C), 121.0 (s, 6-C), 111.6 (t, 7′-C), 52.8 (d, 5′-C), 48.2 (d, 1′-C), 36.2 (t, 4′-C), 35.7 (t, 1′′-C), 31.7 (t, 3′′-C), 31.0 (t, 2′′-C), 29.2 (q, 1′-C), 23.8 (t, 4′′-C), 20.5 (q, 8′-C), 20.4 (2×OAc), 14.3 (q, 5′′-C) ppm. ESI-MS: m/z = 435 $[M + Na]^+$.

Anhydrocannabimovone (12): Pyrrolidine (60 μL, 13 equiv.) was added to a solution of **11a** (100 mg, 0.24 mmol) in CH_2Cl_2 (1.0 mL). After having been stirred at room temperature for 12 h, the reaction mixture was worked up by dilution with brine and extraction with CH_2Cl_2. The organic phase was dried with Na_2SO_4, filtered, and concentrated, and the residue was purified by gravity column chromatography on silica gel (hexane/EtOAc 9:1 as eluent) to yield **12** (63 mg, 80%) as a yellow, amorphous solid. $[\alpha]_D^{22}$ = –17 (c = 0.02, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ_H = 6.15 (s, 1 H, 4-H), 6.11 (s, 1 H, 2-H), 5.54 (dd, J = 3.5, 3.5 Hz, 1 H, 2′-H), 4.87 (s, 1 H, 7′a-H), 4.71 (s, 1 H, 7′b-H), 4.68 (br. s, 1 H, OH), 3.93 (dd, J = 3.5, 1.5 Hz, 1 H, 1′-H), 3.23 (m, 1 H, 3′-H), 2.85 (br. s, 1 H, 5′-H), 2.46 (d, J = 7.0 Hz, 2 H, 1′′-H), 2.32 (s, 3 H, 10′-H), 2.07 (m, 1 H, 4′a-H), 1.84 (s, 3 H, 8′-H), 1.81 (m, 1 H, 4′b-H), 1.54 (m, 2 H, 2′′-H), 1.29 (m, 4 H, 3′′-H, 4′′-H), 0.89 (t, J = 7.3 Hz, 3 H, 5′′-H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ_C = 206.1 (s, 9′-C), 161.4 (s, 1-C), 157.9 (s, 5-C), 148.3 (s, 6′-C), 145.5 (s, 3-C), 115.7 (s, 6-C), 109.7 (t, 7′-C), 108.2 (d, 4-C), 102.3 (d, 2-C), 91.2 (d, 2′-C), 57.4 (d, 3′-C), 56.0 (d, 1′-C), 50.6 (d, 5′-C), 35.7 (t, 1′′-C), 32.3 (t, 3′′-C), 31.1 (t, 2′′-C), 28.5 (q, 10′-C), 27.4 (t, 4′-C), 23.6 (t, 4′′-C), 20.7 (q, 8′-C), 14.3 (q, 5′′-C) ppm. ESI-MS: m/z = 351 $[M + Na]^+$.

TRPV1, TRPM8, TRPA1 Receptor Assays: HEK293 (human embryonic kidney) cells were grown as monolayers in minimum essential medium supplemented with nonessential amino acids, fetal calf serum (10%), and glutamine (2 mM), maintained under CO_2 (5%) at 37 °C, and plated on Petri dishes (100 mm diameter). The cells were transfected at approximately 80% confluence with Lipofectamine 2000 (Invitrogen, Carlsbad, CA) with the aid of a plasmid pcDNA3 (Invitrogen) containing human TRPV1-cDNA, rat TRPA1-cDNA, or rat TRPM8-cDNA according to the manufacturer's protocol. Stably transfected clones were selected by use of G-418 (Geneticin, 600 μg mL^{-1}). The effect of the substances on $[Ca^{2+}]_i$ was determined by use of Fluo-4, a selective intracellular fluorescent probe for Ca^{2+}. For this purpose, on the day of the experiment, cells overexpressing the TRPV1, or the TRPM8, or the TRPA1 channels were loaded for 1 h at room temperature with the methyl ester Fluo4-AM (4 μM, Invitrogen) in minimum essential medium without fetal bovine serum. After the loading, cells were washed twice in Tyrode's buffer [NaCl (145 mM), KCl (2.5 mM), $CaCl_2$ (1.5 mM), $MgCl_2$ (1.2 mM), D-glucose (10 mM), and HEPES

(10 mM), pH 7.4], resuspended in Tyrode's buffer, and transferred (50–60000 cells) to the quartz cuvette of the spectrofluorimeter (Perkin–Elmer LS50B; Perkin–Elmer Life and Analytical Sciences, Waltham, MA) with continuous stirring. Intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) was determined before and after the addition of various concentrations of test compounds by measuring cell fluorescence at 25 °C (λ_{EX} = 488 nm, λ_{EM} = 516 nm). Curve fitting (sigmoidal dose/response variable slope) and parameter estimation were performed with GraphPad Prism® (GraphPad Software Inc., San Diego, CA). Potency was expressed as the concentration of test substances exerting a half-maximal agonist effect (i.e. half-maximal increases in $[Ca^{2+}]_i$, EC_{50}), calculated with use of GraphPad®. The efficacies of the agonists were first determined by normalizing their effects to the maximum Ca^{2+} influx effect on $[Ca^{2+}]_i$ observed with application of ionomycin (Sigma, 4 μM). The effects of TRPA1 agonists are expressed as percentages of the effect obtained with allyl isothiocyanate (100 μM). In the case of TRPM8 the experiments were carried out at 22 °C with a Fluorescence Peltier System (PTP-1, Perkin–Elmer): varying doses of the compounds were added directly to the quartz cuvette with stirring 5 min before stimulation of cells with icilin (0.25 μM). Data were expressed as the concentrations exerting half-maximal inhibition of agonist $[Ca^{2+}]_i$ increasing effect (IC_{50}), which was again calculated with the aid of GraphPad Prism® software. The effect on $[Ca^{2+}]_i$ exerted by icilin (0.25 μM) was taken as 100%. All determinations were performed at least in triplicate. Statistical analysis of the data was performed by analysis of variance at each point with the aid of ANOVA, followed by Bonferroni's test. At higher concentrations (>10 μM), compound **12** caused an effect on $[Ca^{2+}]_i$ in cells transfected both with and without any TRP construct, and the values obtained from the latter were taken as baselines to be subtracted from the former.

CB_1 and CB_2 Receptor Binding Assays: Membranes harvested from human recombinant CB_1 (B_{max} = 2.5 pmol per mg protein) or CB_2 (B_{max} = 4.7 pmol per mg protein) receptor transfected HEK-293 cells were incubated with the high-affinity ligand [^{3}H]-CP-55,940 (0.14 nM, K_d = 0.18 nM, or 0.084 nM, K_d = 0.31 nM for CB_1 and CB_2, respectively), and displaced with 10 μM of the heterologous competitor for nonspecific binding (WIN 55212-2, K_i values 9.2 nM and 2.1 nM for CB_1 and CB_2, respectively). All compounds were assayed according to the manufacturer's (Perkin–Elmer, Italy) instructions. Increasing concentrations of compounds were incubated with [^{3}H]-CP-55,940 for 90 min at 30 °C to generate displacement curves. IC_{50} values of the test compounds for the displacement of the bound radioligand were obtained by use of GraphPad Prism® and used to calculate K_i values with the aid of the Cheng–Prusoff equation. Data are represented as means ± SEMs of at least n = 3 experiments.

Supporting Information (see also the footnote on the first page of this article): Selected NMR spectra.

Acknowledgments

We gratefully thank Aniello Schiano Moriello (for TRP receptor assays) and Marco Allarà (for CB_1 and CB_2 receptor binding assays). Mass and NMR spectra were recorded at the "Centro di Servizio Interdipartimentale di Analisi Strumentale", Università di Napoli "Federico II". The assistance of the staff is acknowledged.

[1] a) C. E. Turner, M. A. Elsohly, E. G. Boeren, *J. Nat. Prod.* **1980**, *43*, 169–234; b) M. A. ElSohly, D. Slade, *Life Sci.* **2005**, *78*, 539–548.
[2] R. G. Pertwee, *Brit. J. Pharmacol.* **2006**, *147*, S163–S171.
[3] M. Ben Amar, *J. Ethnopharmacol.* **2006**, *105*, 1–25.
[4] In terms of enzymology, CBD and THC are unrelated, because their formation is the result of two distinct enzymes (CBDA synthase and THCA synthase) that share GBG as their substrate and generate the same *p*-menthenyl cation, but that differ in the way this cation is quenched: either by deprotonation (CBDA synthase) or by intramolecular oxygen trapping (THCA synthase). F. Taura, S. Morimoto, Y. Shoyama, R. Mechoulam, *J. Am. Chem. Soc.* **1995**, *117*, 9766–9767.
[5] E. P. M. de Meijer, *J. Int. Hemp Assoc.* **1995**, *2*, 66–73. The ancient variety *Carmagnola* is named after the town of Carmagnola (Piemont), where its cultivation thrived until the second half of the 20th century. The name of the famous French revolutionary song "*La Carmagnole*" was in fact inspired by the trade of this variety of hemp, characterized by very long fibers and unrivaled for the manufacture of naval ropes, from Carmagnola to Marseille; see: http://architettura.supereva.com/image/festival/1998/en/bonino.htm.
[6] From the Latin *abeo* = I move.
[7] a) A. A. Izzo, F. Borrelli, R. Capasso, V. Di Marzo, R. Mechoulam, *Trends Pharmacol. Sci.* **2009**, 515–527; b) L. De Petrocellis, V. Di Marzo, *J. Neuroimm. Pharmacol.* **2009**, in press.
[8] N. M. Kogan, R. Rabinowitz, P. Levi, D. Gibson, P. Sandor, M. Schlesinger, R. Mechoulam, *J. Med. Chem.* **2004**, *47*, 3800–3806.
[9] R. Mechouolam, Y. Shvo, *Tetrahedron* **1963**, *19*, 2073–2078.
[10] H. Takikawa, S. Sano, K. Mori, *Liebigs Ann./Recueil* **1997**, 2495–2498.

Received: December 15, 2009
Published Online: February 11, 2010

