Journal of Computer-Aided Molecular Design, 6 (1992) 191–201
ESCOM



J-CAMD 148

# Structure–activity relationships of pyrethroid insecticides. Part 2. The use of molecular dynamics for conformation searching and average parameter calculation

Brian D. Hudson[a,*], Ashley R. George[a], Martyn G. Ford[b] and David J. Livingstone[c]

[a]*Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS, U.K.*
[b]*School of Biological Sciences, Portsmouth Polytechnic, Portsmouth, Hants PO1 2DY, U.K.*
[c]*SmithKline Beecham Pharmaceuticals, The Frythe, Welwyn, Herts AL6 9AR, U.K.*

Received 21 March 1991
Accepted 21 August 1991

*Key words:* Molecular dynamics; Conformational analysis; Conformation; Pyrethroid; Insecticide; Quantitative structure–activity relationship; QSAR; Multivariate analysis; Computer-aided molecular design

## SUMMARY

Molecular dynamics simulations have been performed on a number of conformationally flexible pyrethroid insecticides. The results indicate that molecular dynamics is a suitable tool for conformational searching of small molecules given suitable simulation parameters. The structures derived from the simulations are compared with the static conformation used in a previous study. Various physicochemical parameters have been calculated for a set of conformations selected from the simulations using multivariate analysis. The averaged values of the parameters over the selected set (and the factors derived from them) are compared with the single conformation values used in the previous study.

## INTRODUCTION

Pyrethroids are an important class of nerve poisons having broad spectrum insecticidal activity combined with low mammalian toxicity [1,2]. The chemical structure of deltamethrin, one of the most active of this class of compounds, is shown in Fig. 1. Structure–activity studies of related compounds have revealed that the stereochemical requirements for activity can be summarised by a set of empirical rules [2,3] involving the stereochemistry at carbons 1 and 3 of the cyclopropane ring and the presence of the geminal dimethyl groups at the 2 position of the cyclopropane ring.

Quantitative structure–activity studies, involving steric and electronic parameters, have recent-

* To whom correspondence should be addressed.

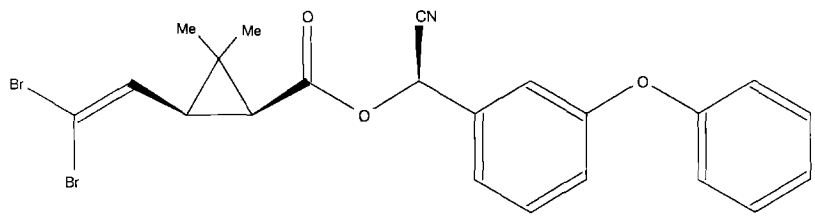



Fig. 1. Chemical structure of deltamethrin.

ly been performed on a series of pyrethroids with a range of activity spanning 7 orders of magnitude [4]. This series differs from deltamethrin in having the opposite configuration at the C3 atom of the cyclopropane ring, in lacking the benzylic cyano group and in having substituents other than 3′-phenoxy in the aromatic ring. The substituents on the aromatic ring were specifically preselected, using the program SELECT [5], to give a set of compounds amenable to rigorous QSAR study. Statistical analysis of a range of physicochemical parameters suggested that common molecular features (namely a partial positive charge on the alcohol moiety and a low overall dipole character) are associated with neurotoxic activity in insects [4].

Problems remain with this study however, not least of which is the necessity to decide upon a single conformation for which to calculate the required physicochemical parameters. It is well known that pyrethroids are very flexible molecules [6,7] and that the choice of conformation can affect the values of the calculated physicochemical parameters [4]. The previous study assumed the crystal structure of deltamethrin, with minor modifications, to be relevant to the receptor-bound conformation of both deltamethrin and the series under study. This ignores other equally accessible conformations for the pyrethroid molecules, possibly having very different values of the calculated physicochemical parameters. What is ideally required is knowledge of the conformation at the receptor. In the absence of such, a putative conformation has therefore been chosen and the considerations governing this choice are given in Ref. 4.

Whilst this approach is often successful it is interesting to consider what the effect of using all the possible conformations accessible to each compound to generate the required physicochemical parameters would be. The simplest way of doing this would be to take a value for each parameter averaged over all accessible conformations. Whilst this would undoubtedly include conformations which are definitely not active at the receptor, it may be less misleading than choosing an arbitrary conformation which may or may not be the active one.

The first stage in testing this idea is to develop a method for generating the required conformations. One possibility would be to use an exhaustive conformation search for each molecule, using a program such as the SEARCH algorithm in SYBYL [8], or the MULTIC algorithm in MACROMODEL [9]. Other possibilities include the Monte Carlo type conformational search techniques [10–12]. The approach adopted here has been to use molecular dynamics (MD) as a conformation searching tool. This technique has been applied to conformation searching mainly in the area of small peptides [13] but has also been applied to a small molecule 18-crown-6 [14] and appears to work well in both cases.

The issues assessed in this preliminary study are 3-fold. Perhaps the most important is whether or not MD is suitable as a conformation searching tool for small molecules. In particular does it sample all the accessible conformational space and if so what are the conditions required to a-

chieve this. Secondly, do the results of the dynamics simulations tell us anything about the conformation used in the previous study and its applicability for QSAR analysis? Thirdly, can we use MD to select representative conformations for each compound and hence generate averaged values of the required physicochemical parameters and, if so, are these of any greater utility than the static values?

## METHODS

The initial structures of all compounds were as used in Ref. 4. The MD simulations were performed using the package DISCOVER [15]. Structures were minimised and simulations performed using the DISCOVER CVFF force field, using harmonic potentials and neglecting cross terms. Additional parameters required were obtained either by comparison with existing DISCOVER parameter values or by conversion from the TRIPOS force field [8]. A new atom type was defined for iodine, the required parameters being obtained by comparison with the SYBYL values. Values for all the additional parameters used are given in Table 1.

The MD simulations were performed using a canonical ensemble i.e. constant temperature, constant volume and a constant number of molecules, with an integration time step of 1 fs. The structures were initialized at 300 K followed by 20 ps of equilibration and 200 ps of simulation also at 300 K. Torsion angles were calculated every 50 fs and a conformation recorded every 200 fs.

Data analysis was performed using RS/1 [16] for the graphical representations and ARTHUR [17] for the multivariate analysis. All dynamics simulations were performed on a CRAY XMP/28.

## RESULTS AND DISCUSSION

Several MD simulations were performed on representative compounds in the series for different time periods (30 ps, 50 ps and 200 ps). It was found to be necessary to run the simulations for 200 ps in order to get consistent results i.e. all conformations accessed by each compound. No advantage was seen by using higher temperature simulations as the conformational energy differences are very small ($\sim$0.5 kcal [4]). These simulation conditions gave consistent results which were quick and simple to calculate whilst also being long enough to give a degree of energy weighting of the final parameters, i.e. the averaged parameters should favour lower energy conformations due to their longer lifetime.

Figure 2 defines the flexible torsion angles in all members of the series. Figures 3A–D are plots of the values of torsion angles $T_1$, $T_2$, $T_4$ and $T_5$ against time throughout the simulation period for

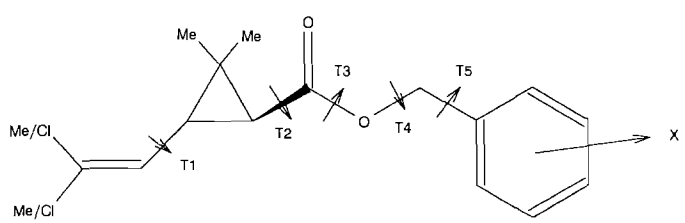
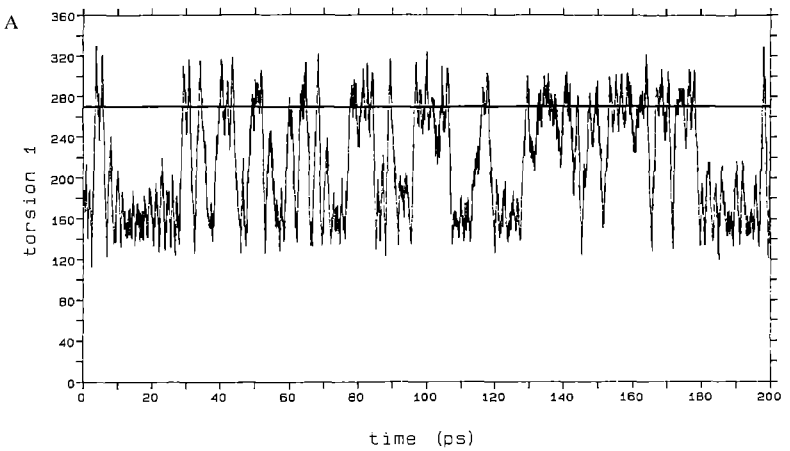
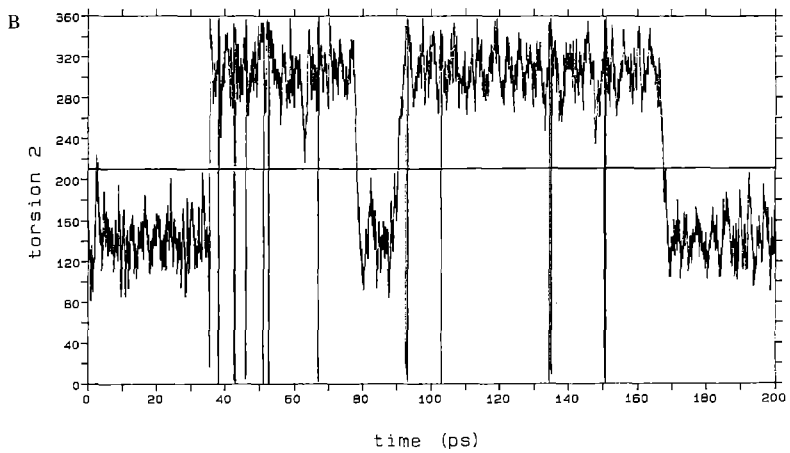
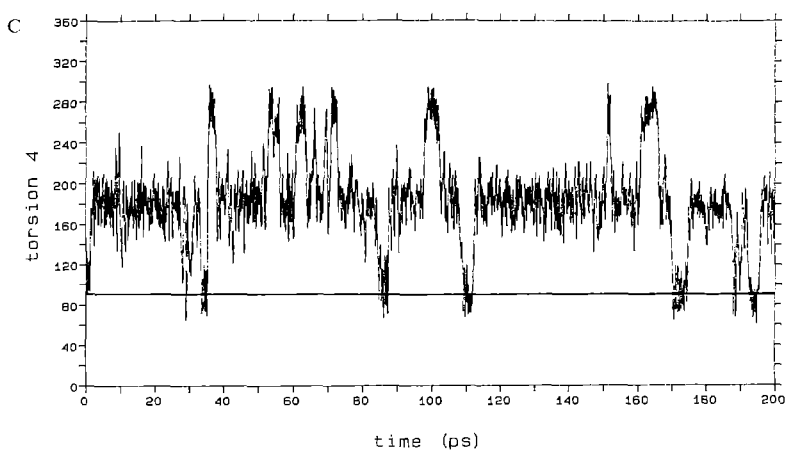



Fig. 2. Chemical structure of QSAR02 showing the flexible torsion angles.


A
torsion 1
time (ps)


B
torsion 2
time (ps)


C
torsion 4
time (ps)

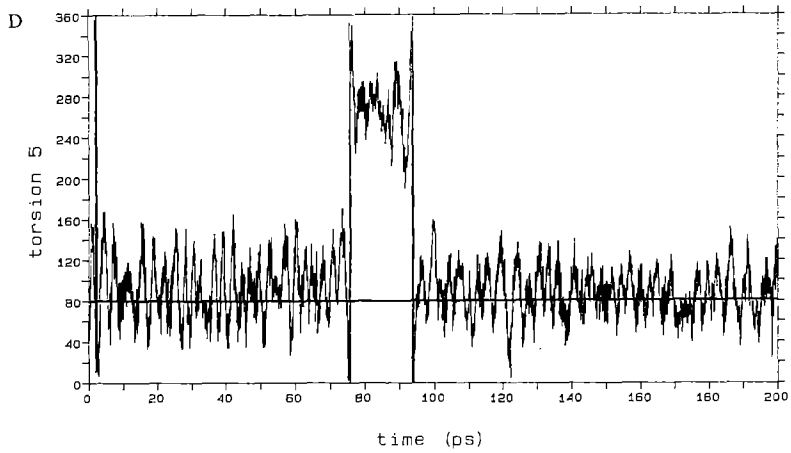



Fig. 3. (A) Plot of $T_1$ for QSAR02 against time; (B) plot of $T_2$ for QSAR02 against time; (C) plot of $T_4$ for QSAR02 against time; (D) plot of $T_5$ for QSAR02 against time.

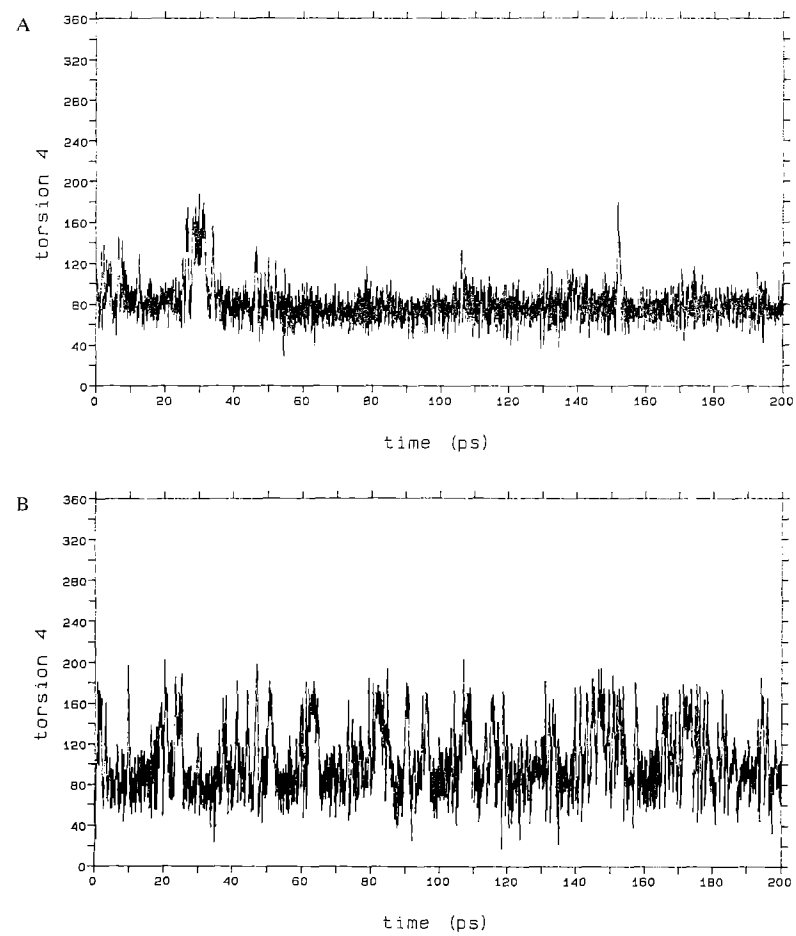



Fig. 4. (A) Plot of $T_4$ for deltamethrin against time at 300 K; (B) plot of $T_4$ for deltamethrin against time at 600 K.

TABLE 1
ADDITIONAL DISCOVER PARAMETERS USED

| Bond | $K_b$ (kcal/mol) | $R_e$ (Å) | Comparison |
|---|---|---|---|
| br c= | 206.06 | 1.920 | DISCOVER cp br |
| cl ct | 320 | 1.458 | SYBYL C.3 C.1 |
| ct nt | 800 | 1.158 | SYBYL C.1 N.1 |
| cp o | 384 | 1.370 | DISCOVER cp oh |
| c= cl | 260 | 1.75 | SYBYL C.2 Cl |
| n o- | 310 | 1.4 | SYBYL Npl3 O.2 |
| cp i | 245 | 2.05 | SYBYL C.ar I |

| Angle | $\theta_e$(°) | $H_d$ (kcal/mol) | Comparison |
|---|---|---|---|
| o c cp | 110.5 | 46.6 | DISCOVER c c cp |
| c c c= | 110.5 | 46.6 | DISCOVER c c cp |
| c c= c | 120.0 | 50.0 | DISCOVER c c= c′ |
| br c= br | 120 | 32.828 | SYBYL Br C.2 Br |
| br c= c= | 120 | 59.09 | SYBYL Br C.2 C.2 |
| o cl ct | 109.5 | 36.11 | SYBYL * C.3 * |
| ct cl cp | 109.5 | 32.828 | SYBYL C.2 C.3 O.3 |
| ct cl h | 109.5 | 26.26 | SYBYL * C.3 H |
| cl ct nt | 180 | 65.66 | SYBYL C.3 C.1 N.1 |
| cp o cp | 110 | 32.828 | SYBYL C.ar O.3 C.ar |
| c= c= cl | 120 | 59.09 | SYBYL C.2 C.2 Clcl |
| c= cl | 122 | 49.242 | SYBYL Cl C.2 Cl |
| cp n o- | 120 | 65.66 | SYBYL * Npl3 * |
| o- n o- | 127 | 98.48 | SYBYL O.2 Npl3 O.2 |
| cp cp i | 120 | 59.09 | SYBYL C.ar C.ar I |

| Out of plane | $K_x$ (kcal/mol) | $n$ | Phase | Comparison |
|---|---|---|---|---|
| c c= c c= | 11.1 | 2 | 180 | DISCOVER c c= c′ c= |
| c= c= br br | 10.00 | 2 | 180 | SYBYL C2 |
| c= c= cl cl | 10.00 | 2 | 180 | SYBYL C2 |
| cp n o- o- | 10.00 | 2 | 180 | SYBYL C2 |
| cp cp cp i | 10.00 | 2 | 180 | SYBYL C2 |
| cp cp cp f | 10.00 | 2 | 180 | SYBYL C2 |
| cp cp cp cl | 10.00 | 2 | 180 | SYBYL C2 |
| cp cp cp n | 10.00 | 2 | 180 | SYBYL C2 |

| Lennard–Jones | A (kcal·Å$^{12}$/mol) | B (kcal·Å$^6$/mol) |
|---|---|---|
| i | 7722540 | 2495 |

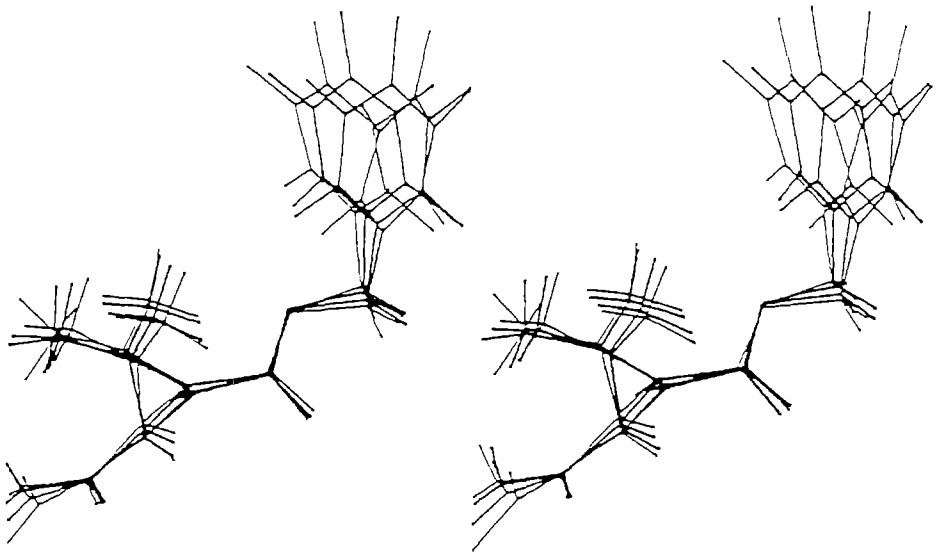


Fig. 5. Stereoview of the overlay of the 12.0, 12.2, 12.4 and 12.6 ps minimised structures of QSAR02.

QSAR02 (the pentafluoro derivative). $T_3$ not unsurprisingly stays constant throughout the simulation. These plots clearly show the conformational preferences of the 4 torsion angles. There are two possible values for $T_1$ (160° and 280°), two for $T_2$ (140° and 300°), three for $T_4$ (90°, 180° and 270°), and two for $T_5$ (90° and 270°). Thus there is a maximum of 24 possible conformations of these compounds sampled during the simulation.

The plots for QSAR02 are typical of the plots obtained for the other members of the series, the only exception being QSAR14. This compound has chloro groups at both ortho positions and shows only one conformation for $T_5$ (280°) probably as a result of a high barrier to rotation due to the steric bulk of the ortho chlorines. This result is consistent with NMR studies [4]. Despite this, the fact that similar features are seen for all other structures suggests that the simulation conditions used are sufficient to search the conformational space of these molecules.

It is interesting to compare the results of these simulations with the static structure used in the previous study [4]. The horizontal lines on Figs. 3A–D represent the values of the torsion angles of this static structure. It can clearly be seen that whereas torsions $T_1$, $T_4$ and $T_5$ can easily access the static structure, $T_2$ does not. This could be due to crystal packing forces in the static structure which do not exist in vacuo giving rise to a conformation not accessed in the dynamics simulations. It is also possible that the crystal structure, being an average structure itself, is actually an average of the two conformations accessed in the MD simulations since it lies approximately midway between these.

However, a referee has pointed out that the most likely explanation for this is the known preference for cyclopropyl groups to adopt bisected conformations [18]. This is not likely to be reproduced in these calculations as standard sp3 carbons were used for the cyclopropyl ring. The calculations could be improved by using atom types specific for cyclopropyl carbons with correct torsional potentials.

It is also of interest to compare these simulations with the equivalent simulation performed on

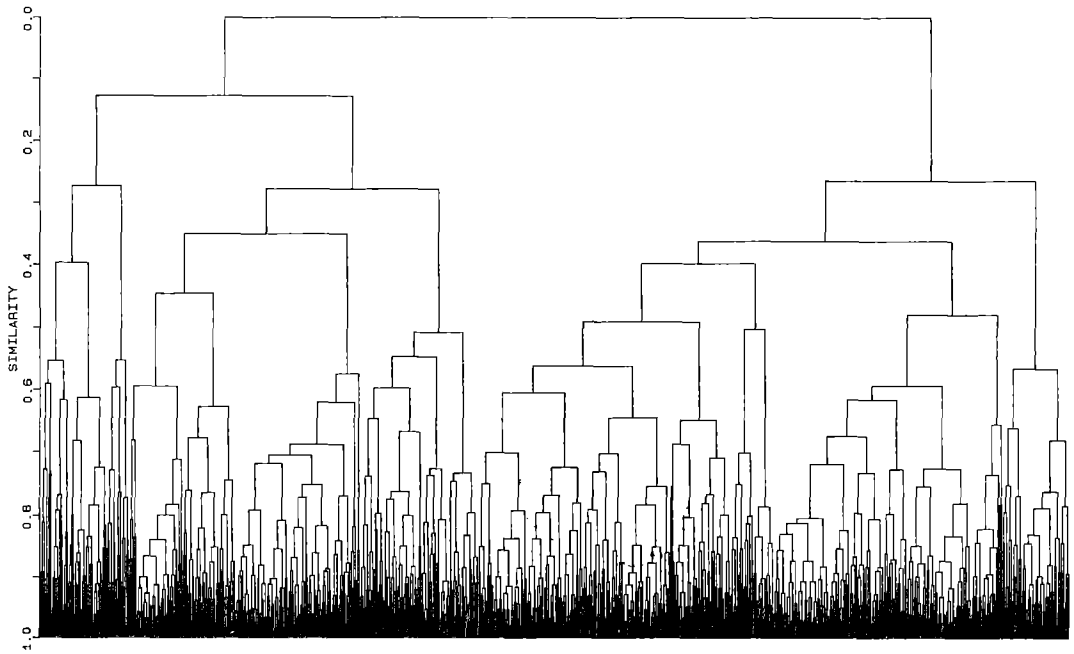



Fig. 6. Cluster analysis dendogram for QSAR02.

the much more active compound deltamethrin. The only significant difference between these is that deltamethrin shows only two values for $T_4$ (Fig. 4A) as compared to the three for the present series. A higher temperature simulation (600 K) was performed for deltamethrin to confirm that the whole conformational space was being explored (Fig. 4B). This gave a faster interconversion between the two sets of conformations but still showed no evidence of $T_4 = 270°$ conformations. The absence of these conformations in deltamethrin is due to the cyano group and could have important consequences for the receptor-active conformation. Note also that the equivalent of the $T_4 = 180°$ conformations have $T_4 = 150°$ again due to the presence of the cyano group.

Also worthy of note is the large variation in the torsion angles ($\pm 20°$) within basically the same conformation. Since one of the main aims of this work is to select different conformations for subsequent property calculation it was necessary to confirm that structures with similar torsion angles correspond to the same conformation after minimisation. In order to check this, 4 conformations were extracted from the simulation of QSAR02. The structures chosen were the 12.0 ps, 12.2 ps, 12.4 ps and 12.6 ps structures where the molecule is not undergoing any gross conformational change but where there are considerable variations in the torsion angles. Each of these structures was minimised in DISCOVER. The resulting structures are shown overlaid in Fig. 5 which clearly shows that even with the large variations in torsion angle the sampled structures all minimize to similar conformations.

Because of the large number of conformations recorded (1000 per simulation) it is impractical to calculate physicochemical parameters for each one and average over the whole simulation. A method for selecting a subset of structures from the simulation was therefore necessary. One approach to this would be to take structures at defined time intervals although this may not sam-

TABLE 2
MEAN VALUES OF STATIC AND AVERAGED PARAMETERS

| Property | Static | Averaged |
|---|---|---|
| DM | 3.705 | 3.017 |
| DVX | 1.231 | 0.574 |
| DVY | −0.513 | −0.232 |
| DVZ | 2.360 | −0.435 |
| DCA | 7.9 | 8.4 |
| SA | 386.8 | 397.2 |
| MIX | $2.1 \times 10^3$ | $2.8 \times 10^3$ |
| MIY | $1.1 \times 10^4$ | $9.0 \times 10^3$ |
| MIZ | $9.9 \times 10^3$ | $8.0 \times 10^3$ |
| PAX | 11.2 | 9.7 |
| PAY | 3.2 | 3.5 |
| PAZ | 4.3 | 5.0 |

ple all the accessible conformations. The approach adopted here was to perform a multivariate cluster analysis, using the values of the torsion angles as parameters, to define groups of similar conformations and to take one structure from each of these clusters. This necessitated making a modification to the distance matrix routine in ARTHUR to take account of the periodic nature of torsion angles (i.e. $0° = 360°$).

Figure 6 shows the resulting cluster analysis for QSAR02. In order to extract conformations from this, a method of taking one structure from each cluster at a given similarity level was initially considered. A much simpler method was however eventually adopted. This was to take 25 structures at evenly spaced intervals across the dendogram. Twenty-five was chosen so as to adequately cover the 24 possible conformations (larger sample sizes of up to 40 structures did not significantly alter the averaged parameter values). Each of these selected structures was fully minimised in DISCOVER and its parameters calculated using MOPAC [19] and PROFILES [20]. A data matrix consisting of the members of the series as rows and the averaged values of each parameter over the 25 selected structures as columns was then constructed.

Table 2 shows the mean values over the whole series obtained for a selection of the calculated properties compared with the means of the values used in the previous study. Many of the properties show little difference between the averaged and the static values. Whilst this is not surprising for the properties which show little conformational dependence (such as the partial atomic charges) larger differences might have been expected for the overall size and shape parameters (such as the moments of inertia and the principal ellipsoid axes). The reason for this becomes clear on inspection of the data through interactive viewing of the simulations. Most of the conformations accessed can be classified as 'extended' ones with the 'folded' conformations occurring infrequently. Since the crystal structure is an extended conformation the similarity between the static and the average values of the overall shape parameters is easy to explain. The only large differences are

seen in the electronic parameters (the dipole moment and its components) showing the high degree of conformational dependence of this parameter.

With this data matrix it is now possible to repeat the previous analysis to assess the performance of these new parameters in the statistical analysis. The factor analysis was therefore rerun with the averaged values of the parameters. The main feature of this analysis was found to be the generation of 9 orthogonal factors with eigenvalues greater than unity (a commonly accepted significance criterion [21,22]) compared to only 8 generated from the static values. This indicates that there is more information in the matrix of averaged values than in the static data matrix. Four of these factors were highly correlated with the equivalent (i.e. associated with the same variables) factors in the static analysis and, of these 4, 3 correspond to the 3 factors associated with insecticidal potency in the previous study. Additionally the data were shown to correlate with one of these 3 factors in such a way that increased lifetime in the extended form favours insecticidal potency. Full details of this analysis will be reported elsewhere.

## CONCLUSIONS

MD is a simple method for the study of conformational effects in small molecules. It is also reasonably efficient provided, as is the case for pyrethroids [4], that the barriers to rotation are small. In the present study all the expected conformational features can be seen in a 200 ps simulation at 300 K. The main advantage of this method of conformation searching is that it produces a sample of the available conformational space rather than all valid conformations as would be produced by a conformational search program. Hence there are many fewer coordinate sets to deal with. It allows the study of concerted conformation changes, i.e. different torsions changing together. Furthermore the plots of torsion angle against time provide a very clear and easy to interpret picture of the conformational effects.

MD also gives us more information about the choice of the static conformation. For the present series the choice of a basically extended conformation (the crystal structure of deltamethrin) appears to be reasonable since the simulations show that the most common forms of the pyrethroids are extended. Comparison of the simulations with those of deltamethrin, especially for $T_2$, gives further information about the 'receptor-active' conformation of this series.

Regarding the calculation of properties it does appear that MD can produce a representative sample of conformations from which to calculate averaged values of the molecular properties. However, the procedure used here could be improved in a number of ways. The main problem is the need to run a MOPAC calculation for each of the representative conformations. This is expensive of computer time and means that a rather small sample is being used to calculate the averaged values. A possible solution to this would be to use an electronegativity-based scheme [23–25] to calculate the atomic charges and dipole moments. These methods are much faster than MOPAC and would allow many more conformations to be used in the averaging.

Another problem is the method used for the selection of representative conformations. The cluster analysis method seems reasonable but the use of torsion angles as the parameters could prove a problem. This is because quite a small change in one torsion angle in a large flexible molecule can lead to large differences in shape particularly if the torsion is near the centre of the molecule. The cluster analysis does not take account of this and could easily group together conformations similar in terms of their torsion angles but very different in shape. Initial attempts to address

this suggest that it is not a problem in the current series but may become so for larger molecules.

The actual values of the averaged parameters are in this case very similar to those from the static conformation used previously. This is primarily because throughout the simulations the compounds are in conformations very similar to the crystal structure. This is further support for the use of the crystal structure as the static conformation in the previous study. The use of the averaged values in a QSAR analysis seems to have some advantages over the static data matrix in that whilst the overall results are similar, there appears to be additional information relating to conformation in the data.

In conclusion MD is a useful tool for the study of small molecule conformational effects and is suitable for the generation of conformations from which averaged values of QSAR parameters can be calculated.

## ACKNOWLEDGEMENTS

We would like to thank CRAY Research Ltd. and Biosym Technologies Inc. for the provision of computer time to perform these calculations.

## REFERENCES

1 Crombie, L. and Elliott, M., Fortschr. Chem. Org. Naturst., 19 (1961) 120.
2 Davies, J.H., In Leahey, J.P. (Ed.) The Pyrethroid Insecticides, Taylor and Francis, London, 1985, pp. 1–41.
3 Elliot, M., Farnham, A.W., Janes, N.F. and Khambay, B.P.S., Pestic. Sci., 23 (1988) 215.
4 Ford, M.G., Greenwood, R., Turner, C.R., Hudson, B. and Livingstone, D.J., Pestic. Sci., 27 (1989) 305.
5 Livingstone, D.J., Ford, M.G. and Buckley, D.S., In Lunt, G.G. (Ed.) Molecular Basis of Drug & Pesticide Action (Int. Congr. Ser., Vol. 832), Elsevier, Amsterdam, 1988 pp. 483–496.
6 Elliot, M. and Janes, N.F., In Elliot, M. (Ed.) Synthetic Pyrethroids (ACS Symp. Series, Vol. 42) ACS, Washington, DC, 1977, pp. 29–36.
7 Byberg, J.R., Jorgensen, F.S. and Klemmensen, P.D., J. Comput.-Aided Mol. Design, 1 (1987) 181.
8 SYBYL, Tripos Associates, St. Louis, MO.
9 MACROMODEL, Still, W.C., Mohamadi, F., Richards, N.G.J., Guida, W.C., Liskamp, R., Lipton, M., Caufield, C., Chang, G. and Hendrickson, T., Department of Chemistry, Columbia University, New York, NY.
10 Saunders, M.J., J. Am. Chem. Soc., 109 (1987) 3150.
11 Chang, G., Guida, W.C. and Still, W.C., J. Am. Chem. Soc., 111 (1989) 4379.
12 Ferguson, D.M. and Raber, D.J., J. Am. Chem. Soc., 111 (1989) 4371.
13 Osguthorpe, D.S., Dauber-Osguthorpe, P., Sessions, R.B., Paul, P.K.C. and Burney, P.A., In Leeming, P.R. (Ed.), Topics in Medicinal Chemistry (RSC Special Publ. No. 65), Royal Society of Chemistry, London, 1988.
14 Lybrand, T.P., McCammon, A. and Wipff, G., Proc. Natl. Acad. Sci. U.S.A., 83 (1986) 833.
15 DISCOVER, Biosym Technologies Inc., 10065 Barnes Canyon Rd, San Diego, CA 92121.
16 RS/1, BBN Software Products, 10 Fawcett St, Cambridge, MA 02238.
17 ARTHUR 81, Version 3, B & B Associates, PO Box 85505, Seattle, WA 98105.
18 Rapport, Z. (Ed.), The Chemistry of the Cyclopropyl Group, Wiley, New York, 1987.
19 MOPAC, QCPE No. 455, QCPE, Bloomington, IN.
20 Rose, V.S. and Glen, R.C., J. Mol. Graphics, 5 (1987) 79.
21 Lukovits, I., J. Med. Chem., 26 (1983) 1104.
22 Kubota, T., Hanamura, J., Kano, K. and Uno, B., Chem. Pharm. Bull., 33 (1985) 1488.
23 Abraham, R.J. and Hudson, B., J. Comp. Chem., 5 (1984) 562.
24 Abraham, R.J. and Hudson, B., J. Comp. Chem., 6 (1985) 173.
25 Gasteiger, J. and Marsili, M., Tetrahedron, 36 (1980) 3219.