ELSEVIER

Journal of Pharmaceutical and Bio9medical Analysis
18 (1999) 947–956

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Structure elucidation and conformational properties of synthetic cannabinoids (-)-2-(6a,7,10,10a-tetrahydro-6,6,9-trimethyl-1-hydroxy-6H-dibenzo [b,d]pyranyl)-2-hexyl-1,3-dithiolane and its methylated analog

T. Mavromoustakos *, E. Theodoropoulou, M. Zervou, T. Kourouli, D. Papahatjis

Institute of Organic and Pharmaceutical Chemistry, National Hellenic Research Foundation, Vas. Constantinou 48, Athens 11635, Greece

Received 7 October 1997; received in revised form 23 February 1998; accepted 8 March 1998

Abstract

The synthetic cannabinoid (-)-2-(6a,7,10,10a-tetrahydro-6,6,9-trimethyl-1-hydroxy-6H-dibenzo[b,d]pyranyl)-2-hexyl-1,3-dithiolane (AMG-3) is a cannabimimetic molecular probe with one of the highest binding affinities reported to date. Therefore, due to its potential pharmacological importance, its structure was sought to be elucidated and its conformational properties were studied using a combination of 1D, 2D NMR spectroscopy and molecular modelling. The structure of its methylated analog (-)-2-(6a,7,10,10a-tetrahydro-6,6,9-trimethyl-6H dibenzo [b,d]pyranyl-1-methoxy)-2-hexyl-1,3 dithiolane (AMG-18), was also studied and its conformational properties were compared with AMG-3. AMG-18 lacks of the phenolic hydroxyl group a strict requirement for cannabimimetic activity and is almost devoid of any biological activity. The conformational analysis studies showed that 1′,1′ dithiolane ring restricted the orientation preferences of alkyl chain. This may account for the high binding affinity of AMG-3 to cananbinoid receptors. Grid scan search studies showed different preferences of possible adopting dihedral values of phenolic hydroxyl group and its methyl ether. These observations may account for their differences in biological activity.

Keywords: Cannabinoids; Conformational analysis; NMR spectroscopy; Molecular modelling

1. Introduction

Hitherto, two cannabinoid receptors have been identified. The cannabinoid receptor (CB1) in mammalian brains and a peripheral cannabinoid receptor (CB2). CB1 receptor was characterized as a G-protein-coupled and was cloned from rat and human cDNA libraries while CB2 receptor was cloned and expressed in macrophages in the marginal zone of the spleen [1–6].

In an effort to obtain information on the regiochemical and stereochemical requirements for productive binding at the active site of the cannabinoid receptors CB1 and CB2 within a specific

* Corresponding author. Fax: +30 1 7247913 or 7253821, e-mail: tmavro@eie.gr

0731-7085/99/$ - see front matter
PII S0731-7085(98)00100-9

class of cannabimimetic (CBMM) agents, a synthetic effort was made in the development of novel ligands possesing high affinity and selectivity.

As a prototype (-)-Δ^8-tetrahydrocannabinol (Δ^8-THC) was chosen, a molecule that has similar pharmacological profile with (-)-Δ^9-tetrahydrocannabinol (Δ^9-THC), the most active consituent of cannabis, but with greater chemical stability. Accumulated evidence indicated that within the cannabinoid structure the aliphatic side chain plays a pivotal role in determining CBMM activity. The synthetic effort has been focused on modifications at the C1′ position of the side chain.

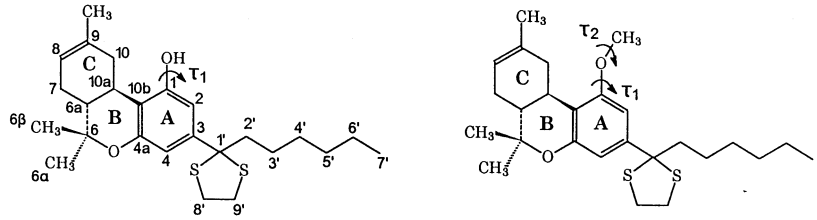

Fig. 1. Molecular structures of the cannabinoids under study.

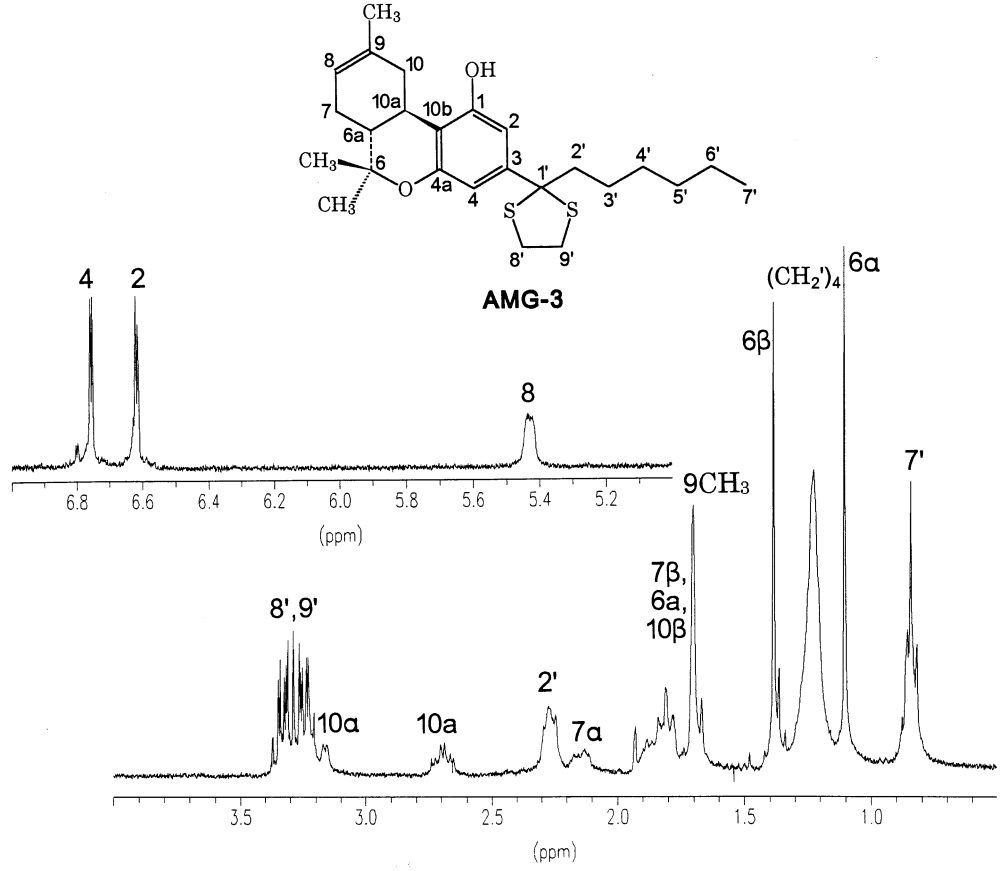

Fig. 2. NMR spectra of AMG-3 in $CDCl_3$ at 298 K recorded on a Bruker AC 300 MHz.

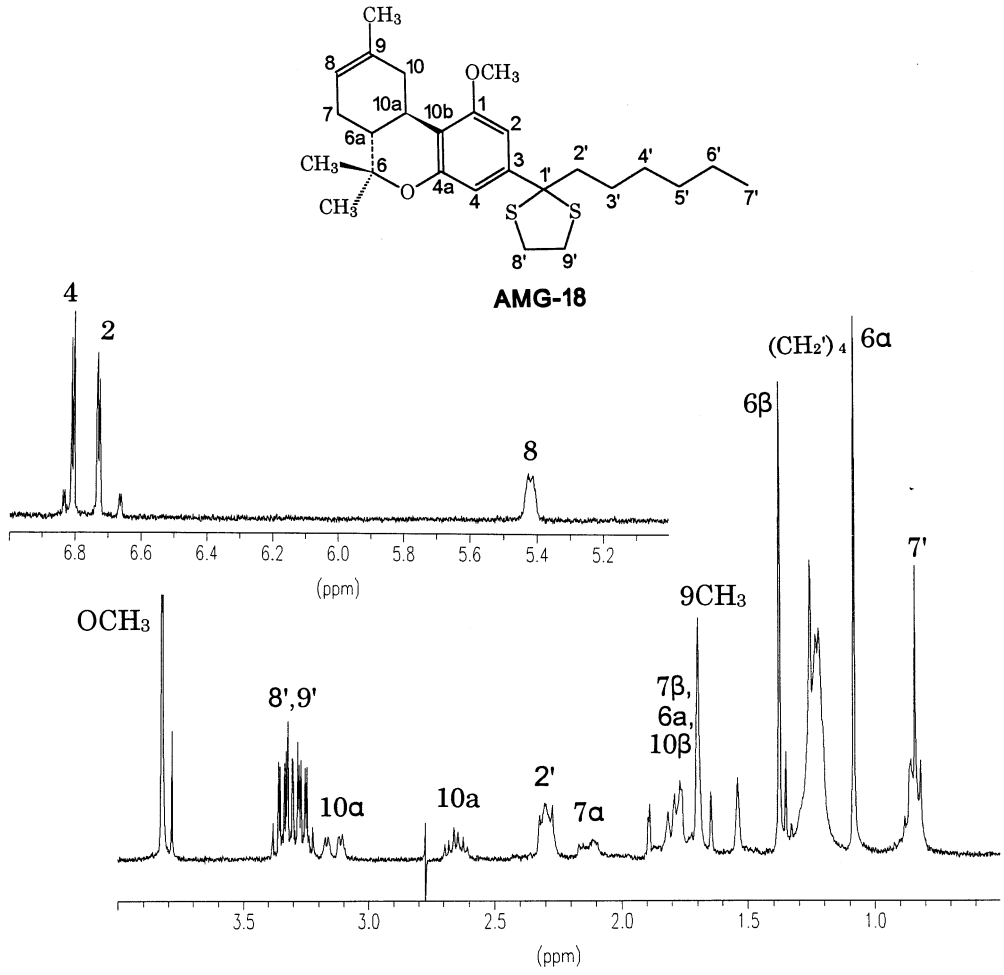

Fig. 3. NMR spectra of AMG-18 in $CDCl_3$ at 298 K recorded on a Bruker AC 300 MHz.

On the basis of earlier literature, the length of the side chain was optimized to seven carbon atoms [7]. No analog synthesized so far, has the side chain pharmacophore in a fully restricted conformation. The drug design of these novel analogs serves to narrow down the conformational requirements at the receptor active sites.

Among the synthesized analogs 2-(6a,7,10,10a-tetrahydro-6,6,9-trimethyl-6H dibenzo [b,d]pyran-1-ol)-2-hexyl-1,3-dithiolane (AMG-3) (Fig. 1) is a cannabimimetic probe and it was found to have high binding affinity (K_i values of 0.32 and 0.52 nM for CB1 and CB2 receptors respectively) [8]. This increase in binding affinity was attributed to two factors. First, its increased hydrophobicity may fit to a corresponding hydrophobic subsite of a receptor at the benzylic side chain carbon. Second, the side chain pharmacophore is conformationally more defined than the prototype cannabinoid (-)-Δ^8-THC.

Previous conformational analysis studies on the non classical cannabinoids CP-47,497 and CP55,940 have shown that the dimethylheptyl chain adopts one of four preferred conformations in all of which the chain is almost perpendicular to the phenol ring. The phenolic O–H bond was found coplanar with the aromatic ring and points away from the cyclohexyl ring [9,10]. This conformation although found by other authors using the classical cannabinoid Δ^9-THC was not considered

the most important one on the premis that another low energy conformation adopted by the active Δ^9-THC with the C1 phenolic hydroxyl torsion angle 155° could not be taken up by the almost inactive 1-methoxy-Δ^9-THC with favourable energy terms [11,12].

As it is mentioned above the dithiolane chain of AMG-3 is anticipated to possess reduced orientations in comparison to the non-classical cannabinoids which possess alkyl chain without any substituent at the C1′ position. To investigate this proposition its structure was elucidated and its conformational analysis properties were studied using a combination of molecular modeling and NMR spectroscopy.

Its methylated analog AMG-18 (Fig. 1), a molecule almost devoid of any biological activity was also synthesized and its conformational properties were compared with AMG-3 using a combination of NMR spectroscopy and molecular modelling.

2. Materials and methods

2.1. Materials

$CDCL_3$ and ultra precision NMR tubes were purchased from Solvents Documentation Synthesis (SDS), Peypin, France.

2.2. NMR spectroscopy

The high resolution spectra were obtained using

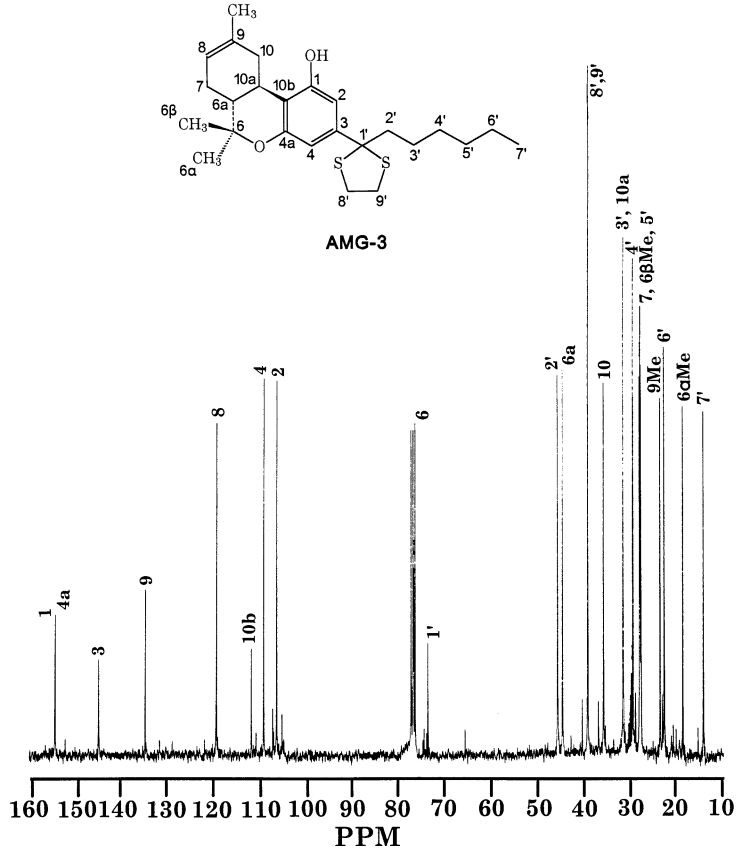

Fig. 4. ^{13}C spectrum of AMG-3 in $CDCl_3$ at 298 K recorded on a Bruker AC 300 MHz.

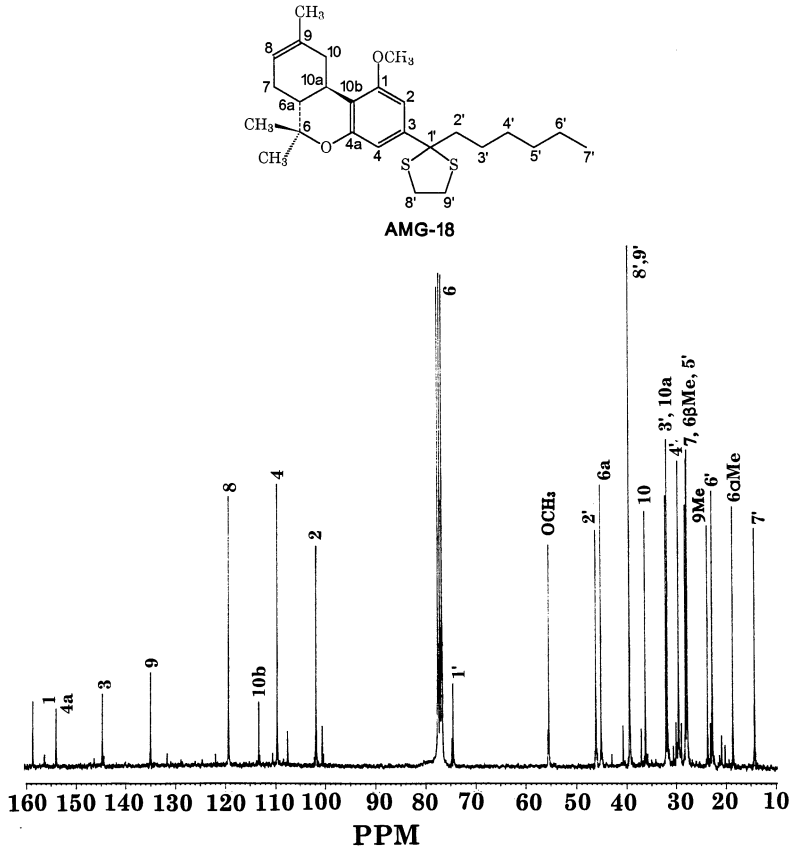

Fig. 5. ^{13}C spectrum of AMG-18 in $CDCl_3$ at 298 K recorded on a Bruker AC 300 MHz.

Bruker AC 300 instrument. All data were collected using pulse sequences and phase-cycling routines provided in the Bruker library of pulse programs. Data processing including sine-bell apodization, Fourier transformation, symmetrization and plotting were performed using Bruker software packages. ^{1}HNMR spectra were recorded using the following acquisition parameters: pulse width (PW) 6.0 μs, spectral width (SW) 6024 Hz, data size (TD) 16 K, recycling delay (RD) 1.0 s, number of transients (NS) 16, and digital resolution 0.735 Hz pt^{-1}. ^{13}CNMR spectra were performed with PW 6.2 μs, SW 20000 Hz, TD 8 K, RD 2.5 s, NS 2000 and digital resolution 2.441 Hz pt^{-1}. 2D phase-sensitive ^{1}H-^{1}H nuclear Overhauser enhancement (NOESYPH) spectra were recorded using the following acquisition parameters: $D_1 = 2.0$ s, $D_0 = 3$ μs, SW in F_2 2551 Hz and F_1 1275.5 Hz.

2.3. Molecular modeling

Computer calculations were performed on a Silicon Graphics 4D/35 using the QUANTA 3.3 version of molecular simulation incorporated (MSI) program. The conformational energy of AMG-3 and AMG-18 were first minimized and then subjected to molecular dynamics experiments to explore their lower energy conformers. The molecular dynamics calculations were run for the two molecules using simulation time 2 ps at a temperature of 1000 K. Family structures were generated using the dihedral angle criterion. The lowest energy

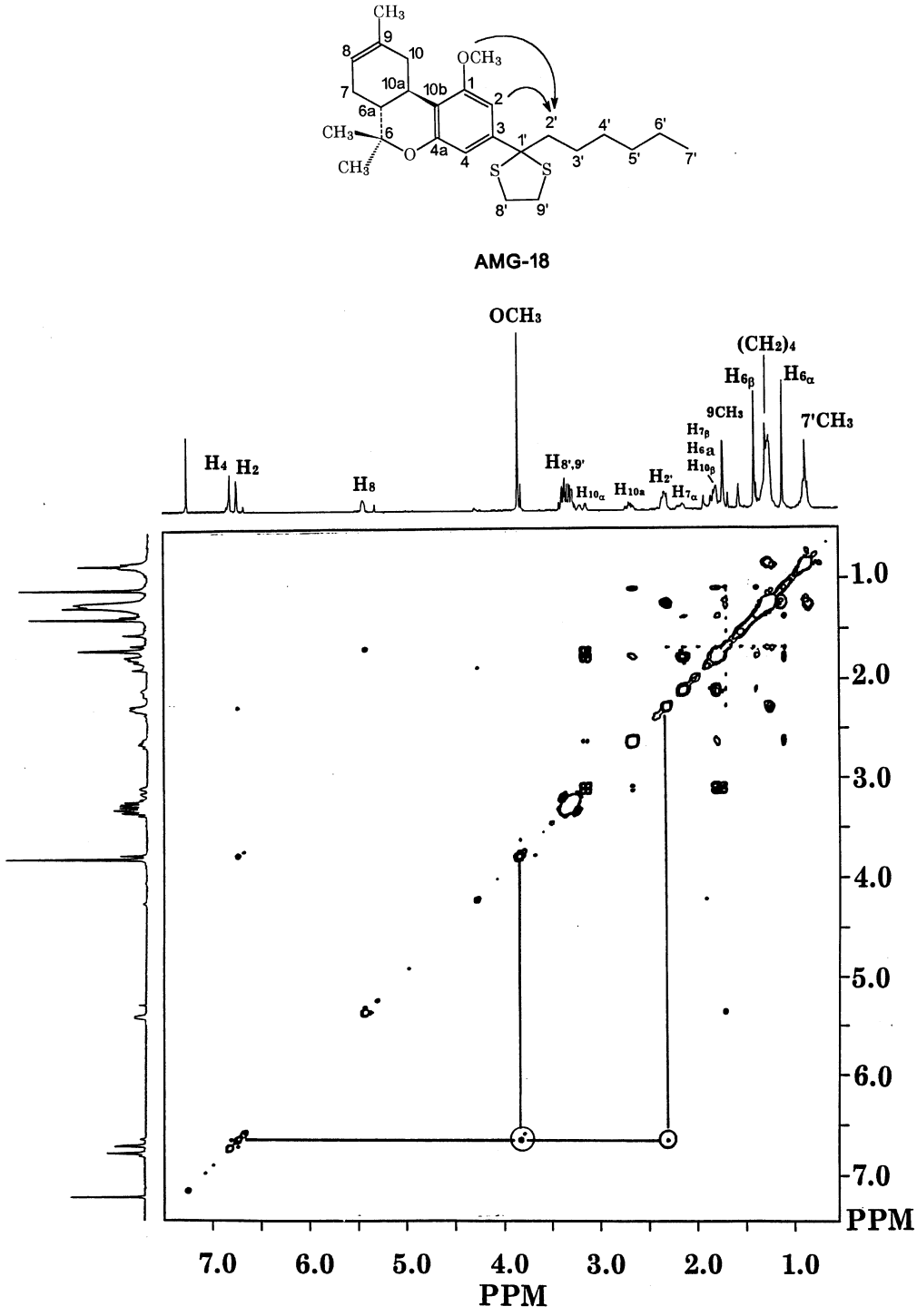

Fig. 6. NOESY spectrum of AMG-18 in $CDCl_3$ at 298 K recorded on a Bruker AC 300 MHz. The lines indicate the most useful ^1H-^1H through space interproton couplings.

conformers from each family were considered as the representative ones [13,14]. Conformational energy profile (kcal mol^{-1}) for AMG-3 as a function of its dihedral angle τ_1 was obtained from a grid search scan with increments of 5°. For the AMG-18 contour plot was generated as a function of τ_1 and τ_2 dihedral values from a conformational grid search analysis with increments of 10°.

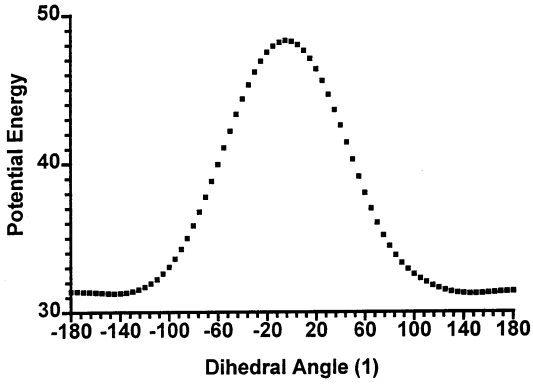

Fig. 7. Conformational energy profile (kcal $mole^{-1}$) as a function of C2–C1–O–H (τ_1 dihedral angle) obtained from a grid search scan with increments of 5° in AMG-18.

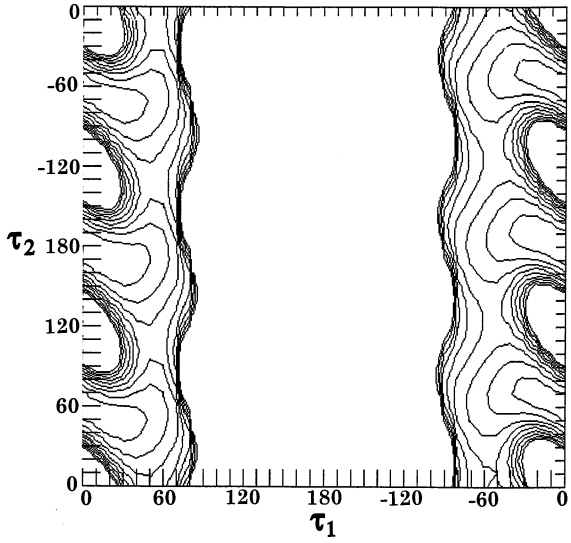

Fig. 8. Contour plot as a function of τ_1 and τ_2 dihedral angles of AMG-18 obtained from a conformational grid search analysis with increments of 10°.

3. Results and discussion

3.1. Structure identification of AMG-3 and AMG-18

Figs. 2 and 3 depict the ^{1}HNMR spectra of AMG-3 and AMG-18 in $CDCl_3$ solvent. Observed peaks are referenced to TMS. The assignment of the peaks is shown on the top of the spectrum. The peak identification was based on ^{1}HNMR data of other synthetic derivatives and was confirmed by integration of the peaks and a 2D COSY spectra.

The ^{13}CNMR signals of the two studied cannabinoids are unambiquously assigned based on other synthetic derivatives and an inverse ^{13}C-^{1}H 2D NMR experiment on the molecule. The ^{13}CNMR spectra of AMG-3 and AMG-18 are shown in Figs. 4 and 5. The assignment of the peaks are shown on the top of the spectra.

3.2. Conformational properties of AMG-3 and AMG-18

The observed NOEs H2′-H2 and H8′-H6β (Fig. 6) are critical for the conformational analysis of the studied molecules because the former establishes spatial vicinity between the alkyl chain and the aromatic ring A while the latter between the non aromatic rings B and C. A small but very important NOE was observed between 6α and $(CH_2)_4$ groups. This NOE imposes the alkyl chain to a spatial proximity with the B ring.

The NOESY spectrum of AMG-18 otherwise identical with that obtained with AMG-3 reveals additionally a spatial vicinity of $-OCH_3$ protons with H_2. This indicates that $-OCH_3$ points towards the side of alkyl chain.

The contour energy profile as a function of τ_1 (C2–C1–O–C) and τ_2 (C1–O–C–H) in AMG-18 is shown in Fig. 7. The low energy values for τ_1 which determines the preference of $-OCH_3$ protons to the alkyl chain range between $\pm 30 - \pm 80°$. At these minimal values for τ_1, the τ_2 dihedral angle adopts all possible dihedral angles. The obtained theoretical calculations strengthen the experimental data as well as supplement them because they give distinct range values within 6

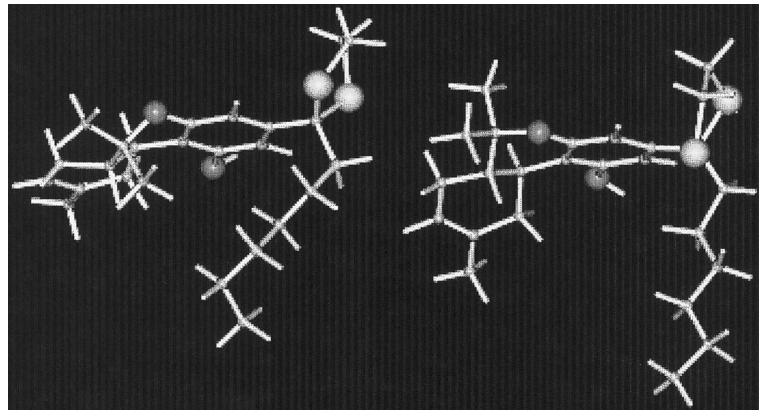

Fig. 9. Representative lowest energy conformers for AMG-3 derived from family structures obtained after performing dynamics at a simulated temperature of 1000 K.

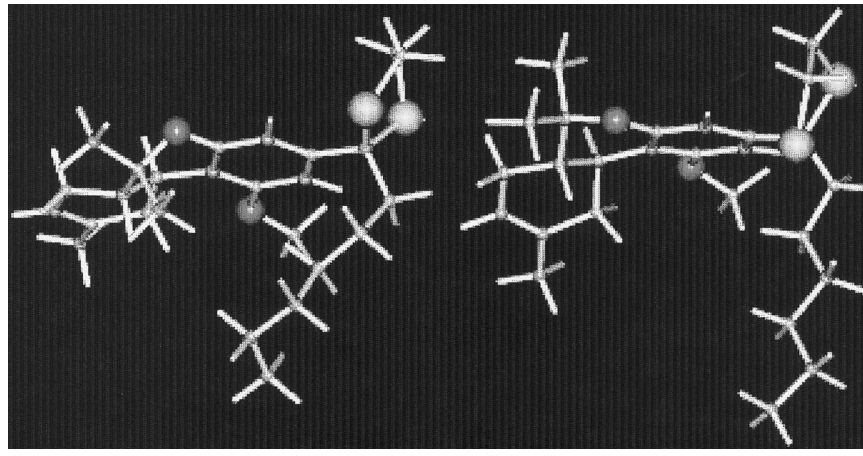

Fig. 10. Representative lowest energy conformers for AMG-18 derived from family structures obtained after performing dynamics at a simulated temperature of 1000 K.

kcal mol^{-1} range of τ_1 in which the molecule adopts low energy conformers.

The potential energy profile as a function of τ_1 (C2–C1–O–H) in AMG-3 is shown in Fig. 8. It appears that a different energy profile is observed with the active cannabinoid. The minimal values for τ_1 are ranged between $\pm 140 - \pm 180°$. Thus, the phenolic hydroxyl group is either coplanar or close to coplanar with the aromatic ring and is pointing to the alkyl chain as with the inactive analog. The observed results agree with those obtained by other authors using conformational analysis on classical and non classical cannabinoids [9–12].

The conformational analysis data point out that the two molecules adopt different τ_1 dihedral angles in their low energy structures. This supports the premis by Reggio et al. [13,14] that these differences may be responsible for their different biological activity. It has also been hypothesized in the literature that the difference in activity between the two molecules is due to the fact that phenolic hydroxyl group must be involved in any receptor/membrane hydrogen bonding interaction. It has been determined by molecular mechanics energy calculations and volume map determinations that the proton of the hydroxyl group may be involved in hydrogen bonding at

the putative receptor site [15]. Biophysical studies showed that Δ^8-THC orients in an awkward way in membrane bilayer probably because in this way maximizes its amphipathic interactions while the more hydrophobic molecule O-methyl-Δ^8-THC is embedded deeper in the hydrophobic core of the bilayer and is governed mainly with non-polar interactions [16–21]. Which of the two factors is the decisive one for the different biological activity exerted by AMG-3 and AMG-18 remains to be further elaborated.

To explain the NOE between $(CH_2)_4$ and 6α the alkyl chain has to form some gauche conformers. This is not prohibited energetically as it is depicted from applied theoretical calculations. Such structure is governed by compact conformation (Fig. 9, left). When the alkyl chain is in all trans conformation the molecule has lower energy but the proximity between $(CH_2)_4$ and 6α is missed (Fig. 9, right). The same applies for the almost inactive molecule AMG-18. Two low energy conformers generated by the dynamics experiments and which show the flexibility of alkyl chain are shown in Fig. 10.

From the low energy conformers and the observed NOE between $(CH_2)_4$ and 6α it is apparent that the presence of dithiolane ring restricts alkyl chain to orient only to one phase of the molecule. This supports the preposition that dithiolane reduces the flexibility in orientation of the alkyl chain. However, it does not affect its capability to form various gauche conformes within the alkyl chain.

This reduce in the flexibility of orientation of the alkyl chain may be of biological importance because it may result in a better fitting to the canabinoid receptor.

4. Conclusions

The synthetic analogs AMG-3 and AMG-18 contain dithiolane ring at the benzylic position of the side chain. This group contributes to the decreased capability of alkyl chains to adopt various orientations relative to the tricylic part of the molecule. This may explain the high biological activity of AMG-3. Its phenolic hydroxyl group was found to be almost coplanar or coplanar with the aromatic ring and to point towards the alkyl chain. AMG-18 is almost devoid of any biological activity because it lacks the phenolic hydroxyl group. The methoxy group seems to prefer an orientation towards the alkyl chain rather than the cyclohexane C ring as it is depicted from the observed NOE between H_2 and methoxy hydrogens. Theoretical calculations support these data and depict a limited range of values for τ_1 for the molecule to adopt low energy values. These differences in the preferences of the critical dihedral angle τ_1 between the two molecules may in part contribute to their different biological activity.

References

[1] W.A. Devane, F.A. Dysartz III, R.M. Johnson, L.S. Melvin, A.C. Howlett, Mol. Pharmacol. 34 (1988) 605–613.
[2] A.C. Howlett, Mol. Pharmacol. 27 (1985) 429–436.
[3] L.A. Matsuda, S.J. Lolait, M.J. Browstein, A.C. Young, T.I. Bonner, Nature 346 (1990) 561–564.
[4] C. Gerard, C. Mollereau, G. Vassart, Nucleic Acids Res. 18 (1990) 7142.
[5] W.A. Devane, L. Hanus, A. Breuer, R.G. Pertwee, L.A. Stevenson, G. Griffin, D. Gibson, A. Mandelbaum, A. Etinger, R. Mechoulam, Science 258 (1992) 1946–1949.
[6] S. Munro, K.L. Thomas, M. Abu-Shaar, Nature 365 (1993) 61–65.
[7] A. Makriyannis, R.S. Rapaka, Life Sci. 47 (1990) 2173–2184.
[8] D.P. Papahatjis, T. Kourouli, V. Abadji, A. Makriyannis, J. Med. Chem. 41 (1998) 1195–1200.
[9] X.Q. Xie, L.S. Melvin, A. Makriyannis, J. Biol. Chem. 271 (1996) 10640–10647.
[10] X.Q. Xie, D.P. Yang, L.S. Melvin, A. Makriyannis, J. Med. Chem. 37 (1994) 1418–1426.
[11] P.H. Reggio, K.V. Kreer, S.M. Kox, J. Med. Chem. 32 (1989) 1630–1635.
[12] P.H. Reggio, Structure-Activity Relationships of the Cannabinoids, in: R.S. Rapaka, A. Makriyannis (Eds.), NIDA, 79, 1987, pp. 82–95.
[13] J. Matsoukas, J. Hondrelis, M. Keramida, R. Yamdagri, Q. Wu, T. Mavromoustakos, A. Makriyannis, G. Moore, J. Biol. Chem. 269 (1994) 5303–5312.
[14] T. Mavromoustakos, D.-P. Yang, E. Theodoropoulou, A. Makriyannis, Eur. J. Med. Chem. 30 (1995) 227–234.
[15] S.F. Semus, B.R. Martin, Life Sci. 46 (1990) 1781–1785.
[16] T. Mavromoustakos, D.P Yang, A. Charalambous, L.G. Herbette, A. Makriyannis, Biochim. Biophys. Acta 1024 (1990) 336–344.

[17] T. Mavromoustakos, D.P. Yang, W. Broderick, D. Fournier, L.G. Herbette, A. Makriyannis, Pharmacol. Biochem. Behav. 40 (1991) 549–552.

[18] A. Makriyannis, D.P. Yang, T. Mavromoustakos, in: R.S. Rapaka, A. Makriyannis, M.J. Kuhar (Eds.) The combined use of solid state NMR, small angle x-ray diffraction and differential scanning calorimetry in studies of cannabinoid:membrane interactions, Emerging Technologies and New Directions in Drug Abuse Research, US Department of Health and Human Services, Drug Abuse and Mental Health Administration, Rockville USA, 1991, pp. 106–128.

[19] D.P. Yang, T. Mavromoustakos, K. Beshah, A. Makriyannis, Biochim. Biophys. Acta 1103 (1992) 25–35.

[20] D.P. Yang, T. Mavromoustakos, A. Makriyannis, Life Sci 53 (1993) 117–122.

[21] P. Martel, A. Makriyannis, T. Mavromoustakos, K. Kelly, K.R. Jeffrey, Biochim. Biophys. Acta 1151 (1993) 51–58.