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QSAR studies of the pyrethroid insecticides Part 3. A putative pharmacophore derived using methodology based on molecular dynamics and hierarchical cluster analysis

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Abstract

Previous studies of the conformational behaviour of a group of synthetic pyrethroid insecticides have been extended to a more structurally diverse set. This includes compounds with different backbones and differing stereochemistry, with both Types I and II biological activity. These compounds also encompass a large range of biological activities. A parameterisation of the CHARMM force field for these compounds has been performed and the extra parameters are reported. Conformational sampling, using molecular dynamics (MD), has been performed for each of the 41 active structures. The accessible conformations of each have been characterised by the values of the common torsion angles using hierarchichal cluster analysis (HCA). A further CA, based on the centroids derived from the conformational sampling, identified a conformation common to at least 39 of the 41 structures. The critical torsion angles of this conformation lie at the centre of the molecule about the ester linkage and are defining an extended conformation, which differs from the minimum energy conformation of deltamethrin used previously. This may represent a putative pharmacophore for kill.

The methods used here improve significantly on those used previously. The CHARMM force field was parameterised for the compounds and an improved method of conformational sampling, based on centroid clustering, has also been used.

Keywords: QSAR; Pyrethroid insecticides; Molecular dynamics (MD); Cluster analysis; Conformational analysis

1. Introduction

The pyrethroids, synthetic analogues of the naturally occurring esters of chrysanthemic acid, possess broad-spectrum insecticidal activity combined with low mammalian toxicity [1]. The structure–activity relationships (SARs) have been extensively studied using static representations [2–4]. However, any study of the SARs of pyrethroid insecticides should take full account of their flexibility and low internal barriers to rotation. New methodology is, therefore, required to address this problem.

1.1. Structure–activity relationships (SARs) of pyrethroid insecticides

The structural requirements for insecticidal activity in the pyrethroids are well known [1]. Naumann [5] draws attention to the importance of the following features: an aromatic or olefinic π-system linked to a small lipophilic anchor group, e.g. cyclopropanoyl, a spacer group of three atoms, e.g. ester, ether and, optionally via a flexible pivot –O– or $–CH_2–$, a *meta*-substituted phenyl group. In addition, there are two crucially important centres of asymmetry (Fig. 1), that can modify insecticidal activity, viz. C1 of the cyclopropane ring (only the (*R*) configuration is active) and the α-carbon (C8) of the alcohol moiety (only the (*S*) configuration is active in Type II compounds that subtend a cyano-substitutent at this atom). A third asymmetric centre can also modify insecticidal activity slightly, viz. the C3 of the cyclopropane ring for which the (*R*) and (*S*) configurations (*cis*- and *trans*-, respectively) show small changes in activity, usually differing by a factor <2.

These stereochemical requirements are strongly suggestive that the compounds act at a chiral receptor, or at least need to be able to adopt a defined shape. If this is the case it is also highly likely that they will exhibit common conformational features. In addition the large range of biological activities (five orders of magnitude) within similar series suggests that molecular properties are also of crucial significance. Since some of these are likely to be highly

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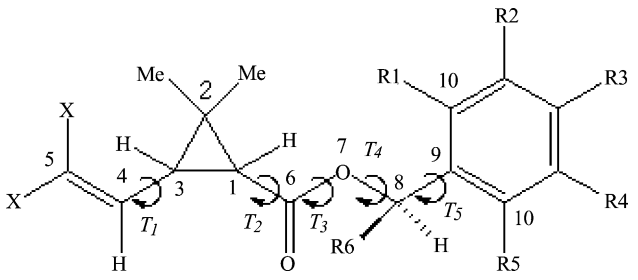


Fig. 1. General structure of pyrethroid insecticides and torsion angle nomenclature (where torsion angle T_1 is C=C–C3–C1; T_2 is C3–C1–C–O; T_3 is C–C–O–C; T_4 is C–O–C–C and T_5 is O–C–C–C).

dependent on stereochemistry, the conformational behaviour of these compounds is extremely important. Given that the structural, conformational and physicochemical properties of these compounds are likely to affect their biological activities, it is of interest to apply modern three-dimensional QSAR techniques to attempt to elucidate the basis of their activity. Several studies have attempted to establish such three-dimensional SARs using a variety of techniques [3,4,6,7].

Although pyrethroids are considered to act at a site on the voltage-dependent sodium channel, the exact site of binding is not known with certainty. Indeed, there is evidence to suggest that the nerve cell membrane itself may play a role in pyrethroid action [8,9]. Given this lack of information about the receptor site, it is necessary to develop methods for identification of a putative pharmacophore that rely only on the variation in structure, property and dynamic behaviour of compounds of known insecticidal activity.

1.2. Molecular modelling and QSAR studies of pyrethroids

Our interest started with a study of a set of compounds known, by us, as the QSAR series [3]. These materials were chrysanthemate and chlorsanthemate esters derived from (1*RS*)-*trans*-3-(2,2-dimethylvinyl)-2,2-dimethylcyclopropane-1-carboxylic acid and (1*RS*)-*trans*-3-(2,2-chlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid, respectively. The set was specifically designed to be amenable to QSAR studies using a selection routine based on literature values of molar refraction, log *P*, and substituent constants for inductive and resonance effects [10] for substituents in the benzylic ring. The compounds were synthesised and biological data obtained for ten activities and responses including killing activity (KA) and knockdown activity (KDA) [11]. A set of 13 compounds was selected based on the range of their biological activities. These were modelled to produce a three-dimensional structure from which a set of 70 molecular descriptors was calculated [3]. A number of methods for reducing this dimensionality was examined, Factor Analysis eventually being used to identify eight factors (F1–F8). Regression techniques were used to obtain a set of QSAR equations for each of the measured biological activities.

The results of this study were encouraging in that they showed the validity of using three-dimensional QSAR methods for this series of compounds. The QSAR equations were in accord with experience and identified important features. The problems were with the low number of compounds used and, more importantly, the necessity of choosing a conformation upon which to base the calculation of the molecular descriptors. This is important as some of these are highly dependent on conformation. As an example, the QSAR equations identify a negative coefficient for factor 7 in both KA and neurotoxicity (NT). Factor 7 is most closely correlated with the magnitude of the dipole moment and its component in the *Z*-direction. Both of these are critically dependent on the choice of conformation and have been previously identified as important to the biological activity [3].

The initial study used a model-built structure for the QSAR compounds based on the crystal structures of deltamethrin and other pyrethroids [12]. This model uses a number of rather bold assumptions, necessary because a reliable structure for the QSAR compounds was not available. An approach towards identification of this structure was subsequently investigated [4]. This involved using molecular dynamics (MD) simulations to sample the conformational space of each member of the series. MD was shown to be well suited to these compounds due to the very low rotational barriers. Thus, short MD simulations were able to sample all of the accessible conformations, with little dependence on the starting conformation. In addition, MD resulted in a small set of "fuzzy" but distinct local conformations centred around particular values of torsion angles but having rather large ranges around these; this is in contrast to the very many conformations that a full conformational search would have identified.

After suitable parameterisation of the MD methodology to ensure that it reliably sampled the conformational space, parameters were calculated for samples of the conformations of each of the compounds and averaged across the simulations. The purpose of this procedure was to smooth out any spurious parameter values arising solely from the choice of the static conformation. Subsequently, repetition of the factor analysis followed by regression against the biological activities gave results which were broadly in agreement with the previous study.

Successful as they were in identifying useful parameter-based QSAR equations for use with the pyrethroid insecticides, neither study properly addressed the real problem with these compounds, namely that of their high degree of flexibility and the lack of knowledge of the "receptor active" conformation. The present study attempts to address this. The previously developed methodologies of MD and HCA are here applied in a systematic way to attempt to identify a conformation suitable for future three-dimensional QSAR studies on these compounds. The group of 41 compounds (cf. 13 compounds used previously) comprises the full QSAR series together with additional pyrethroids generously supplied by Dr. Bhupinder Khambay, IACR Rothamsted.

2. Methods

2.1. Molecular modelling

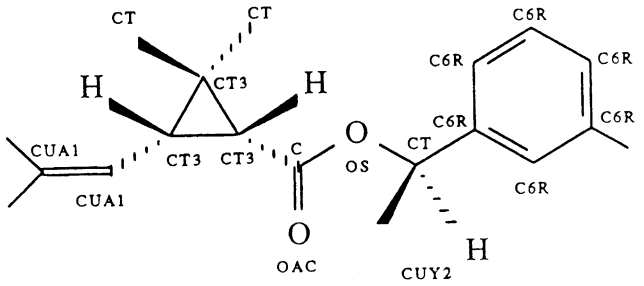
The expanded set of structures used in this study is given in Table 1. Modelling studies were based on the active (1*R*)-configuration at C1 of the cyclopropane ring shown in Fig. 1, which also defines the nomenclature used for the torsion angles. Simulations were performed using CHARMM Version 21.3 [13]. The CHARMM force field was parameterised for missing values (Table 2) using an implementation of AM1 within MOPAC [14]. calculations on simplified structures as a reference. For example, the torsional parameters for T_1 (Fig. 1) were adjusted to match the MOPAC energy profile for rotation about the equivalent bond in vinylcyclopropane, an approach based on semi-empirical methods and commonly used for investigation of rotational barriers [15–18]. A feature of rotation at T_1 is the energy minima associated with conjugation of the vinyl group with the cyclopropane ring which is not predicted by MNDO calculations. An ab initio study of this region using a 6-13G* basis set as employed in Gaussian 90 also showed the additional minima as did parameter values supplied by molecular simulations incorporated (MSI) (Fig. 2).

The results for torsion T_2 are also of interest. The parameterisation accurately reproduces the torsional preferences in cyclopropane carboxylic acid, i.e. two minima with the carbonyl oxygen lying above and below the cyclopropane ring. This is also observed in the crystal structures of pyrethroids [12]. However, when the derived parameters are applied to

Table 1
Structures of the pyrethroid insecticides investigated

1	QSAR01	2,3,4,5,6-Pentafluorobenzyl-(1*RS*)-*trans*-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropane carboxylate
2	QSAR02	2,3,4,5,6-Pentafluorobenzyl-(1*RS*)-*trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylate
3	QSAR03	2,3,4,5,6-Pentamethylbenzyl-(1*RS*)-*trans*-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropane carboxylate
4	QSAR04	2,3,4,5,6-Pentamethylbenzyl-(1*RS*)-*trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylate
5	QSAR05	3-Iodobenzyl-(1*RS*)-*trans*-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropane carboxylate
6	QSAR06	3-Iodobenzyl-(1*RS*)-*trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylate
7	QSAR07	2-Aminobenzyl-(1*RS*)-*trans*-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropane carboxylate
8	QSAR08	2-Aminobenzyl-(1*RS*)-*trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylate
9	QSAR09	4-Dimethylaminobenzyl-(1*RS*)-*trans*-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropane carboxylate
10	QSAR10	4-Dimethylaminobenzyl-(1*RS*)-*trans*-3-(2,2-dichlorovinyl)-2,2-dimthylcyclopropane-1-carboxylate
11	QSAR11	3,5-Dinitrobenzyl-(1*RS*)-*trans*-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropane carboxylate
12	QSAR12	3,5-Dinitrobenzyl-(1*RS*)-*trans*-3-(2,2-dichloro-vinyl)-2,2-dimethylcyclopropane-1-carboxylate
13	QSAR13	2,6-Dichloro-3-nitrobenzyl-(1*RS*)-*trans*-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropane carboxylate
14	QSAR14	2,6-Dichloro-3-nitrobenzyl-(1*RS*)-*trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylate
15	QSAR15	3,5-bis-Trifluoromethylbenzyl-(1*RS*)-*trans*-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropane carboxylate
16	QSAR16	3,5-bis-Trifluoromethylbenzyl-(1*RS*)-*trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylate
17	QSAR17	3-Nitrobenzyl-(1*RS*)-*trans*-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropane carboxylate
18	QSAR18	3-Nitrobenzyl-(1*RS*)-*trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylate
19	QSAR19	2,3,5,6-Tetrachloro-4-hydroxymethyl-(1*RS*)-*trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylate
20	QSAR25	4-Sulphonylmethylbenzyl-(1*RS*)-*trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylate
21	QSAR26	2,6-Dichloro-4-nitrobenzyl-(1*RS*)-*trans*-2,2-dimethyl-3-(2,2-dichlorovinyl)cyclopropane carboxylate
22	Benzyl chrysanthemate	Benzyl-(1*RS*)-*trans*-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropane carboxylate
23	ABC (NRDC100)	4-Allylbenzyl-(1*RS*)-*trans*-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropane carboxylate
24	DMABC (NRDC101)	4-Allyl-2,6-dibenzyl-(1*RS*)-*trans*-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropane carboxylate
25	NRDC105	5-Benzyl-2-methyl-3-furylmethyl-(1*R*)-*trans*-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropane carboxylate
26	Bioresmethrin (NRDC107)	2-Benzyl-4-furylmethyl-(1*R*)-*trans*-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropane carboxylate
27	Cismethrin (NRDC119)	5-Benzyl-3-furylmethyl-(1*R*)-*cis*-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropane carboxylate
28	Deltamethrin (NRDC161)	α-Cyano-3-phenoxybenzyl-(1*RS*)-*trans*-2,2-dimethyl-3-(2,2-dibromovinyl)cyclopropane carboxylate
29	1*R*-*Cis*-cypermethrin (NRDC998)	α-Cyano-3-phenoxybenzyl-(1*RS*)-*cis*-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropane carboxylate
30	1*R*-*Trans*-cypermethrin (NRDC999)	α-Cyano-3-phenoxybenzyl-(1*R*)-*trans*-(1*R*,*3S*)-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropane carboxylate
31	δ-Phenothrin (NRDC900)	3-Phenoxybenzyl-(1*R*)-*trans*-(1*R*,3S)-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropane carboxylate
32	1*R*-*Trans*-benzylcrysanthemate (NRDC1000)	Benzyl-(1*R*)-*trans*-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropane carboxylate
33	Dimethrin	2,4-Dimethylbenzyl-(1*RS*)-*trans*-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropane carboxylate
34	Pyrethrin I	NRDC501 (+)-pyrethronyl-(1*R*)-*trans*-(1*R*,3S)-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropane carboxylate
35	Pyrethrin II	NRDC504 (+)-pyrethronyl-(1*R*)-*trans*-3-((2-carboxymethyl)-2-dimethylvinyl)-2,2-dimethylcyclopropane-1-carboxylate
36	Cinerin I	(+)-Cineronyl-(1*R*)-*trans*-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropane carboxylate
37	Jasmolin I	(+)-Jasmolonyl-(1*R*)-*trans*-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropane carboxylate
38	*S*-Bioallethrin	(+)-Allethronyl-(1*R*)-*trans*-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropane carboxylate
39	Kadethrin (NRDC600)	5-Benzyl-3-furylmethyl-(*E*)-(1*R*,*3S*)- 2,2-dimethyl-3-(2-oxothiolan-3-ylidenemethyl)cyclopropane carboxylate
40	1*R*-*Cis*-permethrin	3-Phenoxybenzyl-(1*R*)-*cis*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylate
41	1*R*-*Trans*-permethrin (NRDC702)	3-Phenoxybenzyl-(1*R*)-*trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylate

Table 2
Additional parameters for CHARMM force field



Atom type	K_b (kcal mol^{-1} Å^{-2})	R_0 (Å)
Bond length		
CT3 CT3	268.0	1.50
Atom type	K_θ (kcal mol^{-1} rad^{-2})	θ_0 (°)
Bond angle		
C CT3 CT3	55.5	120.0
C CT3 HA	59.4	115.5
CT CT3 CT	40.0	116.0
CT3 C OAC	45.0	116.0
CT3 C OT	54.0	110.1
CT3 CT HA	37.5	109.47
CT3 CT3 CT	45.0	117.0
CT3 CT3 CUA1	45.0	119.0
CT3 CUA1 CUA1	55.0	121.15
HA CT3 CT3	31.0	114.0
HA CUA1 CT3	35.0	119.0
OS C CT3	60.0	114.4
Atom type	K_ω (kcal mol^{-1})	Periodicity	Phase
Torsion angle			
CT3 CT3 C OAC	0.05	1	0
CT3 CT3 C OS	0.05	1	180
CT3 CT3 C OT	0.05	1	180
X CT3 CUA1 X	0.00	3	0
X CT3 CUA1 X	0.05	2	0

pyrethroids themselves, two different conformations, rotated by 90°, are predicted probably as a result of the substitutions at positions 2 and 3 of the cyclopropane ring. The force field could be further modified to take this into account but this would entail a loss of generality in the parameterisation.

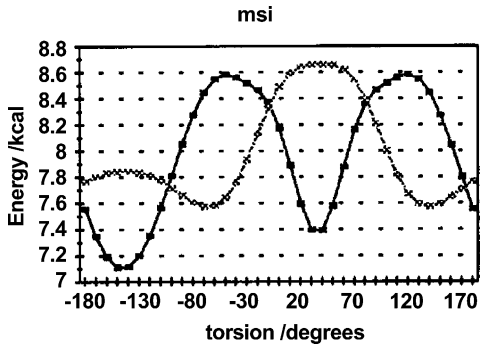


Fig. 2. Energy profiles for the torsional bond (T_1) in vinyl cyclopropane and the torsional bond (T_2) in the ester cyclopropane: (■) rejected MSI profile; (×) MNDO profile.

As the energy surface flattens, the degree of variation within the clusters would be expected to increase and, for pyrethroids, the surface is very flat (the average range of values of the conformations within each cluster for deltamethrin is $T_1 = 46°$, $T_2 = 81°$, $T_3 = 7°$, $T_4 = 30°$, $T_5 = 78°$). For any compound, a cluster centroid, therefore, represents a fuzzy set of accessible conformations rather than a single conformation. A cluster can include any particular conformation, for example the crystal structure, even though that structure may be some distance from the centroid. This fuzziness may aid in identifying a loosely defined pharmacophore.

More controversially, it could be argued that the above comments provide an advantage of the current approach over those based on localised energy minima or full conformational searching. Since the MD procedure will sample the accessible conformations, even if they are not local minima, it allows all reasonable possibilities to be considered. It also provides a degree of "independence" from the parameterisation of the force field. Thus, in cases like like this, a sampling methodology decreases the reliance on the accurate parameterisation of the force field. It should, however, be re-iterated that the force field used here does not give the same conformations for T_2 as are shown in the crystal structures and readers should form their own opinions.

The derived parameters were validated by comparing averaged values of the calculated dipole moment (using previously derived methodology) against measured values [19]. Full details of the parameterisation procedure are given elsewhere [20].

Initial co-ordinates for the structures were based on those used previously [3,4]. MD simulations were performed using CHARMM at a constant temperature of 300 K. The MD was initialised at 300 K, equilibrated for 20 ps and simulated for 800 ps with an integration time step of 1 fs. These conditions have been shown [4,20] to be sufficient to sample the conformational space of these molecules where the rotational barriers are known to be very low. Each pyrethroid, however, produced a different number of structures per simulation reflecting differences in conformational accessibility between compounds. Structures were saved at 1 ps intervals. Each dynamic simulation resulted in a file containing 800 (simulation time × sampling frequency = 800/1) individual structures, each with its set of torsional parameters. Random selections of structures were, therefore, made to provide representative, but manageable, samples ($n = 200$, sample fraction = 25%) on which statistical analysis could be performed.

2.2. Multivariate statistics

Cluster analyses were performed using BMDP [21] with the similarity matrix modified to account for the periodic nature of torsion angle space [20]. Descriptive statistics were calculated using BMDP [21] and MINITAB [22]. Graphical representations were performed using SYBYL [23] with

Table 3
Centroids of the largest clusters for deltamethrin

Cluster	Cluster membership	T_1	T_2	T_3	T_4	T_5
S1	73	−156	124	180	76	56
S2	20	−169	−50	178	167	43
S3	12	−156	33	−178	74	47
S4	21	86	137	179	78	52

torsion angles set to representative structures sampled during the dynamics simulations.

3. Results and discussion

3.1. Molecular dynamics simulations of deltamethrin

The general structure of insecticidal pyrethroid esters is presented in Fig. 1. Initial studies centred around MD simulations of deltamethrin. The aim here was to confirm that the simulation conditions were adequate to sample fully the conformational space accessible to these compounds. Previously [4], the simulations had been run for 800 ps which, despite the low rotational barriers, is quite short. Running the simulation at a higher temperature (900 K) suggested that the simulation length was sufficient but such high temperature simulations are not an ideal way of investigating this [20]. In addition, it was important to identify whether the different force field and new parameters gave results consistent with those of the previous study (which used the DISCOVER force field with unknown torsion angle parameters set to zero).

The simulation of deltamethrin was, therefore, repeated using the new force field with the significantly longer simulation time of 3.2 ns. The results obtained are in accord with those obtained previously indicating that the simulation conditions are sufficient to sample the conformational space. Thus, T_1, T_2 and T_5 each exhibit two distinct values. T_4 shows only two of the potential three values due to the presence of the cyano group. T_3 is invariant remaining in the anti-conformation (*trans*-) throughout the simulation.

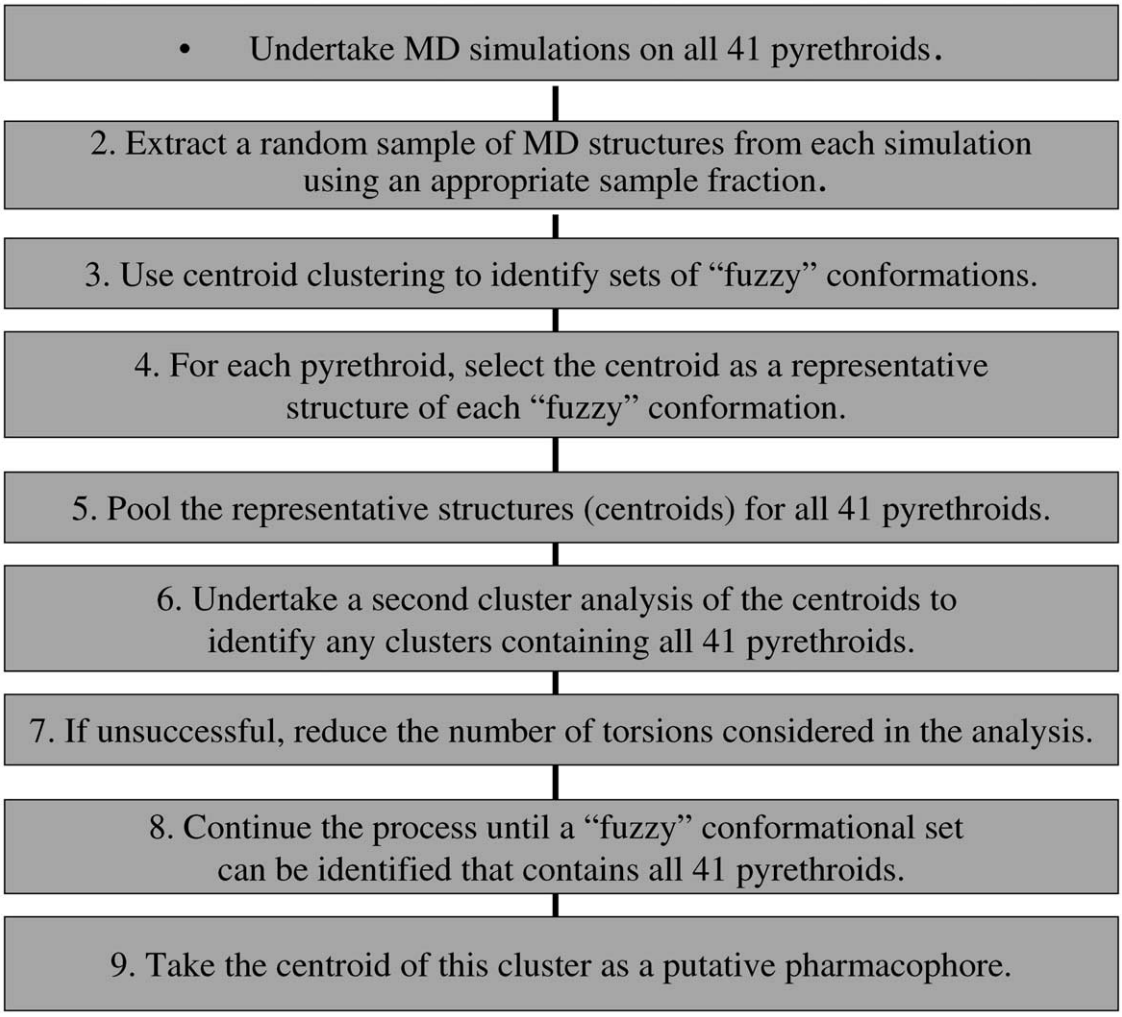


Fig. 3. Procedure for identifying a putative pharmacophore based on a 'fuzzy' conformation common to all members of a set of biologically-active pyrethroid insecticides.

Not all of these combinations are energetically possible. It is, therefore, necessary to devise a methodology to extract the conformations from the simulations. Cluster analysis (CA) is well suited to this task as it is able to group together similar structures on the basis of their torsion angles. The previous study [4] used a CA method to cluster the structures but, due to computational limitations, the "representative" structures were sampled from the CA on the basis of their position in the resulting dendrogram. This method is far from ideal and a method based on the cluster centroids has, therefore, been developed. A range of hierarchical CA methods was examined to perform this analysis. All produced broadly similar classifications of the conformations of deltamethrin. The *centroid* link method produced both the smallest number and the most distinct clusters and was used for all subsequent analyses. Table 3 shows the centroids of the largest clusters identified for deltamethrin using this methodology.

3.2. Extended study of a diverse set of insecticidal pyrethroids

This methodology has been applied to the MD simulations of all of the 41 compounds in the study. The centroid for each cluster was selected as its representative structure, thus, generating 20–30 centroid conformations per compound. The next step was to identify a putative active conformation. For QSAR purposes, this should be one which is accessible to all structures. The approach for this was again to use a CA technique. Thus, the sets of centroid structures obtained for each of the 41 compounds were pooled to give a total of 535 representative conformations which were analysed using a second CA. The resulting clusters were then examined to determine the number of structures in each. The reasonable expectation was that an identifiable active conformation would be within a cluster containing a large number of the active structures and, therefore, readily accessible to a diverse set of biologically-active compounds. The strategy is summarised in Fig. 3.

Initially this CA was performed using all the torsion angles T_1–T_5. This resulted in 60 clusters, or putative pharmacophores, but none of these contained large numbers of compounds. Thus, the three largest clusters contained only 26, 24 and 21 compounds, respectively. Analysis of the clusters suggested that too many torsional degrees of freedom had been included, leading to unnecessarily high dimensionality and, hence, to statistical noise. Thus, T_3 does not change from 180° in any of the simulations and should not, therefore, be included as a variable. T_5 varies between two values corresponding to a flipping of the rapidly rotating aromatic ring. This flipping does not fundamentally affect the conformation since it only inverts the position of the *ortho*- and *meta*-substituents, an effect which this methodology would not be expected to identify. Thus, T_5 is also effectively just adding noise. Most importantly, this torsion is invariant for certain cyclic analogues such as the naturally occurring cyclopentenolone esters (compounds 34–38). A more difficult problem occurs with T_1 in that this is affected

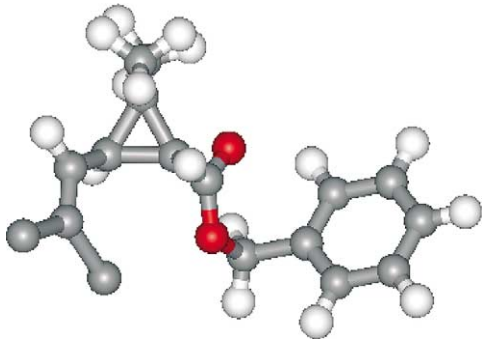


Fig. 4. The 'active' conformation.

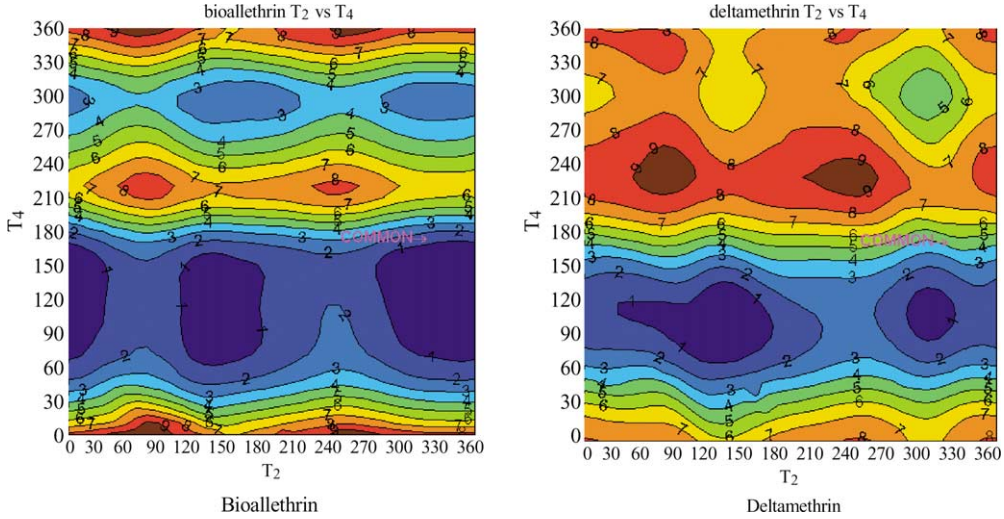


Fig. 5. Ramachandran plots of the critical torsions T_2 and T_4 for the pyrethroid insecticides bioallethrin (Type I) and deltamethrin (Type II)—the torsion angles in these plots have been incremented by 180° compared with the values reported in the text.

by the different stereochemistries at C1. This makes the definition of T_1 inconsistent across the series. However, the relatively small differences in activity arising from different substitutions on the vinyl group (cf. chrysanthemates and chlorsanthemates) and from the different stereochemistries at C1, suggest that this torsion has relatively little biological significance. Reducing the number of torsion angles focuses the investigation and gives the distinct advantage that only those bonds involved in eliciting the biological response are used to define the pharmacophore.

The analysis was, therefore, repeated without these parameters, i.e. with T_2 and T_4 only. These are better-behaved in a statistical sense and, since they are the central torsion angles, are in any case expected to have the dominant effect on the overall conformation [3,18]. This analysis did produce one large cluster containing 39 of the 41 active structures examined. The values of the centroid torsion angles in this cluster were $T_2 = -38^\circ$ and $T_4 = 175^\circ$.

The two structures which are not represented in this cluster are cinerin I (36) and pyrethrin II (35). These compounds are closely related to pyrethrin I (34), which is a member of the cluster, and their absence probably arises as a result of sampling only 200 structures for the initial clustering procedure rather than from an inability to adopt the appropriate conformation (perhaps a larger sampling fraction would have been appropriate). This was further suggested by energy minimisation studies. To test this, both were energy minimised, starting from the common conformation. Both minimised to conformations well within the standard deviation of the torsion angles of the common conformation, lending support to the view that 'fuzzy' conformations have sufficient precision to represent a pharmacophoric structure in a reliable manner.

3.3. A putative pharmacophore?

A conformation common to all the biologically-active pyrethroids investigated has been identified by this new methodology and is shown in Fig. 4. This structure is interesting in that it differs from that of the minimum energy (most abundant) conformation of cypermethrin. Thus, the new 'active' conformation is an extended structure whereas the corresponding minimised molecular mechanics structure of cypermethrin is folded to form a 'clam' [24,25]. It also differs from the crystal structure of deltamethrin used previously [3] and the preferred conformation of pyrethrin I [26]. These results suggest that, for a given compound, the most abundant conformation is unlikely to be the biologically-active structure or pharmacophore.

Pyrethroid insecticides have been classified (Types I and II) on the basis of the biological symptoms that they induce. Response to Type I compounds involves the behavioural sequence: hyperactivity, incoordination, knockdown, paralysis and death; all but the last of these states are transient and, therefore, reversible, although the possibility of recovery diminishes along the sequence. In contrast, Type II responses involve the relatively slow onset of paralysis followed by death; hyperactivity, incoordination and 'true' knockdown are not observed. Both types of response, with knockdown occurring within minutes and kill taking place over days and even weeks, are important in pest control. The knockdown and kill responses are also differentiated by their temperature dependence, which is positive for knockdown (exclusively Type I), but negative for kill (Types I and II).

In general and with few exceptions, Type II but not Type I compounds contain an α-cyano group subtended by the α-carbon of the benzylic methylene and adopting the *S*-configuration. An important consequence of this substitution is that rotation is strongly constrained in an identifiable region of the torsional space defined by the critical torsions T_2 and T_4 (Fig. 5) due essentially to repulsion between similarly-directed dipoles, viz. CN, CO. This constraint does not occur in Type I compounds suggesting that KDA is promoted by free rotation about T_4 giving access to an appropriate conformation inaccessible to Type II compounds. A possible conformation for kill, as indicated in Fig. 4, is that already identified as common to all the pyrethroids investigated here.

Although Type I compounds can kill, their activity in this respect tends to be low compared with the most potent Type II compounds such as cypermethrin and deltamethrin. Moreover, Type I compounds show a weak but significantly positive association between knockdown and killing potencies [11]. We, therefore, suggest that these observations follow from a requirement for pyrethroids to adopt different conformations for knockdown (a transient state) and kill (an absorbing state), such that the 'kill' conformation can be accessed by both Types I and II but the 'knockdown' conformation is only possible for Type I. Further, the 'knockdown' conformation will not be the most stable, reflecting its higher probability at elevated temperatures as indicated by the positive temperature dependence of the knockdown response noted earlier. The energy profiles in Fig. 5 also allow the possibility that free rotation about torsions T_2 and T_4 is a requirement for knockdown. Again, this would be consistent with the observed positive temperature coefficient for this response. By contrast, the 'kill' conformation may be the most stable unconstrained structure. Thus, the 'kill' conformation is represented by the only structure (Fig. 4) observed during MD simulations to be accessible to pyrethroid Types I and II.

4. Conclusions

A new methodology based on MD and CA has been developed and applied to a study of pyrethroid insecticides. A putative "active" conformation for the pyrethroid insecticides has been identified and related to the killing potency of these insecticides based on its accessibility to all of the compounds under investigation. This pharmacophore differs from previous conformations used and may be more useful for QSAR because it is common amongst a diverse series.

It is also proposed that a different structure with access restricted to Type I pyrethroids is required for knockdown action. This structure is differentiated from that for kill by rotations about the critical torsions T_2 and T_4.

The methodology developed here has wider application and advantages over previously published approaches. Thus, the method is not computationally intensive compared with a conformational searching procedure used in an earlier study [25] and deals only with accessible conformations. Moreover, it avoids over definition and the associated loss of degrees of freedom that characterise certain 4DQSAR procedures. A particularly useful feature is the ability to identify a putative pharmacophore for a series of flexible molecules without prior knowledge of the receptor or its geometry. Moreover, diverse compounds with different degrees of torsional freedom can be included in the same analysis. Finally, the approach has identified a low dimensional pharmacophore for use in QSAR studies based on two critical torsion angles. This pharmacophore has been used successfully to predict the knockdown and killing potencies of non-cyclopropane carboxylate pyrethroids [8], i.e. pyrethroids of different chemical class to that for which the pharmacophore has been defined. That study will be the subject of a subsequent publication.

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References

[1] L. Crombie, M. Elliott, Chemistry of the natural pyrethrins, Fortrschritte de Chemie Organischer Naturstoffe 19 (1961) 1–120.
[2] M. Elliott, Synthetic pyrethroids, in: M. Elliott (Ed.), Synthetic Pyrethroids, ACS Symposium Series, Vol. 42, 1977, pp. 1–28.
[3] M.G. Ford, R. Greenwood, C.R. Turner, B. Hudson, D.J. Livingstone, The structure–activity relationships of pyrethroid insecticides. 1. A novel approach based upon the use of multivariate QSAR and computational chemistry, Pestic. Sci. 27 (1989) 305–326.
[4] B.D. Hudson, A.R. George, M.G. Ford, D.J. Livingstone, The structure–activity relationships of pyrethroid insecticides. 2. The use of molecular dynamics for conformation searching and average parameter calculation, J. Comput. Aided Mol. Design 6 (1992) 191–201.
[5] K. Naumann, in: K. Naumann (Ed.), Chemistry of plant protection. 4. Synthetic Pyrethroid Insecticides: Structure and Properties, Springer, Berlin, 1990.
[6] J.R. Byberg, F.S. Jorgenson, P.D.A. Klemmensen, J. Comput. Aided Mol. Design 1 (1987) 181–195.
[7] A. Mullaley, R.J. Taylor, Conformational properties of pyrethroids, Comput. Aided Mol. Design 8 (2) (1994) 135–152.
[8] M.G. Ford, N.E. Hoare, B.D. Hudson, T.G. Nevell, J.A. Wyatt, Putative pharmacophores for flexible pyrethroid insecticides, in: K. Gundertofte, F.S. Jorgensen (Eds.), Molecular Modeling and Prediction of Bioactivity, Academic Publishers, New York, 1999, pp. 303–304.
[9] C. Chiang, A.L. Devonshire, Changes in membrane phospholipids, identified by Arrhenius plots of acetylcholinesterase and associated with pyrethroid resistance (kdr) in houseflies (*Musca domestica* L.), Pestic. Sci. 13 (1982) 156–161.
[10] D.J. Livingstone, B.D. Hudson, A. George, M.G. Ford, The use of molecular dynamics in QSAR studies, in: C. Silipo, A. Vittoria (Eds.), Proceedings of the 8th European Symposium on Quantitative Structure–Activity Relationships. QSAR: Rational Approaches to the Design of Bioactive Compounds, Elsevier, Sorrento, Napoli, Italy, 1991, pp. 557–560.
[11] D.S. Buckley, M.G. Ford, L.D. Leake, D.W. Salt, P.E. Burt, M.D.V. Moss, C.J. Brealey, D.J. Livingstone, A neurophysiological investigation of the action of synthetic pyrethroids against adult houseflies *Musca domestica* L, in: Proceedings of the 6th European Symposium on Quantitative Structure–Activity Relationships, Portorose: QSAR in Drug Design and Toxicology, Yugoslavia, 1987, pp. 336–339.
[12] (a) J.D. Owen, J. Chem. Soc. Perkin I (1975) 1865–1868;
(b) J.D. Owen, J. Chem. Soc. Perkin I (1976) 1231–1235;
(c) J.D. Owen, The structure of rel-(α,*R*)(1*R*, *trans*)-α-cyano-3-phenoxybenzyl-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate, Acta Chrysta. B37 (1981) 1311–1314.
[13] B. Brooks, R.E. Bruccoleri, B.D. Olafson, D.J. States, S. Swaminathin, M. Karplus, CHARMM—a program for macromolecular energy, minimisation and dynamics calculation, J. Comput. Chem. 4 (1983) 187–217.
[14] M.J.S. Dewar, E.G. Zoebisch, E.F. Healey, J.J.P. Stewart, The development and use of quantum mechanical molecular models. 76. AM1—a new general purpose QM molecular model, J. Am. Chem. Soc. 107 (1985) 3902–3909.
[15] A.A. Mohamed, Conformations and rotational barriers of 2,2-bithiazole and 4,4-dimethyl-2,2-bithiazole semi-empirical, ab initio and density functional theory calculations, Int. J. Quant. Chem. 79 (2000) 367–377.
[16] L. Ernst, E.L. Riek, M. Soliven, Preferred conformation and barriers to internal rotation of *ortho*-disubstituted cyclopropane benzenes, Can. J. Chem. 77 (1999) 1697–1706.
[17] T. Sato, M. Kataoka, Molecular structure and electron spectrum of taspine: semi-empirical calculations, J. Heterocyclic Chem. 36 (1999) 1091–1093.
[18] X.-Q. Xie, X.W. Han, J.Z. Chen, M. Eissenstat, A. Makriyannis, High resolution NMR and computer modelling studies of cannabimimetic aminoalkylindole prototype WIN-55212-2, J. Med. Chem. 42 (1999) 4021–4027.
[19] M.G. Ford, N.E. Hoare, B.D. Hudson, T.G. Nevell, J.J. Robinson, J. Wyatt, The use of dipole measurement in molecular dynamics studies of pyrethroids, Pestic. Sci. 36 (1992) 83–84.
[20] N.E. Hoare, The Use of Molecular Dynamics Simulations in QSAR Studies of Pyrethroid Insecticides, Ph.D. Thesis, University of Portsmouth, Portsmouth, 1995.
[21] M.B. Brown, W.J. Dixon, in: L. Engleman, M.A. Hill, R.I. Jennrich (Eds.), BMDP Statistical Software Manual, University of California Press, Berkeley, USA, 1988.
[22] Minitab, http://www.minitab.com/support/docs/index.htm, 2000.
[23] M. Clark, R.D. Cramer III, N. van Opdenbosch, Validation of the general-purpose TRIPOS 5.2 force-field, J. Comp. Chem. 10 (1989) 982–1012.
[24] A.J. Hopfinger, D. Malhotra, R.B. Battershell, A.W. Ho, Conformational behaviour and thermodynamic properties of phenothrin analog insecticides, J. Pestic. Sci. 9 (1984) 631–641.
[25] H. Chuman, S. Goto, M. Karawasi, M. Sasaki, U. Nagashima, K. Nishimura, T. Fujita, Three-dimensional structure–activity relationships of synthetic pyrethroids. 1. Similarity in bioactive conformations and their structure–activity pattern, Quant. Struct. Act. Relat. 19 (2000) 10–21.
[26] M. Elliott, N.F. Janes, Preferred conformations of pyrethroids, in: M. Elliott (Ed.), Synthetic Pyrethroids, ACS Symposium Series, Vol. 42, 1977, pp. 29–36.