Application of Molecular Modelling Techniques to Pheromones of the Marine Brown Algae *Cutleria multifida* and *Ectocarpus siliculosus* (Phaeophyceae). Metalloproteins as Chemoreceptors?

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Dedicated to Professor Hans Rudolph on the occasion of his 60th birthday

Algal Pheromones, Conformational Analysis, Molecular Mechanics, Cambridge Structural Data Base, Receptor Interaction

The conformation of the two algal pheromones ectocarpene (**1**) and multifidene (**2**) was studied by the active analogue approach using molecular mechanics calculations within the software package SYBYL. A common conformation for both pheromones and all active analogues was filtered out by superposition- and energy minimizing procedures starting from crystallographic data of the Cambridge Structural Data Base (CSD). The interaction of the algal receptor systems with their olefinic pheromones and heteroanalogues is in excellent agreement with the assumption of a receptor-bound metal cation acting as the binding side.

Introduction

The evaluation of the active conformation of pheromones in binding to their chemoreceptors is a major challenge in modern pheromone chemistry. Up to now, this problem was mainly approached by *(semi-)*quantitative structure-activity studies using modified pheromone molecules. Particularly analogues with restrained conformational freedom resulted in valuable informations on the interaction of a flexible achiral ligand with the macromolecular receptor environment [1–3]. More recently several attempts have been made to assess conformational problems by computer calculations, mainly based on the MM2 program developed by Allinger and coworkers [4, 5]. Their main approach is the identification of the minimum energy conformation of a lead structure with the active conformation. Enclosed is the assumption, that the calculated minimum energy conformation largely corresponds to the biologically relevant conformation of the signal molecule. However, within flexible ligands a large number of conformers exist within only 1 kcal/mol of the energy of the thermodynamically most stable one. The problem of this approach is the simple fact, that the binding geometry is strongly influenced by the interaction with the active site of the receptor with may cause large geometrical changes. For this reason, the active analogue approach [6, 7], using molecular mechanics in combination with synthetic probes of restricted conformational freedom and subsequent correlation with biological data is nowadays most frequently employed. For example, in the case of the pheromone system of the turnip moth, *Agrotis segetum* this method recently resulted in a remarkable accurate coincidence between measured biological activity and the calculated conformational energies of the active conformation defined by the model [8].

To apply the active analogue approach to the pheromone system of marine brown algae is particularly promising, since the molecular prerequisites are more favorite. In most cases alicyclic structures of *eo ipso* restrained conformational mobility, having only one or two olefinic substituents like *e.g.* ectocarpene (**1**) or multifidene (**2**) are involved (Fig. 1a).

Both molecules have been previously used in extensive structure activity studies with androgametes of *Ectocarpus siliculosus* and *Cutleria multifida,* and a large set of biological data is available for the pheromones and analogues [9, 10].

Furthermore, in laboratory experiments it has been shown, that the two pheromones, ectocarpene

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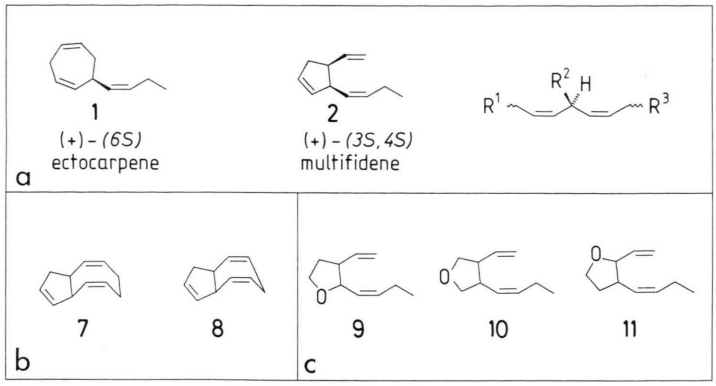

Fig. 1.

(**1**) and multifidene (**2**) are also able to trigger mutual cross reactions between both species at somewhat higher concentrations, *e.g.* multifidene (**2**) with male gametes of *E. siliculosus*, and ectocarpene (**1**) with *Cutleria* males [11]. Since, in general, the interaction of all algal pheromones with their receptors is mainly due to weak dispersion forces between the double bonds of the ligands and appropriate functional groups on the receptor side, a common mode of binding must exist for the structurally different types of pheromones.

We now describe the application of molecular modelling techniques for the evaluation of the biologically relevant conformation of the two pheromones of the marine brown algae *Ectocarpus siliculosus* and *Cutleria multifida*, namely ectocarpene (**1**) and multifidene (**2**). The approach is based on active analogues, where the binding geometry is filtered out by superposition of different active compounds.

An attempt is made to go beyond the active analogue approach by making plausible assumptions on the nature of the pheromone receptor.

Materials and Methods

Chemicals

The synthesis of pheromones and analogues has been described elsewhere [12–14]. The absolute configuration of ectocarpene (**1**) is (+)-(6*S*), and that of multifidene (**2**) was determined to be (+)-(3*S*,4*S*). To warrant comparability with the analogues **3**–**6** and **9**–**11**, this work is based on the biological data of racemic compounds in all cases. For simplification of modelling and superposition of structures, the absolute configurations of the natural compounds served as a starting basis. Since the bioassays gave no evidence for synergistic or antagonistic effects between the enantiomers of a racemic mixture [15], this procedure is justified as a first approach.

Calculations

Geometrical models of the pheromones and analogues were constructed by combining information from the Cambridge Structural Data Base (CSD) [16, 17] and molecular mechanics methods [4, 5] using the software package SYBYL [18]. All used software runs on a VAX 8300 under VMS.

Biological activity tests

The response of male gametes of *Ectocarpus siliculosus* and *Cutleria multifida* to the pheromones and related compounds was tested using the fluorocarbon-droplet technique (FC 41 or FC 72, 3M Company, Düsseldorf, F.R.G.) as described previously [19]. The apparent threshold concentrations were corrected for the aqueous interface by the experimentally determined partition coefficients ($K_{\text{FC 72/water}}$ [9]). Most of the biological data presented here are given in the references [9, 10].

Results

*I. Evaluation of the active conformation of multifidene (**2**) and ectocarpene (**1**)*

The first step to evaluate the active conformation of multifidene (**2**) in binding is the comparison of the pheromone with the functional group topology and possible conformations of the most active analogues **3** and **4**. In a second step this conformation will be

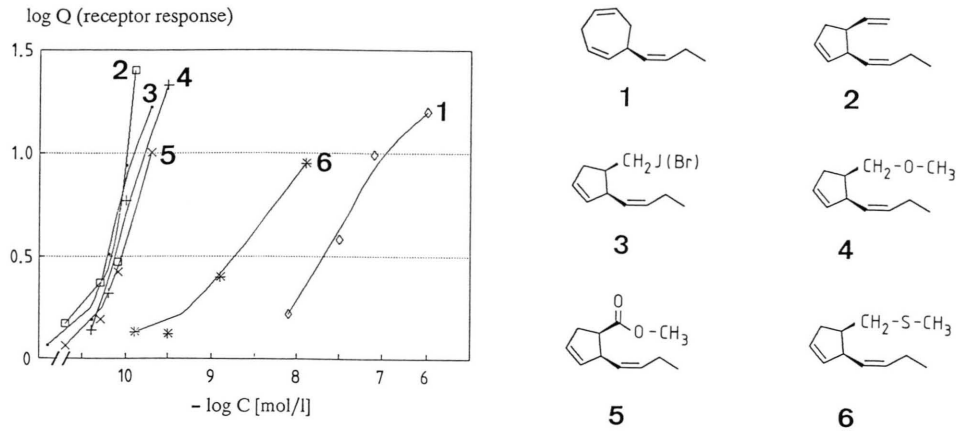

Fig. 2. Dose-response profiles for ectocarpene (**1**), multifidene (**2**) and the multifidene analogues **3**–**6** tested with *Cutleria* males. Plotted are log Q-values (Q-value = biological response [9]) *versus* log concentration of the test substances. The concentrations are corrected for the aqueous interphase using the intrinsic partition coefficients of individual compounds [9]. Insignificant Q-values at low concentrations are omitted.

mapped onto ectocarpene (**1**), to account for the biological activity of this compound on the *Cutleria* receptor.

Since there are no hetero-atoms in the pheromones, only the double bonds remain as candidates for functional groups [9]. It will be shown that there exists a common geometric arrangement of these functional groups, *i.e.* the double bonds, for **1**, **2**, **3**, **4** and **5**, respectively.

The comparison of **2** and its active analogues makes sense only, if there is indeed a common mode of action. There exists no rigorous proof for such a common mechanism, but the very similar dose-response curves of multifidene (**2**) and the most active compounds **3**, **4** and **5** of Fig. 2 strongly support such an assumption.

The common interesting fragment in **1**, **2**, **3**, **4** and **5** containing most of the functional groups is the divinylmethane substructure (Fig. 1a). In multifidene (**2**) this fragment is contained once, whereas in ectocarpene (**1**) it can be formally found twice: first, by combination of an acyclic double bond with one endocyclic double bond (identical to **2**), and second, as combination of the two endocyclic double bonds. Geometrical models of **1**, **2** and the analogues **3**–**5** have been constructed by using crystallographic data from the Cambridge Structural Data Base (CSD) [16] and refinement using force field techniques [4]. Most of the graphics operations have been performed using the program SYBYL [18]. The structures **1**–**5** contain partially unsaturated homocyclic rings having non-planar geometries. Irrespective of their absolute configurations, they may have at least two types of conformations related by a reflection of the ring. This deviation from planarity is small for the cyclopentenes (**2**–**5**) and large for the cycloheptadiene (**1**) (Fig. 3).

Starting from conformations extracted from the CSD, the SYBYL feature RINGSEARCH has been applied to the rings; RINGSEARCH evaluates in a crude way the possible conformations for a given ring. It shows, that for all cases only two types of conformations exist, transferable into another for a given ring by a reflection (*cf.* Fig. 3). The class of substances which has been studied most extensively is the class of the multifidene analogues. In addition, the aforementioned uncertainty connected with deformations of the rings is also smallest for the cyclopentenes. Both facts suggest to start to establish a

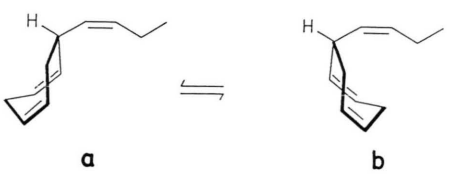

Fig. 3. Schematic drawing of the conformational equilibrium of (6*S*)-ectocarpene (**1**) resulting from inversions of the ring. The substituent may occupy either an equatorial (a) or axial (b) position.

model with multifidene (**2**) and the analogues **3**, **4** and **5**.

For **3** and **4** and **5** the same absolute stereochemistry is used for modelling and energy minimizing as for multifidene (**2**) itself. Two torsion angles alpha and beta, respectively, can be defined for structures **2**, **4** and **5** (*cf.* Fig. 4). For structure **5** the ester group has been fixed to the predominant geometry as derived from the CSD *(vide infra)*. Legal values for the combination of alpha and beta are derived by the SYBYL feature SEARCH. SEARCH treats atoms as hard spheres with adjustable radii. The radii are scaled down in the above examples to values considerably less than the van-der-Waals radii to ensure that no possible conformations are overlooked. Given a set of possible combinations of alpha and beta, SEARCH looks on the basis of these radii for collisions between unbonded atoms. Since all analyzed structures **2**, **3**, **4** and **5** are highly biologically active (*cf.* Fig. 2), it is plausible to regard only those conformations (*i.e.* combinations of alpha and beta) as possible candidates for the active conformation, which are legal for all biologically active compounds. This set of conformations is shown in Fig. 4a/b as clusters in the twodimensional plane of the torsions alpha and beta.

According to Fig. 4b only a very limited number of conformations fulfills the above requirements. Furthermore, the clusters do not depend significantly on the chosen type of ring conformation. Thus, the legal conformations of multifidene (**2**) can be limited to those, which are depicted in Fig. 4b.

As to be taken from Fig. 4b, there is a small uncertainty in the orientation of the butenyl moiety. The vinyl group shows a higher degree of rotational freedom (relative positions of substituents *e.g.* A(A′,B′,C′) or B(A′,B′,C′)). From these combinations the subset A/C′ can be immediately ruled out, since the bicyclic pheromone analogs **7** and **8** from Fig. 1b showed no biological activity at all.

Further confinement can be done, if we next examine the mode of interaction of ectocarpene (**1**) with the *Cutleria* receptor. For this purpose it is reasonable to assume, that ectocarpene (**1**) interacts *via* its double bonds with exactly the same functionalities of the receptor as multifidene (**2**) itself. Therefore, a mapping of the corresponding fragments of both molecules onto each other has to be performed. Starting from the already filtered out conformations of multifidene (**2**) only those mappings are considered as relevant, which ensure a significant overlap of all double bonds. In principle, two possibilities exist, which are schematically drawn in Fig. 5.

The result is, that a mapping of type **b)** leads to a good overlap of the double bonds, while a mapping of type **a)** does not. Particularly the unfavourite sterical interaction of the methyl group of the butenyl side chain and the ring methylene group of **1** (Fig. 5a) prevents the correct orientation of the side chain, which is necessary to mimic the cyclopentene system. Furthermore, in (3*S*,4*S*)-multifidene (**2**), the tertiary hydrogens are oriented downwards, whereas for (6*S*)-ectocarpene (**1**) the opposite orientation is required for optimum mapping of the double bonds of both molecules onto each other. The conformations of type **b)** are simply transferable into each other by

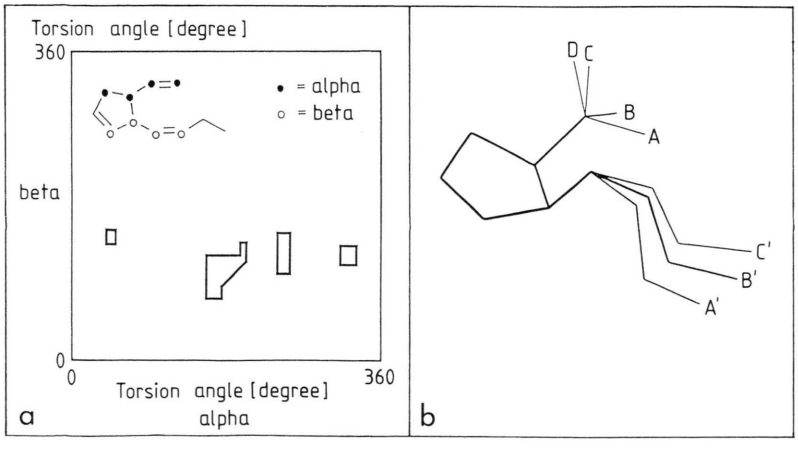

Fig. 4. a) Clusters of simultaneously allowed conformations for the compounds **2**, **3** and **5** in the twodimensional plane of alpha (●) and beta (○). The respective carbon atoms defined as alpha (●) or beta (○) are indicated. The clusters in the twodimensional plane of alpha and beta are the result of the optimization of the cyclopentenes **2**, **3**, **4** and **5** and represent legal conformations which are common to all four compounds. b) Conformations of multifidene (**2**) as derived from the simultaneously allowed torsion angles alpha and beta of Fig. 4a.

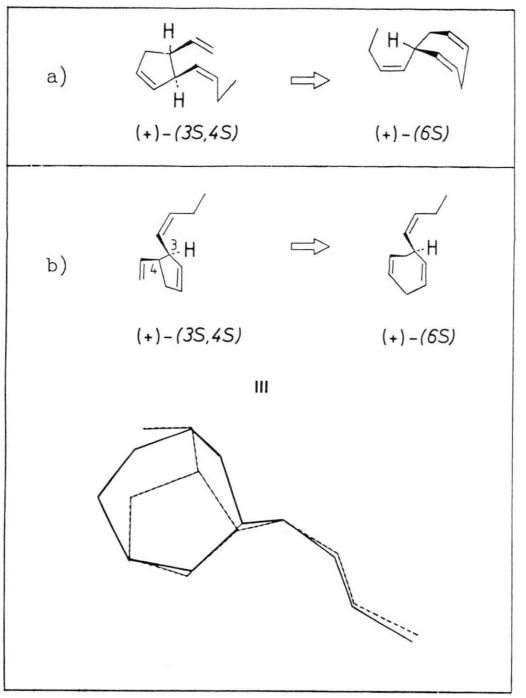

Fig. 5. Mappings of multifidene (**2**) onto ectocarpene (**1**). Fig. 5a identifies the two alkenyl substituents of multifidene with the cycloheptadienyl moiety of ectocarpene (**1**). The superposition is incomplete with respect to the projection of the butenyl side chain of ectocarpene (**1**) onto multifidene (**2**). The mapping of type b) involves projection of the cyclopentene- and vinyl double bond of multifidene (**2**) onto the cycloheptadiene backbone of ectocarpene (**1**) and leads to an acceptable overlap of the π-electron systems of both compounds as shown in the superposition of both compounds (broken line = multifidene (**2**), bold line = ectocarpene (**1**); double bonds are not shown).

disconnecting the C(3)–C(4) σ-bond of multifidene and attaching C(3) to the terminus of the vinyl group to give ectocarpene (**1**). This conformation is roughly identical with the D(A′,B′,C′)-combinations of Fig. 4b. At this point we have filtered out exactly one type of conformation being a candidate for the active conformation by extracting information from structures **1**, **2**, **3**, **4** and **5**. This process of filtering is successful for several reasons:

- The structures are of restricted conformational mobility.
- The stereochemistry is known for all compounds.
- There exists an unambiguous identification of the relevant functional groups.

Nevertheless, the derived model can serve only as a first guess for the active conformation(s). The criterion of optimum match of comparable groups is nothing but a substitute of the real fact of a similar interaction with the unknown receptor. Further experiments with bicyclic pheromone mimics, derived from the first model are necessary to confirm or disprove the deduced conformations.

II. Metalloproteins, possible candidates for pheromone binding?

The double bonds obviously play an important role in binding to the receptor. Thus, one may speculate on the complementary parts of the receptor involved in the binding of the double bonds. Two mechanisms which have been already discussed previously seem to make sense [9]:

- Binding to a receptor-bound metal cation by interaction with the double bonds (and/or heteroatoms).
- An energetically favourable interaction between a double bond and complementary conjugated systems on the receptor surface.
- Combination of both types of interaction with the three double bonds in distant parts of the molecule.

Here, only the first case will be discussed. It has been studied by analyzing the CSD. The situation is much simpler than in the case of interactions between two π-systems, because one finds precise positions of the complementary receptor part, *i.e.* the metal cation, for the different interaction modes. The superposition of the positions relative to the double bond(s) gives a probability distribution for a bound metal cation. A more sophisticated superposition of the active compounds themselves can be established by using the regions of highest probability for a metal cation bound to the double bond(s) as reference points instead of using the double bonds themselves. Furthermore, this scheme allows for interpreting active compounds where double bonds have been functionally replaced by hetero-atoms by taking into account the different metal cation binding mode of hetero-atoms.

To get a preliminary probability distribution for the location of a metal relative to a *cis* double bond, all entries of the CSD having a *cis* double bond and a metal cation have been extracted. Fig. 6a/b shows the result of this procedure.

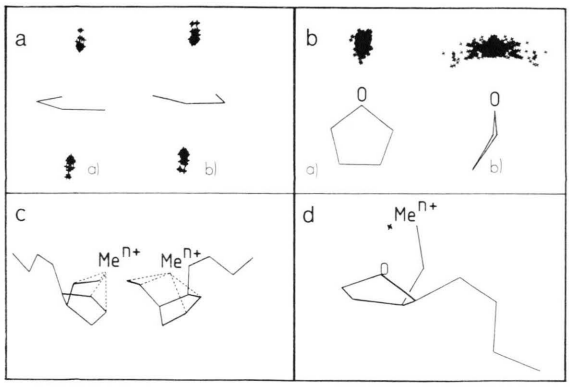

Fig. 6. The drawing shows the spatial distribution of whatever metal ligands relative to *Z*-double bonds (Fig. 6a) or tetrahydrofurans (Fig. 6b) independend of the structural environment. Each cross denotes a specific entry in the Cambridge Structural Data Base (CSD). a) Front view, b) side view. Fig. 6c and 6d present perspectivic drawings of the interaction of multifidene (**2**) (Fig. 6c) or the tetrahydrofurane (**9**) (Fig. 6d) with a receptor-bound metal. In each case the metal occupies positions which are in full accord with the relative positions extracted from the CSD for other structures. Bonds to the metal are indicated by broken lines. Optimal binding can be achieved only, if the cyclopentene- (or furane oxygen) and the vinyl group are used for complexation.

The crosses show positions of a metal cation relative to the double bond. The superposition contains contacts between the double bonds and metals in different chemical contexts, *i.e.* double bonds in cyclic systems like cyclopentene, cycloheptadiene and acyclic double bonds. It gives a good overview on the overall preferred positions. This arrangement can be superimposed to the double bonds in the first guess for the active conformation of structures **1** and **2**. The most interesting fact is the coincidence of regions with high probability (energetically favoured) for a metal cation for the two endocyclic double bonds in ectocarpene (**1**). The real existence of such complexes can be seen by inspection of some specific examples from the CSD [21–23]. Following our assumption, two ligand sites of the metal cation are occupied by the pheromone, the remaining ligand sites fix the metal itself to the receptor surface.

The next step starting from our derived first guess of the active conformation (Fig. 4b) is the interpretation of multifidene (**2**) within this scheme. First, only those structures are extracted from Fig. 6a, where the double bond is part of a cyclopentene ring. This determines a region of high probability for a metal cation similar to the region for structure **1**. The interesting question is, how the second double bond of one of the two side chains may interact with the metal cation to occupy both binding sides. The obvious way (which has been implicitly assumed in the derivation of the first model) is to move the vinyl group in a position to bind to the metal cation in an optimum manner. In the first step, the double bonds of multifidene (**2**) and ectocarpene (**1**) themselves have been superimposed. This rough model is now refined by introducing the metal cation and mapping the metal positions instead of the double bonds. To achieve a simultaneous favorite binding of the cyclopentene- and the vinyl group in multifidene (**2**), a moderate twisting of the vinyl group is necessary (Fig. 6c).

A similar arrangement of the cyclopentene- and the butenyl side chain is highly improbable due to strong sterical interactions between the ethyl hydrogens and the $-CH_2-$ group of the ring.

The validity of the assumption of structures **1–5** interacting with a receptor-bound metal in the derived way can be checked by discussing the consequences of this hypothesis for other structures with known biological activities. It has been shown, that among the tetrahydrofurane analogues of multifidene **9**, **10** and **11** compound **9** is active, while the isomeric compounds **10** and **11** are less active [20] (Fig. 1c).

This experimental result is understandable in the context of our hypothesis. Assuming a binding from the ring system to the metal and fixing compounds **9–11** by identifying the butenyl groups with those of **2–5**, the binding function of the cyclic double bond in multifidene (**2**) has to be fulfilled by the oxygen atom in the tetrahydrofurane system. Filtering all structures from the CSD which have a tetrahydrofurane moiety and a transition metal bound to that ring gives a region of high probability for the transition metal as shown in Fig. 6b. Again, each cross refers to a hit in the CSD giving the relative position of the metal atom. The highest probability of finding a metal atom is in the plane determined by the ring oxygen and its carbon neighbours (Fig. 9). Going back to our model for structures **2–5**, this region can be brought to coincidence with the assumed metal position by rotating the tetrahydrofurane ring relative to the butenyl group around the single bond connecting the two fragments. In this geometry the tetrahydrofurane ring is nearly perpendicular to the cyclopentene ring of compound **2** (Fig. 6d).

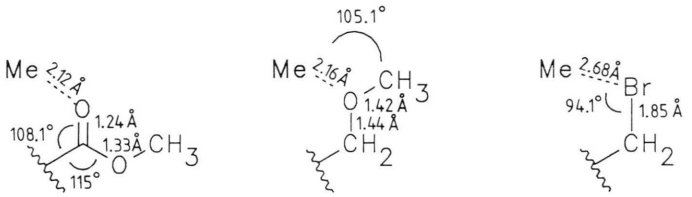

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Fig. 7. Relative positions of a metal in complexes with esters, ethers and bromides. The position, bond length and bond angles are statistical average values from entries of the Cambridge Structural Data Base (CSD).

The vinyl group does not contribute to the binding as perfect as it does for multifidene (**2**) itself and thereby accounts for the overall much lower activity of this compound (energetic and entropic terms for the abstraction of a hydrate shell from the tetrahydrofurans **9**–**11** have to be also regarded; however, these terms should be largely comparable for all three isomers).

The interesting fact is, that this procedure is only tractable for compound **9**. Keeping the butenyl side chain fixed for the other tetrahydrofurans **10** and **11** the region of high probability for a metal atom cannot be brought into coincidence with the assumed metal position.

The hypothesis of interaction with a receptor-bound metal cation is also compatible with the exceedingly high activity of the structures **3**, **4** and **5** (*cf.* Fig. 1). Stable complexes can be found for each case; the average values for the most interesting geometrical parameters are given below for these compounds (Fig. 7).

A first crude fit is perfect for compounds **4** and **5**, and acceptable for compound **3**. At this stage the simultaneous optimization of the active structures can be restarted using the assumed metal position and the common butenyl substructure for each structure as reference points. The metal position is frozen relative to its binding fragment in a structure during the optimization. The result is shown in Fig. 8; the metal position is denoted by a cross. Note that the general type of the superposition is the same as derived in the first step of the active analogue approach (combinations D(A′,B′,C′ of Fig. 4b)). Essential details like the embedding of structures containing hetero-atoms instead of double bonds, or the precise position of the vinyl group in multifidene (**2**) are now intrinsically coupled to the assumption of binding to a metal.

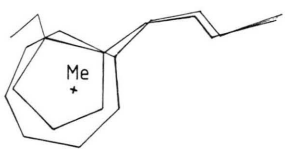

Fig. 8. Top view onto the superimposed structures of multifidene (**2**) and ectocarpene (**1**) showing the relative position of the metal and the double bonds in binding of the two pheromones. The simultaneous optimization of both structures interacting with a receptor-bound metal cation results in virtually the same conformations as derived from Fig. 5b.

Using this model some surprising experimental facts can be explained in a straightforeward manner. One of these facts is the low biological activity of the thioether **6** (Fig. 2).

This fact is surprising, because the related oxygen analogue **4** is a highly active mimic of multifidene (**2**). Once again the crucial step is the investigation of the CSD. Fig. 9 shows typical arrangements of metals above ethers and thioethers.

The difference is obvious: For X = O it is preferable to have the metal in the CH_2-O-CH_2 plane, where maximum interaction between the unoccupied d-orbitals of the metal and the oxygen lone pairs is possible (torsion angle = 170–180°; Fig. 9). A deviation of the torsion angle from planarity of 40 degrees is possible. In contrast, for X = S it is essential, to have the metal in a certain position above (or below) the plane determined by the lone pairs. This leads to different orientations of the methyl group in **4** and **6**; in the case X = O obviously no problem arises. In the case X = S the CH_2-S-CH_3 moiety has to be turned out of the original plane by approximately 40–50 degrees. This moves the methyl group into regions used by no other active analogue, and this may be responsible for the low biological activity of the thioether **6**. Thus, the assumption of an inter-

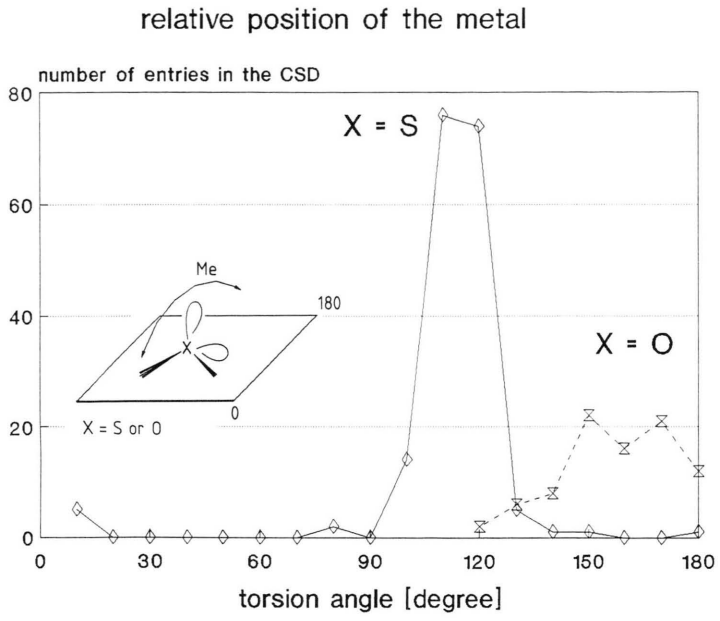

Fig. 9. Frequency curve of the relative position of a metal in complexation of ethers and thioethers. Plotted are the number of entries in the Cambridge Structural Data Base (CSD) *versus* the torsion angle of the metal relative to the plane $-CH_2-X-CH_3$ (X = S or O). The torsion angle is illustrated by the insert.

action with a receptor-bound metal cation explains the experimental facts and has to be probably considered as one of the potential keys in understanding the receptor properties of (algal) chemoreceptors.

Conclusions

The conformations of algal pheromones as derived from the active analogue approach and energy minimizing, provide a convincingly homogeneous basis for the interpretation of the biological data of many different pheromone analogues. Particularly, the observed cross reactions between multifidene (**2**) and ectocarpene (**1**) on the *Cutleria*- or *Ectocarpus* receptor have found a reasonable establishment.

The still hypothetical assumption of a receptor-bound metal cation acting as a co-ordinating center in binding of pheromones is promising because of several major reasons:

1. The conformations derived from this model are identical with those obtained from the active analogue approach and energy minimizing.
2. Complexation by a receptor-bound metal cation is compatible with all functional groups of the pheromone analogues tested so far. Each of these cases can be documented by specific entries of the CSD.
3. Complexation by a receptor-bound metal provides a reasonable argument for the low biological activity of the thioether **6**. Such arguments cannot be found on the basis of bulkiness and polarizability alone, since oxygen containing structures like **4** and **5** possess full biological activity (*cf.* Fig. 2).

The results give the motivation to develop further tests with (bi)cyclic pheromone analogues, designed according to the model of metal complexation. In the future, experiments with correspondingly modified analogues of insect pheromones (*e.g.* ethers, thioethers or spirodithioacetals) may help to verify or falsify this hypothesis and to prove its transferability to other biologically important communication systems.

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