Eur J Med Chem (1995) 30, 227–234

Studies of the conformational properties of the cannabimimetic aminoalkylindole pravadoline using NMR and molecular modeling

T Mavromoustakos[1], DP Yang[2], E Theodoropoulou[1], A Makriyannis[1,2]

[1]*National Hellenic Research Foundation, Institute of Organic and Pharmaceutical Chemistry, Athens 116 35, Greece;*
[2]*University of Connecticut, Department of Pharmaceutical Sciences and Institute of Materials Science, Storrs, CT 06269, USA*

(Received 6 June 1994; accepted 7 November 1994)

Summary — The conformation of the cannabimimetic aminoalkylindole (AAI) pravadoline was studied using a combination of 2D-NMR techniques and computational molecular modeling. This conformation of pravadoline was then compared with that of the analgesic nonclassical cannabinoid (NCC) CP-55940. The pair of more potent and more conformationally restrained analogs WIN 55212-2 and CP-55944 were also included in the comparison study. The results showed that the apparent structural dissimilarities between the AAIs and the NCCs can be reconciled when a closer examination of their preferred conformations is undertaken. Such a study revealed that there is a good correspondence between their respective pharmacophoric groups when the molecules are properly superimposed. Furthermore, the polar and nonpolar groups that determine their amphipathic properties are arranged on the same side of each of these four molecules.

aminoalkylindoles (AAIs) / pravadoline / WIN 55212-2 / nonclassical cannabinoids (NCCs) / CP-55940 / CP-55244 / NMR spectroscopy / molecular graphics

Introduction

Pravadoline (fig 1) is an aminoalkylindole (AAI) analogue, which was synthesized as a potential nonsteroid antiinflammatory agent acting through the inhibition of PG synthetase [1–6]. However, this molecule was also found to have a moderate affinity for the cannabinoid receptor and to possess antinociceptive activity [7, 8]. This finding initiated a search for other AAI analogues with higher potency and selectivity. To obtain information regarding the stereoelectronic features within the AAI structure, which are required for a productive interaction with the cannabinoid receptor, we studied the conformational properties of pravadoline. Such a study is of special interest because of the apparent dissimilarity between the structures of AAIs and cannabinoids that possess analgesic properties.

The conformational analysis of pravadoline was based on the measurement of interproton vicinal coupling constants and the nuclear Overhauser enhancement (NOE) values obtained from 2D-NMR spectra. In addition, a number of molecular modeling techniques were used to calculate the global minimum conformation of this AAI parent compound, which was subsequently compared with that of the known nonclassical cannabinoid (NCC) CP-55940 (fig 1) synthesized at Pfizer Central Research [9]. This last molecule is a potent cannabimimetic agonist with a high affinity for the cannabinoid receptor [10–12]. Furthermore, there is good evidence that the cannabimimetic AAIs and the NCCs induce their effects by binding at the same site on the cannabinoid receptor [7, 8, 10–12]. The conformational properties of the more potent and conformationally more constrained AAI analog WIN 55212-2 and NCC CP-55244 [13, 14] were also compared (fig 1).

Experimental protocols

Materials

Pravadoline was a generous gift from Sterling Winthrop. $CDCl_3$ (99%+) was purchased from SDS (Solvents, Documentation, Synthesis), Peypin, France.

Nuclear magnetic resonance

NMR spectra were obtained at 295K using an MSL Bruker spectrometer operating at 400 MHz ^1H frequency. COSY and NOESY 2D-NMR experiments were performed using pulse sequence and phase cycling routines available in the Bruker library. The simulated subspectrum of pravadoline was analyzed with the help of LAOCOON-based PANIC software, using an Aspect 3000 computer. This program accepts an input of a maximum of nine nuclear spins.

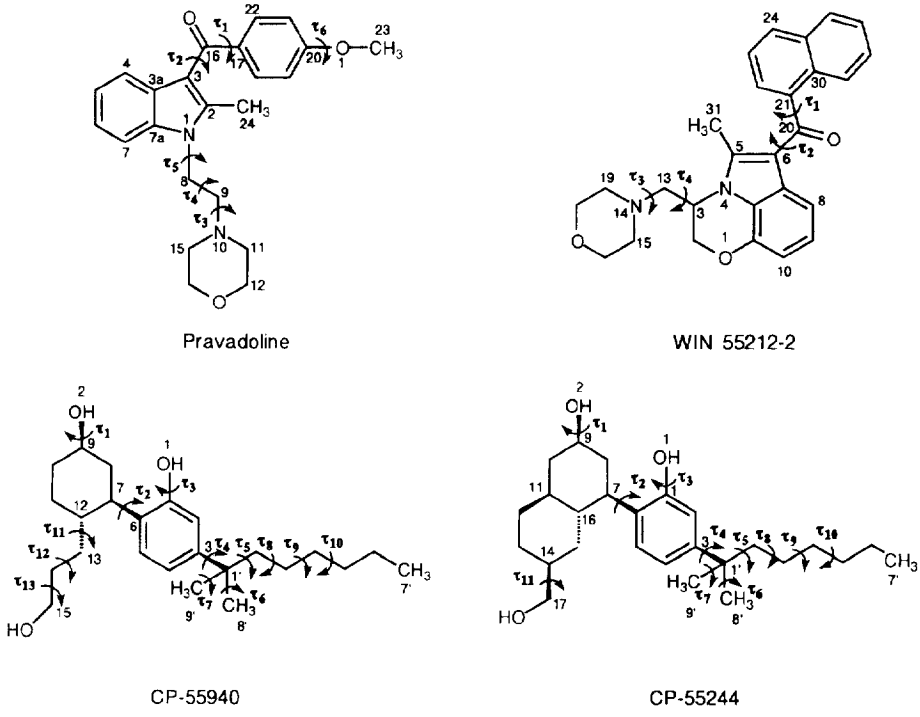

Fig 1. Molecular structures of the AAIs pravadoline and WIN 55212-2 as well as the NCCs CP-55940 and CP-55244.

Molecular modeling

Theoretical calculations were performed on a Silicon Graphics 4D/35 model using the Quanta 3.3 version of molecular simulation incorporated (MSI) program. The structures of all four molecules under study were first minimized using steepest descents (SD) to approach their local minima and the Newton-Raphson (NR) method to reach the local minima. After determining the local minimum for each molecule, four conformational search methods available in the Quanta 3.3 version of MSI to determine the global minimum were applied. (i) The random sampling search procedure in which all selected torsion angles in a structure are changed randomly and a specified number of conformations are produced. As the search progresses CHARMm energy minimization can be undertaken for each randomly altered conformation. (ii) The Boltzmann jump search differs from the random sampling in that a stochastic decision is made to select or reject a new high energy conformation. (iii) The grid search is a systematic search of conformational space, which is achieved by varying one torsion angle (energy diagrams) or two torsion angles (contour plots) over a grid of equally spaced values. The method is of practical value only when the structure has a limited number of variable torsion angles. Thus, this method can be successfully applied on pravadoline, which is conformationally mobile and has only six torsion angles. (iv) Molecular dynamics simulates the natural motion of a molecule and produces a set of coordinates and velocities which describe the atomic motions of the system over time. The resulting dynamics trajectory can be viewed in the dynamics animation and analyzed in the analysis application palette. The superimposition of the two pairs of structures was performed using rigid body fit to target method of molecular similarity software. Rigid body fitting translates and rotates working structures to minimize the RMS of the fit to the target structure.

Results and discussion

Structural identification of pravadoline

Figure 2 depicts of the ^{1}H-NMR spectrum of pravadoline focusing on the two principal regions of the ^{1}H frequencies. The assignment was confirmed by integration of the peaks, the COSY spectrum, and by analogy with the ^{1}H spectra of other structurally related AAIs [1, 2]. Our analysis contradicts previously reported ^{1}H-NMR results with pravadoline [2] in which the peak at $\delta = 7.78$ ppm corresponding to H_{22} was erroneously assigned to H_4.

Conformational results from NMR

Figure 3 is a phase-sensitive NOESY spectrum of pravadoline in chloroform in which the spatial inter-

nuclear ^1H-^1H interactions are highlighted. Two major NOEs (H_{22}-H_4 and H_{23}-H_{11}) are critical for the conformational analysis of this molecule. The NOE between H_{22} and H_4 protons indicates the close spatial relationship between the aromatic indole and anisoyl rings. This was also observed by Bell *et al* [2] using 1D-NOE difference experiments, on which basis a synperiplanar (*s-cis*) conformation of the carbonyl group with the aromatic ring was proposed for pravadoline. Additionally, the observed NOE between the H_{23} and H_{11} hydrogens is evidence for the proximity between the aromatic anisoyl and those of the morpholine ring. Since the synperiplanar conformation proposed by Bell *et al* would not account for such a through-space interaction, we propose a different conformation (fig 4), based on the 2D-NOESY data. This conformation was generated using an energy minimization routine to calculate the global minimum after having imposed distance constraints to reflect the NOE results. These constraints are such that the H_{22} and H_4 hydrogens as well as the H_{23} and H_{11} hydrogens are held within 3.0 Å from each other, respectively. The calculated minimum energy conformation has the above hydrogens at 2.9 and 2.4 Å, respectively. In this conformation, the anisoyl group is nearly (–)-synclinal with the plane of its aromatic ring aligned over that of

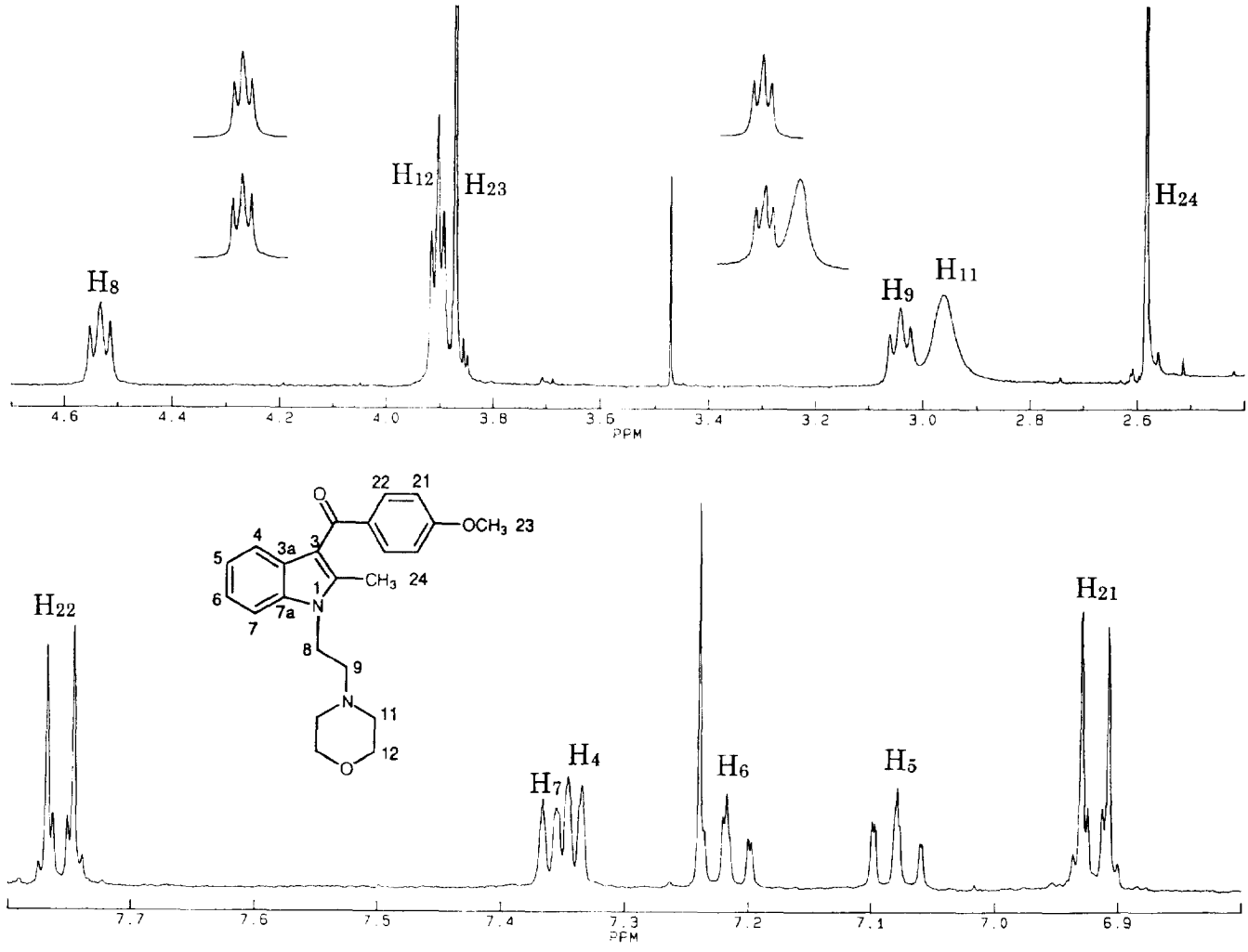

Fig 2. ^1H-NMR spectrum of pravadoline in $CDCl_3$ at 295 K recorded on a Bruker MSL 400 MHz spectrometer. Inserts: simulated subspectra of the H_8 and H_9 multiplets compared with the corresponding experimental subspectra.

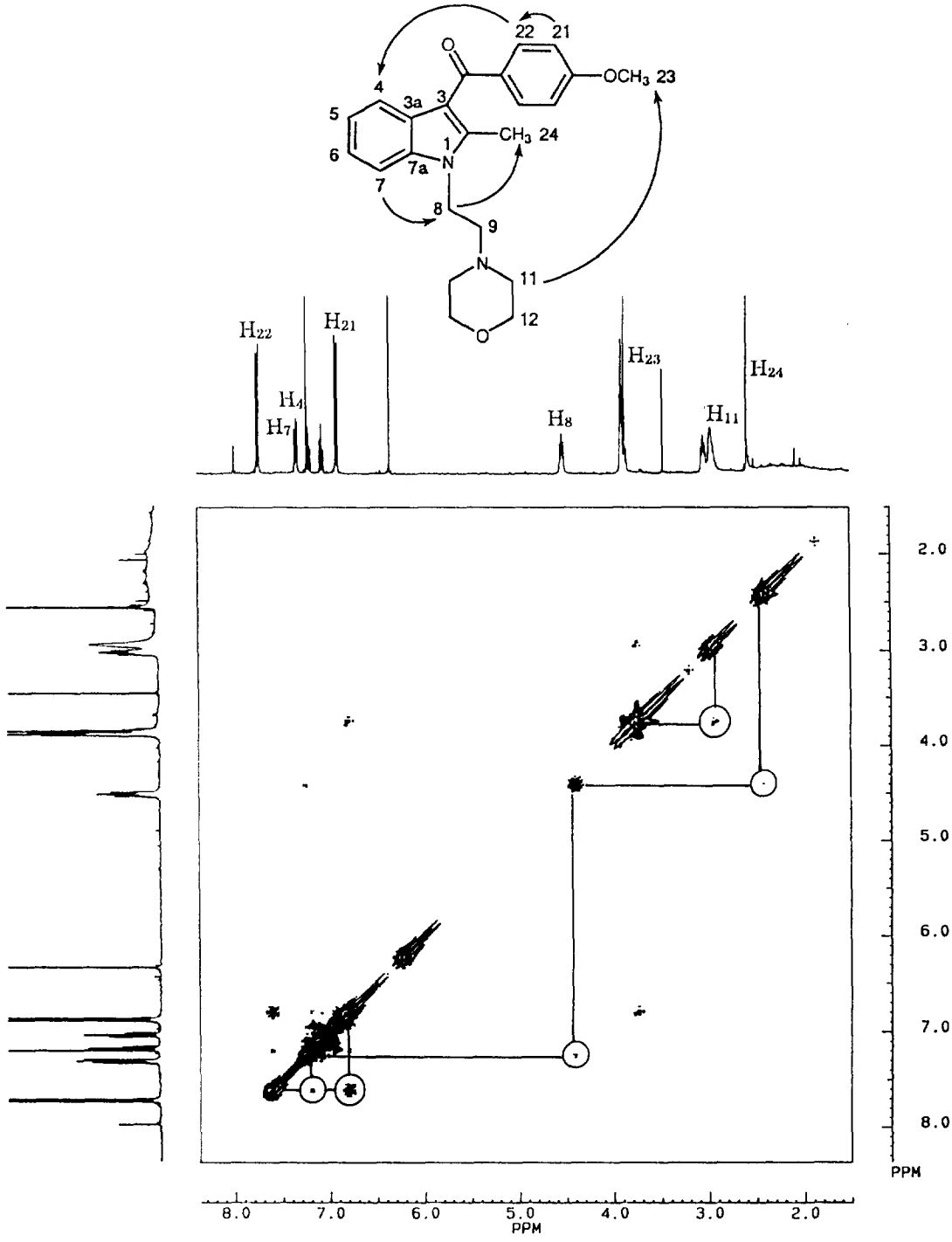

Fig 3. 2D phase-sensitive NOESY spectrum of pravadoline in $CDCl_3$ at 295K at 400 MHz. The circled cross peaks indicate the observed NOE due to through-space interproton couplings as indicated on the molecular structure.

the indole ring while the *O*-methyl group of the anisoyl group is situated directly above the morpholine ring. This represents a folded conformation in which the polar carbonyl and amino groups are pointing outwards on one side of the molecule while the more hydrophobic groups point towards the other side.

Interestingly, the spectrum lacks the expected NOE between H_{24} and H_{22} at ambient temperature.However, when a NOESY experiment at –20°C was run, a positive result was obtained (not shown). Thus, by lowering the temperature the correlation time of the indol methyl group ($\omega\tau_c$) varied and consequently an NOE was observed.

Conformational analysis of the aminoethyl group

The resonance due to the two methylene groups in the 1D ^{1}H-NMR spectrum was first analyzed. The peaks due to H_8 and H_9 are broad, an indication of interconverting antiperiplanar and synclinal conformers at an intermediate rate on the NMR timescale. Subsequently, these subspectra were simulated using a line width of 2.5 Hz (shown as inserts in fig 2) by treating the four hydrogens as an AA'BB' system with the following spin-spin coupling constants, J(AB) = J(A'B') = 6 Hz, J(AB') = J(A'B) = 9 Hz and J(AA') = J(BB') = 12 Hz. These coupling constants represent values averaged over the interconverting antiperiplanar and synclinal conformers. The probabilities of the conformers were obtained using a semiempirical method developed by Abraham and Gatti [15], which is based on the electronegativities [16] of the different substituents for the NCH_2CH_2N spin system:

$$J(\mathrm{AB}) = n_t J_t^t + n_g J_g^g$$

$$J(\mathrm{AB}') = n_t J_t^g + n_g (J_g^t + J_g^{g'})/2$$

where n_t and n_g are the probabilities of being in an antiperiplanar or a synclinal conformer, respectively.

The calculation yielded $n_t = 0.6$ and $n_g = 0.4$ values indicating a significant representation by the synclinal conformers around the NCH_2-CH_2N bond.

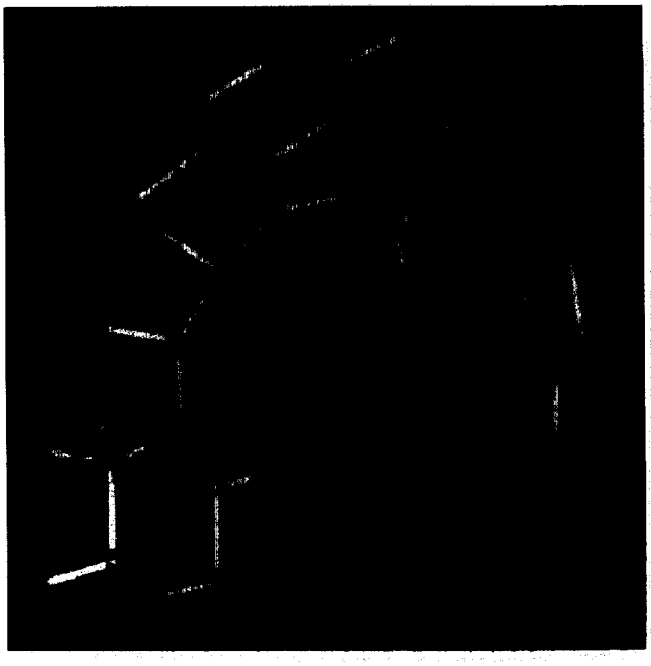

Fig 4. Conformation of pravadoline based on 2D-NOESY constraints between H_{22} and H_4 as well as between H_{23} and H_{11}.

Conformational results from computational methods

The global minimum conformation of all four molecules as well as their least energy conformers within 5 kcal/mol was determined in vacuum without imposing any constraints using the four search methods (random sampling, jump search, grid search and dynamics) described in the *Experimental protocols*. The specific procedure used is described below. First, the energy of the analgesic molecule was minimized using a combination of steepest and Newton-Raphson methods to reach a local minimum conformation. The above-mentioned conformational search methods were then used to arrive to a lower energy conformer which was subjected to a dynamics simulation at 1000 K. The time frames used for the heating, equilibration and simulation steps were 1 ps. One hundred structures of the simulated trajectory files were minimized using 200 iterations and the NR algorithm. The resulting conformers were divided into family structures using the dihedral angle criterion (threshold 10–40, RMS_{min} 0 and RMS_{max} 50–138). Subsequently, the lowest energy structures were further analyzed by applying grid scan on their most important dihedral angles in order to ensure that all of the lowest energy conformers which differed by as much as 5 kcal/mol from the global minimum are included. The results obtained from the theoretical calculations are shown in figure 5. The critical dihedral angles along with their preferred values for each molecule are shown in table I, which also includes the conformational descriptors of the experimentally determined conformer of pravadoline.

The most important features from the conformational analysis of the two aminoalkylindoles are as follows. The anisoyl (or naphthaline) ring is almost perpendicular to the indole ring as defined by the τ_1 and τ_2 dihedral angles (table I). The morpholine and anisoyl rings lie on the same side of the molecule. The

carbonyl group points upward and the methoxy group is coplanar with the aromatic ring. The grid search method was used to obtain the energy minimized conformation of the aminoethyl component of pravadoline by rotating around τ_4 (fig 6). We found that among the rotamers originating from rotation around NC-CN bond, the two synclinal conformers were 5 kcal/mol lower in energy than the antiperiplanar conformer.

In the calculated conformation of pravadoline (fig 5, top left) the distances between the H_4 and H_{22} hydrogens and between the H_{23} and H_{11} hydrogens are respectively 5.18 and 5.36 Å, higher than those imposed as NOE constraints when the first conformation was determined (fig 4). It must be pointed out that the conformation as determined on the basis of the 2D-NOESY data can be converted to the global minimum by applying further energy minimization. It is, thus, possible that in solution the molecule can adopt either of the above two conformations or possibly exists as a mixture of two conformers.

Our calculations on the conformation of CP-55940 and CP-55244 confirm experimentally obtained details [17] on the same molecules and are congruent with the recently published conformation of the NCC prototype [18]. The conformational analysis indicates that the dimethylheptyl side chain is almost perpendicular to the plane of the phenyl ring with the dihedral

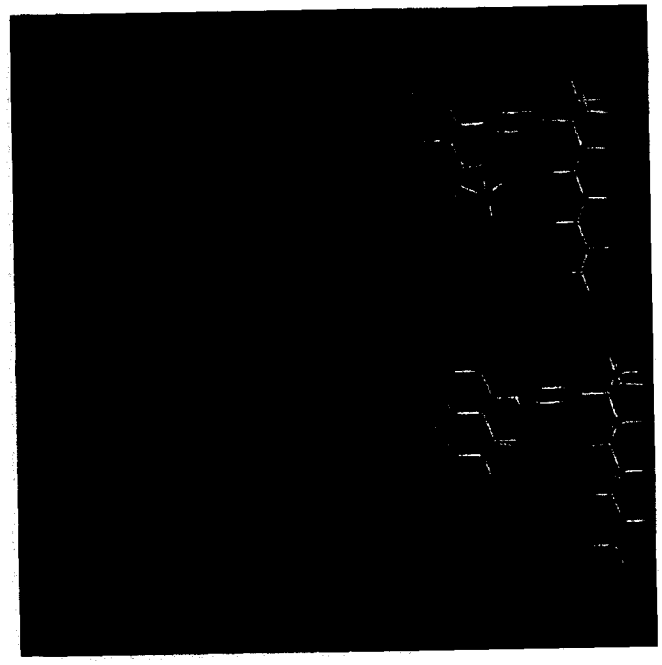

Fig 5. Global minima structures of pravadoline (top, left), CP-55940 (top, right), WIN 55212-2 (bottom, left) and CP-55244 (bottom, right).

Table I. Conformational descriptors of the four molecules under study.

Structures	τ_1	τ_2	τ_3	τ_4	τ_5	τ_6	τ_{11}	τ_{13}
Pravadoline (theoretical)	−140, 44	20	−150, 30	±60, ±180	−120, 70	0, ±180		
Pravadoline (experimental)	−97	60	−125	132	47	−65		
WIN 55212-2	−60, 140	−10, ±170	−100	−55				
CP-55940	±60, ±180	−70, 100	−70, 100	±60, ±120	±60, ±180		−180	−65, ±180
CP-55244	±60, ±180	−70, 100	−70, 100	±60, ±120	±60, ±180		±60, ±180	

Pravadoline τ_1: C22-C17-C16-C3, τ_2: C2-C3-C16-C17, τ_3: C11-N10-C9-C8, τ_4: N1-C8-C9-N10, τ_5: C7a-N1-C8-C9, τ_6: C19-C20-O1-C23. WIN 55212-2: τ_1: C22-C21-C20-C6, τ_2: C5-C6-C20-C21, τ_3: C15-N14-C13-C3, τ_4: N4-C3-C13-N14. CP-55940: τ_1: C8-C9-O2-H2, τ_2: C5-C6-C7-C8, τ_3: C2-C1-O1-H1, τ_4: C4-C3-C1'-C2', τ_5: C3-C1'-C2'-C3', τ_6: C3-C1'-C8'-H8', τ_7: C3-C1'-C9'-H9', τ_8: C1'-C2'-C3'-C4', τ_9: C2'-C3'-C4'-C5', τ_{10}: C3'-C4'-C5'-C6', τ_{11}: C7-C12-C13-C14, τ_{12}: C12-C13-C14-C15, τ_{13}: C13-C14-C15-O3. CP-55244: τ_1: C8-C9-O2-H2, τ_2: C5-C6-C7-C8, τ_3: C2-C1-O1-H1, τ_4: C4-C3-C1'-C2', τ_5: C3-C1'-C2'-C3', τ_6: C3-C1'-C8'-H8', τ_7: C3-C1'-C9'-H9', τ_8: C1'-C2'-C3'-C4', τ_9: C2'-C3'-C4'-C5', τ_{10}: C3'-C4'-C5'-C6', τ_{11}: C15-C14-C17-O3.

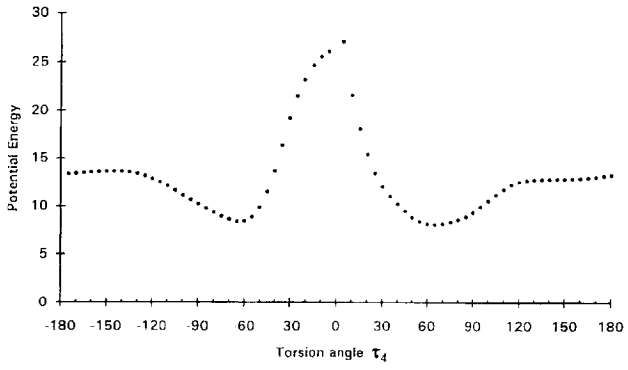

Fig 6. Conformational energy profile (kcal/mol) as a function of NC-CN dihedral angle (τ_4) in the aminoethyl group of pravadoline obtained from a grid search scan with increments of 5°.

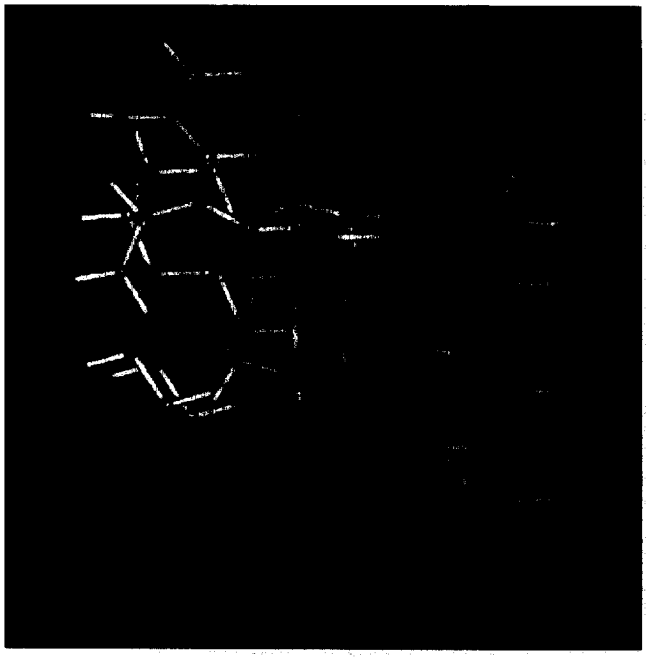

Fig 7. Superimposition of the globally minimized structures of pravadoline with CP-55940.

angles τ_4 and τ_5 to adopt synclinal or anticlinal values. The plane of the phenol ring bisects the C12-C7-C8 angle of the cyclohexane ring. The preferred conformation of the phenolic OH is synclinal with the phenolic ring and points away from the cyclohexyl ring. The τ_5-τ_{10} dihedral angles can adopt any synclinal or antiperiplanar conformation with almost equal probability. The hydroxypropyl group of CP-55940 and hydroxymethyl group of CP-55244 are oriented in such a manner to allow them to undergo hydrogen bonding with the phenolic OH, which serves as the hydrogen acceptor.

Common pharmacophoric features between aminoalkylindoles and nonclassical cannabinoids

To explore the similarities between the pharmacophoric features of the cannabimimetic AAIs and those of the NCCs the preferred conformers of pravadoline and CP-55940 were superimposed (fig 7) so that (a) the phenolic hydroxyl group of CP-55940 coincides with the carbonyl group of pravadoline; (b) the terminal methyl group of the alkyl chain of CP-55940 is overlaid on the methoxy methyl group of the anisoyl ring; (c) the hydroxyl group of the cyclohexanol ring in CP-55940 is in close proximity to the nitrogen of indole ring of pravadoline; and (d) the oxygen of the hydroxypropyl group of CP-55940 overlaps with the oxygen of the morpholine ring and is in the vicinity of the anisoyl ring.

The important features characterizing this superimposition are the following. The phenolic hydroxyl group of CP-55940 and the carbonyl group of pravadoline point upwards in the same direction. Both groups may thus play the same important role during their interaction with the cannabinoid receptor. It is well known that the phenolic hydroxyl group has a pivotal role in determining the pharmacological properties of the cannabinoids and that removal of the hydroxyl hydrogen results in analogs devoid of biological activity [19–21]. It appears that the carbonyl group of pravadoline must also be essential for activity.

Both molecules prefer folded molecular shapes and occupy similar volumes in space. They are amphipathic in character with their polar and hydrophobic groups arranged similarly and occupying different sides of each molecule. Such a separation of the polar and hydrophobic groups within each molecule may be an important pharmacophoric requirement.

To obtain additional support for our pharmacophoric model we have added an NCC and an AAI analog to the superimposition mode. Both of these molecules, namely CP-55244 and WIN 55212-2, are stereochemically better defined than the corresponding CP-55940 and pravadoline analogs (fig 8). If these molecules are superimposed using the same guiding principles as above we find good agreement with our model. Furthermore, the superimposition between the two conformationally more restrained analogs is superior to that of the more flexible pravadoline and the CP-55940 as evidenced by the RMS deviations (1.2 *vs* 3.1 Å) and the distances between the matched atoms (1.1–1.4 *vs* 2.3–3.6 Å).

Conclusion

The above results derive from a combination of 2D NMR spectroscopy and molecular graphics study and demonstrate that the apparent structural dissimilarities between the AAI and the NCC prototypes can be reconciled when closer examination of their preferred conformation is undertaken. The results also show that the computational data calculated in a vacuum are congruent with the experimentally determined conformation in solution. We are currently studying the conformational properties of these molecules in micelles, an environment that more accurately mimics that of biological membrane. A comparative study between the preferred conformation of pravadoline in the two environments is also in progress.

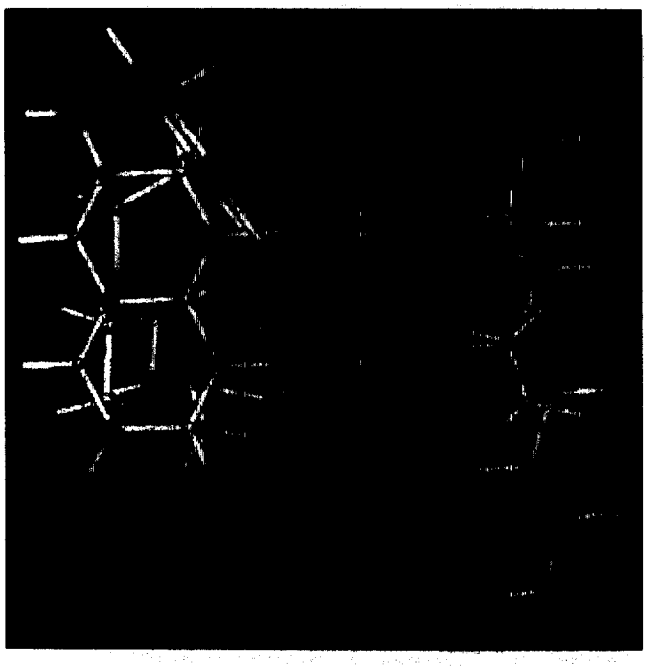

Fig 8. Superimposition of the globally minimized structures of WIN 55212-2 with CP-55244.

Acknowledgments

This work was mainly supported by the National Hellenic Research Foundation and in part by grants from the National Institute on Drug Abuse (DA 3806, DA 152).

References

1 D'Ambra TE, Esterp KG, Bell MR *et al* (1992) *J Med Chem* 35, 124–135
2 Bell MR, D'Ambra TE, Kumar V *et al* (1991) *J Med Chem* 345, 1099–1100
3 Haubrich DR, Ward SJ, Baizman E *et al* (1990) *J Pharmacol Exp Ther* 255, 511–522
4 Ward SJ, Mastriani D, Casiano F, Arnold R (1990) *J Pharmacol Exp Ther* 255, 1230-1239
5 Ward SJ, Miller M, Luttinger D, Eissenstat MA, Bell M (1988) *Neurosci Abstr* 14, 324
6 Pacheco M, Childers SR, Arnold R, Casiano F, Ward SJ (1991) *J Pharmacol Exp Ther* 257, 170–183
7 Kuster JE, Haycock D, Howlett AC, Subramanian B, Ward SJ (1990) *Neurosci Abstr* Abstract 291.14
8 Ward SJ, Baizman E, Bell M *et al* (1990) *NIDA Res Monogr* (Harris LS, ed) 105, 426
9 Devane WA, Dysartz FA, Johnson MR, Melvin LS, Howlett AC (1988) *Mol Pharmacol* 34, 605–613
10 Howlett AC, Bidaut-Russel M, Devane WA, Melvin LS, Johnson MR, Herkenham M (1990) *Trends Neurosci* 13, 420–423
11 Compton DR, Martin BR (1990) *Life Sci* 46, 1575-1585
12 Melvin LS, Johnson MR, Harbert CA, Milne GM, Weissman A (1984) *J Med Chem* 27, 67–71
13 Kuster JE, Stevenson SJ, Ward SJ, D'Ambra TE, Haycock DA (1993) *J Pharmacol Exp Ther* 264, 1352–1363
14 Johnson MR, Melvin LS, Althious JS, Bindra CA, Herbert GM, Milne GM (1981) *J Clin Pharmacol* 21, 271S
15 Abraham RJ, Gatti G (1969) *J Chem Soc* B, 961–968
16 Huggins ML (1953) *J Am Chem Soc* 75, 4123–4126
17 Xie XQ, Yang DP, Melvin LS, Makriyannis A (1994) *J Med Chem* 37, 1418–1426
18 Xie XQ (1993) Thesis Dissertation, University of Connecticut, CT, USA
19 Yang DP, Mavromoustakos T, Beshah K, Makriyannis A (1992) *Biochim Biophys Acta* 1103, 25–35
20 Makriyannis A, Rapaka RS (1990) *Life Sci* 47, 2173–2184
21 Makriyannis A, Mavromoustakos T, Jarrel H, Banijamali A (1988) *In: Marihuana'87* (Chester G, Consroe P, Musty R, eds) Australian Govt Publishing Service, Canberra, Australia, 321–326