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Dedicated to Prof. dr. MERCEDES WRISCHER on the occasion of her 70th birthday.

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STRUCTURE/ACTIVITY CORRELATIONS FOR AUXINS

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Structure-activity correlations for the endogenous phytohormone, indole--3-acetic acid (IAA), and its ring-alkylated and -halogenated derivatives are based on geometric and electronic molecular characteristics, and the resulting physical and chemical attributes. Plant growth-regulating properties are discussed with particular emphasis on molecular (bio)conformation and on substituent effects and their influence on properties such as lipophilicity.

Key words: Auxin, indole-3-acetic acid, quantitative structure-activity relationship, QSAR, molecular recognition, similarity analysis.

Introduction

Structure-activity relationship based on quantitative parameters (QSAR[1]) has been a successful approach in drug design and toxicology (KUBINYI 1993, BÖHM et al. 1996), but deserves more attention in the physiology and pharmacology of plant growth regulation (BURES et al. 1991). Here we focus on the auxins, which control a multitude of aspects of plant development. Widely studied endogenous

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[1]**Abbreviations**: ABP, auxin-binding protein; HPLC, high-pressure liquid chromatography; IAA, indole-3-acetic acid (substituted derivatives are presented by adding the respective chemical symbols, *e. g.* 4-*Cl*-IAA, 4-chloroindole-3-acetic acid); QSAR, quantitative structure-activity relationship.

auxins which function as phytohormones are indole-3-acetic acid (IAA; Figure 1) and its halogenated derivative, 4-chloroindole-3-acetic acid (4-Cl-IAA).

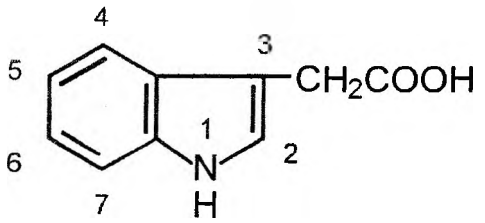

Fig. 1. Structural formula of indole-3-acetic acid (IAA) including the conventional numbering for the ring-positions.

Hundreds of purely synthetic organic acids also have auxin activity (JONSSON 1961), and some of them are routinely used to manipulate the growth and development of crop plants, and to eliminate competing weeds. These particular growth regulators also need to be safe for humans and animals, a requirement which is only gradually understood in its full complexity. The more we know about environmental and health hazards caused by the use of agrochemicals, the more important it appears to invest into the design of less toxic, more efficient, and more selective plant growth regulators. An alternative way of achieving optimal crop performance would include manipulating the mechanisms which mediate the response to endogenous growth hormones. To accomplish such ambitious goals the essential structural elements of both the plant growth regulators and the corresponding receptor proteins must be understood in every relevant detail.

The biological activity of auxins has most frequently been assayed in 'straight growth tests' based on the elongation of stem or coleoptile segments floating in a solution of the test substance. The stimulatory range of concentrations frequently covers 5 and more orders of magnitude; the data are thus conveniently plotted on a logarithmic scale. The dose-response curves obtained (Figure 2) are asymmetrically bell-shaped, ascending gently to a relatively flat top, and descending steeply at supraoptimal auxin concentrations. Two characteristic parameters are commonly used in structure-activity analyses: the optimal stem elongation and the half-optimal auxin concentration. Ideally, the latter reflects affinity to the response-mediating protein (receptor), while the physiological efficacy of the resulting 'complex' is correlated to the optimal elongation response (KATEKAR 1979). A small number of competitive inhibitors of auxin-induced stem (coleoptile) elongation have been classified as antiauxins. Following the systematic studies by MCRAE and BONNER (1952), few additions have been made to their original list of antiauxins (*e. g.* HATANO et al. 1989). Due to the small body of data on this subject, generalized conclusions should be derived with caution.

Once the auxin or antiauxin activities of a compound have been proved, the essential problem is to relate bioactivity to molecular structure. By screening a selection of compounds, characteristic chemical functionalities could be recognized as the essential features for the activity (in pharmacology named pharmacophore and in spectroscopy designated as chromophore). For the auxin class, a

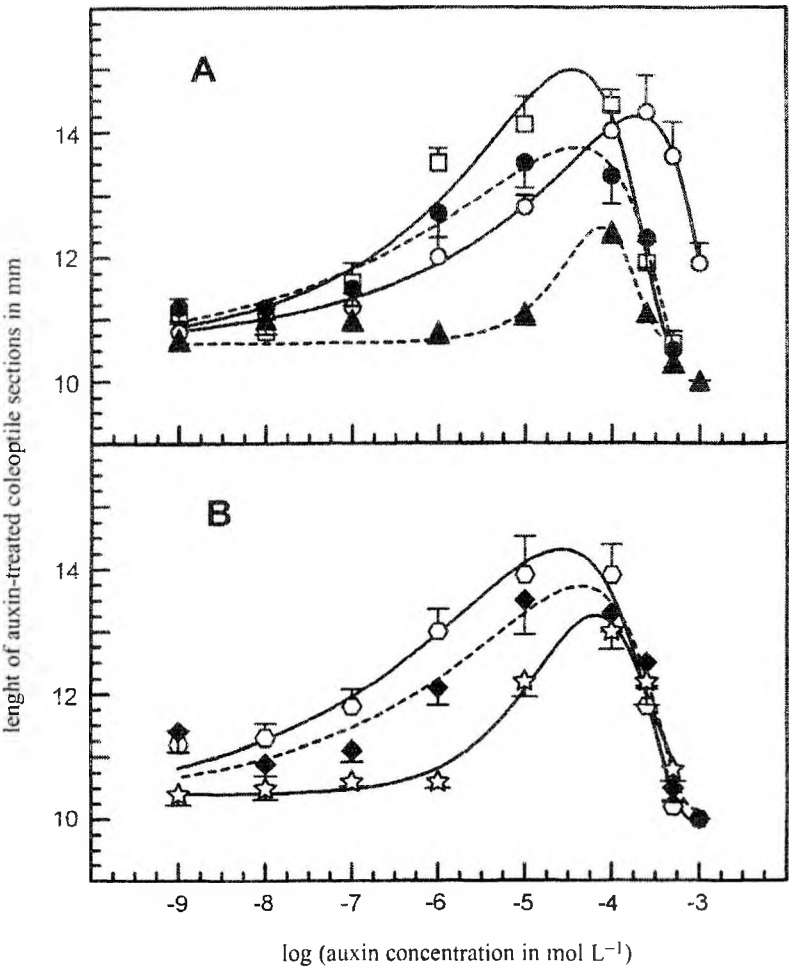

Fig. 2. Examples for dose-response curves obtained in the *Avena* coleoptile straight-growth test for the following auxins: (A) IAA (plain circles, full line), 4,5-Cl_2-IAA (squares, full line), 6,7-Cl_2-IAA (black circles, broken line), and 4,7-Cl_2-IAA (triangles, broken line); (B) 5,6-Cl_2-IAA (hexagons, full line), 4,6-Cl_2-IAA (diamonds, broken line), and 5,7-Cl_2-IAA (stars, full line). Ten-millimeter-long subapical coleoptile sections were incubated overnight in auxin solutions, at the concentrations shown. The error bars represent standard errors of the mean ($n = 10$). The concentration of a particular auxin which causes half the elongation achieved at the optimum level of the same auxin is defined as 'half-optimal'. Further details are presented in an article by ANTOLIĆ et al. (1999).

planar aromatic platform and an anionogenic (acidic) functional group are two characteristic features. The size and the shape of the molecule, including its conformation and electronic properties, influence its bioactivity and physico-chemical properties. To initiate a biological response the molecule in question must be recognized by a target protein, known as a receptor. An 'active' molecule having the function of a substrate (or inhibitor) has to be complementary to the active

site of the receptor. Among nine auxin-binding proteins (ABP) (VENIS and NAPIER 1995), the protein ABP1 is considered a likely candidate for the auxin receptor involved in stem elongation; it was isolated and its gene cloned (NAPIER 1995, NAPIER and VENIS 1995). Interestingly, ABP1 is most abundant in the endoplasmic reticulum, but appears to exert its biological effects at the outer face of the plasmalemma. As the 3-D structures of ABP(s) have not yet been elucidated, the mechanism of auxin binding to the receptor(s) at the molecular level remains unknown (NAPIER 1995). At the present state of knowledge direct interaction of ABP(s) and small molecules can thus not be studied. Therefore, our efforts have been focused on composing a detailed 'identity card' of an auxin molecule using exact experimental methods and computer modelling. By 'inversion' of this model one can gain insight into the topology of the active site of the auxin binding protein. X-ray structure analysis was used to unambiguously define the molecular structures of the natural auxins, indole-3-acetic acid (IAA) (KARLE et al. 1964, CHANDRASEKHAR and RAGHUNATHAN 1982) and 4-Cl-IAA (NIGOVIĆ et al. 1996), and of synthetic hormone analogues, such as other halogenated IAAs (ANTOLIĆ et al. 1996, NIGOVIĆ et al. 1996), dichlorinated IAAs (ANTOLIĆ et al. 1999), and alkylated IAAs (KOJIĆ-PRODIĆ et al. 1991, NIGOVIĆ et al. 1999). Molecular modelling, including *ab initio* calculations, molecular mechanics and molecular dynamics simulations (RAMEK et al. 1995, 1996, 1998 a, b, TOMIĆ et al. 1998 b), was used to detect and to evaluate the stability of the conformers observed *in vacuo* (as an undisturbed ground state) and in aqueous medium (for physiological conditions). These data provide the elements upon which the recognition of auxins (or inhibitors) by specific target proteins is based. The 'molecular recognition' approach then uses all the data available to provide a qualitative or semi-quantitative model for receptor-substrate interaction, whereas QSAR generates a quantitative model.

Molecular recognition

Molecular recognition is a problem fundamental to structural biology. This process includes the selection of a substrate molecule (or inhibitor), its binding to a macromolecular receptor, and the execution of a specific function by the 'complex' formed. The biological response involves 'storage' of information which should be 'recognized' at the molecular level during formation or dissociation of the macromolecule+ligand complex. The first model of the molecular recognition process, the lock-and-key analogy, was proposed by E. FISCHER (1894). This static model was extended to a dynamic one using induced fit, illustrated by the simple analogy hand-glove (KOSHLAND 1994). An approach compromizing the situation at the enzyme active site is known as 'molecular docking'. This approach, covering a spectrum of models, incorporates the lock-and--key and induced-fit theories for ligand binding while taking a more complete view of the dynamic aspects of molecular recognition (GSCHWEND et al 1996).

Molecular structures and modelling

The molecular structures of the compounds studied reveal two conformationally distinct states; a) planar (*P*) – the carboxyl group is coplanar with the

indole ring and b) tilted (*T*) – the carboxyl group is folded with respect to the indole ring (Figures 3a and b). In the crystalline state, the tilted conformation was detected in IAA itself (CHANDRASEKHAR AND RAGHUNATHAN 1982) and in all its chlorinated (NIGOVIĆ et al. 1996) and fluorinated (ANTOLIĆ et al. 1996) derivatives examined so far. However, for the ring-alkylated derivatives studied, both conformations occur with equal frequency (KOJIĆ-PRODIĆ et al. 1991, NIGOVIĆ et al. 1999). This can be explained by the small energy difference between the *P* and *T* conformations, as revealed by computational chemistry methods (RAMEK et al. 1995, 1996, 1998 a, b, ANTOLIĆ et al. 1996, NIGOVIĆ et al. 1996, TOMIĆ et al. 1998 a, b).

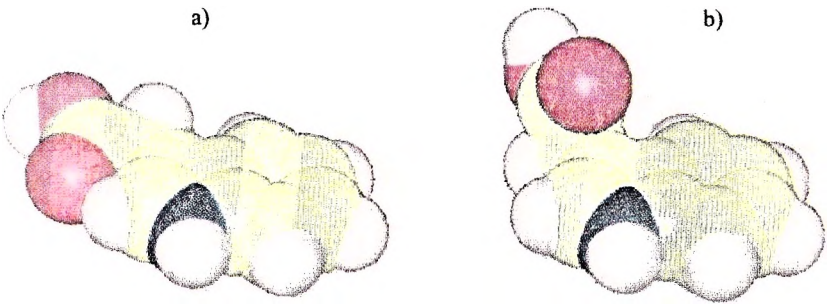

Fig. 3. The molecule of indole-3-acetic acid (IAA) in its two low-energy conformations: the planar (a) and the tilted (b) conformation. The individual atoms are drawn in proportion to their van der Waals radii and are color-coded as follows: green, carbon; white, hydrogen; blue, nitrogen; red, oxygen. The planar (*P*) conformation corresponds to the 'recognition conformation' in KAETHNER'S (1977) conformational change theory, and the tilted (*T*) conformation corresponds to his 'modulation conformation'.

Molecular recognition of auxins has been summarized in two historical hypotheses proposed in the late seventies (KAETHNER 1977, KATEKAR 1979, RAKHAMINOVA et al. 1978). A conformational change of the auxin molecule from a planar 'recognition conformation' to a tilted 'modulation conformation' (Fig.3) on binding to the active site was postulated by KAETHNER (1977). The second hypothesis is based on the topography of the receptor active site (RAKHAMINOVA et al. 1978, KATEKAR 1979). This approach implies complementarity of the active site and the 'ligand' (small active molecule – a substrate or an inhibitor), and is based on analysis of size and shape of the ligand molecule, the orientation of its characteristic chemical functionalities, and lipophilicity. Thus, the conformational change hypothesis proposed by KAETHNER (1977) is incorporated into the topology analyses used by KATEKAR (1979) and RAKHAMINOVA et al. (1978).

Using a more elaborate combined approach, interaction similarity analysis (TOMIĆ et al 1998 a) was employed for the systematic classification of compounds tested for auxin activity. According to the interaction properties of a set of about 50 compounds, four classes were recognized: strongly active, weakly active with weak antiauxin behaviour, inactive, and inhibitory. For this purpose,

molecular modelling (conformational analysis and energy evaluation of the conformers), molecular alignment, and calculation of interaction energies with selected chemical probes (functional groups expected to occur in the receptor) were used. The compounds studied were modeled in their two low-energy conformations, *P* and *T*, in order to obtain the best match to the planar and tilted conformations of indole-3-acetic acid. When the *T* conformers were used, the model permitted to distinguish auxins from antiauxins and inactive compounds. Although the similarity analysis presented cannot provide a definitive ligand binding mechanism, better differentiation of the classes when *T* conformers are used suggests that the *T* conformation might be the active one which was treated as the 'modulation' conformation in the KAETHNER hypothesis. If we thus assume that the conformational change hypothesis (KAETHNER 1977) is realistic, we can speculate from the results obtained, that auxins bind in their planar conformation, and then change to the active – tilted one. In the planar conformation, antiauxins are more similar to auxins than in the tilted conformation. Further support for the conformational change from *P* to *T* is given by the calculated low energy-barriers between these two conformers. One of the practical aspects of this classification would be a guideline for new bioassays required to address the contradictions in the literature regarding auxin activity.

To illustrate the use of topography analysis (RAKHAMINOVA et al. 1978, KATEKAR 1979), let us return to the set of non-natural dichlorinated IAAs referred to in Figure 2 (ANTOLIĆ et al. 1999). According to the dose-response curves presented, 5,6-dichloroindole-3-acetic acid has the smallest optimal and half-optimal concentrations. This large activity, as compared to that of its positional isomers, can be even more pronounced in other test systems (HATANO et al. 1987). In a concept which is difficult to track back to its origin, but was most explicitly formulated by KATEKAR (1979), the active site of the, then largely hypothetical, auxin receptor was subdivided to include separate compartments for the anionogenic group and for the planar aromatic nucleus. The latter compartment was visualized in the shape of a shoe, which loosely fits the indole nucleus, except for a tight section between the 'sole' and the 'heel'. If this concept is correct, then chlorine substitution at positions 5 and 6 can only push the heterocyclic ring system further towards the spacious part of the respective compartment (Figure 4a). The substituents themselves are also well accommodated and have been postulated to be the target of binding interactions, in addition to those involving the aromatic ring system (KATEKAR 1979). In contrast, for the weak auxin, 4,7-dichloroindole-3-acetic acid, both chlorine substituents are forced into the tightly fitting part of KATEKAR'S 'shoe' (Figure 4b). This results in a less favorable energy balance for binding to the auxin receptor and thus decreases growth-promoting activity.

Physico-chemical properties

The physico-chemical and biological properties of any auxin are defined by its molecular and electronic structures. However, due to their dependence on molecular dynamics (vibrations, rotations, etc.), solvation, and similar effects, the resulting correlations are extremely complex. Taking an experimental, rather than a computational, approach we compared (ANTOLIĆ et al. 1996, NIGOVIĆ et

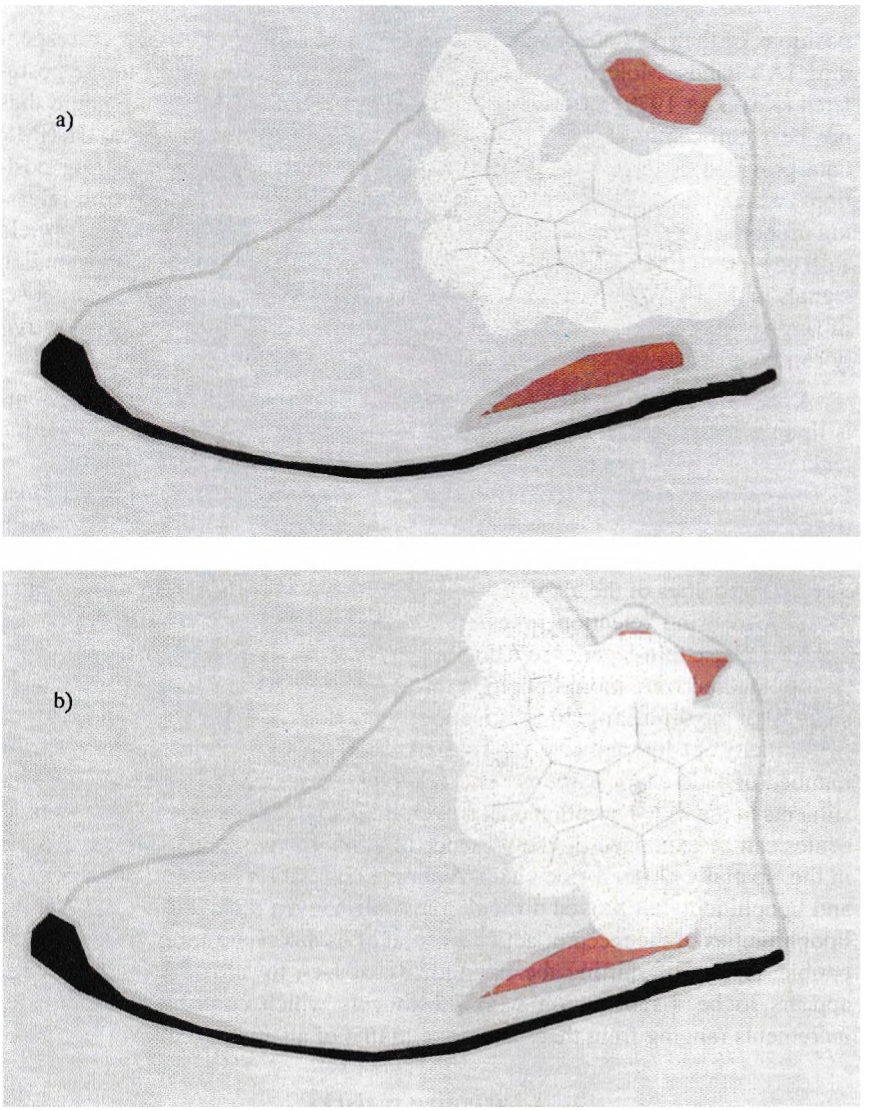

Fig. 4. Deduced orientations of the molecules of 5,6-dichloroindole-3-acetic acid (a) and 4,7-dichloroindole-3-acetic acid (b) in the compartment of the auxin receptor which accomodates the aromatic ring-system. The presentation is based on a model proposed by KATEKAR (1979). The 'shoe' loosely fits the indole nucleus except for two tight spots: between ring-positions 4 and 5 ('the heel') and along positions 1 to 7 ('the back part of the sole'). The carboxyl group is assumed to enter a separate compartment of the auxin receptor.

al. 1996, 1999) the UV and ^{1}H-NMR spectra of IAA to those of its derivatives substituted at the following ring positions (substituents in parentheses): 2 (methyl), 4 (F, Cl, methyl, ethyl), 5 (F, Cl, methyl, ethyl, *n*-propyl, *n*-butyl), 6 (F, Cl, methyl, ethyl), 7 (F, Cl, methyl). All these compounds are active auxins. The

positions of their UV absorbance maxima were within 10 nm of the corresponding IAA bands. Molecular orbital energies, in terms of affinities to the postulated (KATEKAR 1979) π-complexing site of auxin-binding proteins, should thus not be significantly affected. For the above alkyl-substituted IAAs, ^{1}H-NMR data provided estimates for relative electron densities at individual ring positions, which may affect more specific recognition patterns. The electron-releasing properties of the alkyl substituents were clearly reflected by a general upfield shift (Δ) for all ring protons (0.06–0.29 ppm with reference to the corresponding signals in the IAA spectrum; $\Delta_{ortho} > \Delta_{para} > \Delta_{meta}$). ^{1}H-NMR data for a larger set of ring-substituted IAAs are needed before possible correlations to auxin activity can be investigated.

A physico-chemical parameter of particular importance in biological systems is lipophilicity (hydrophobicity), here defined as a quantitative measure for the tendency of a molecule to partition into the lipid phase, at a lipid-aqueous interface. Mathematically, lipophilicity is defined by a set of descriptor variables which are additive in nature. However, fine tuning for positional isomerism (same substituent at different ring positions) requires experimental confirmation. The lipophilicities of the alkylated and halogenated IAAs listed above were thus determined using retention times on reversed-phase HPLC and R_F-values in reversed-phase thin-layer chromatography (NIGOVIĆ et al. 1999). While all compounds studied were more lipophilic than IAA itself: a) substituent effects were smallest at the 4-position, b) fluorination at positions 5–7 had no major effect on lipophilicity, c) lipophilicity for ring-alkylated derivatives increased with the number of side-chain carbons. The relatively small effect of lipophilic substituents in the IAA 4-position on the overall lipophilicities of the respective molecules can be explained by the presence of a highly hydrophilic carboxyl group in the, spatially close, 3-side chain. A simple correlation between auxin activity and lipophilicity has proved difficult to establish, even though it is clear that the lipophilicities of the receptor active site and of its auxin substrates must be compatible. A way of shifting the lipophilicity of IAA towards the optimal range appears to be 4-substitution with substituents which contribute lipophilicity increments ranging from that of fluorine to that of an ethyl group.

Concluding remarks

The general principles of structure-activity relations for auxins have been established two and more decades ago (KAETHNER 1977, THIMANN 1977, SCHNEIDER and WIGHTMAN 1978, KATEKAR 1979). What has been lacking are the structural and physico-chemical particulars required for the design of tailored auxin molecules and tailored auxin receptors. The tools needed to unveil those details have significantly improved, in recent years. X-ray crystallography has become more readily accessible, and methods such as NMR-spectroscopy can now provide a wealth of information on molecular structure in solution. Most importantly, however, contemporary computer design and software now permit to understand the conformational dynamics of auxin molecules in quantitative terms, a subject which used to be addressed using ball-and-stick models and 'intuition', in classical times. Recently refined software also facilitates meaningful structure-activity studies. The interesting results obtained by these new methods make it even

more evident that there will be a long way to go before the structural biochemistry of auxin activity will be understood as completely as contemporary drug design.

We here chose to characterize 'auxin activity' by stem elongation assays based on the enlargement of preexisting cells. This has been a popular approach, for many years, and a large body of published data is available for comparison. Critics use to point out that the auxins and antiauxins applied in stem elongation assays can be modified by plant enzymes before reaching the receptor sites responsible for the growth response. While this possibility must always be kept in mind, documented examples mostly refer to auxin precursors, such as esters, amides or nitriles, which were converted to the corresponding acids by the stem sections used in bioassays (JÖNSSON 1961, THIMANN 1977). When the acids themselves were applied, stem elongation assays, in an impressive number of cases, afforded reasonable structure-activity correlations, without special corrections for metabolic stability. Indeed, if the auxin receptor involved in the stem elongation response is located at the outer face of the cell membrane, as favored by current hypotheses (see above), this is a compartment which only contains a restricted number of metabolic enzymes. The affinity of auxins to putative receptors may be a shaky foundation for structure-activity correlations (EDGERTON et al. 1994), as long as the identity and function of those proteins is not absolutely clear. Also, the respective binding constants contain no information on the efficacy of the 'substrate-receptor complexes' in triggering a stem elongation response and do thus not discriminate auxins and antiauxins, unless bioassays are performed in parallel (RAY et al. 1977).

Auxins also direct the initiation and finalization of morphogenetic patterns in ways which are not obviously related to simple cell enlargement. Some of these processes appear to respond to auxin gradients (TUOMINEN et al. 1997, UGGLA et al. 1998) generated by polar transport. The latter is accomplished by a system of carrier proteins which 'import' auxins at one end of a plant cell and 'export' them at its opposite end (RUBERY 1987). While the natural auxin, IAA, is *both* polarly transported *and* sustains cell enlargement, this is not true for all its synthetic analogues. 2,4-Dichlorophenoxyacetic acid (2,4-D), for example, is actively imported into plant cells, but can leave only by diffusion. The opposite is true for naphthalene-1-acetic acid: Both compounds are thus more slowly transported from cell to cell than is IAA (RUBERY 1987). Polar transport is an essential element of auxin physiology, but the small set of existing experimental data is not yet sufficient to discuss its structural base.

References

ANTOLIĆ, S., KOJIĆ-PRODIĆ, B., TOMIĆ, S., NIGOVIĆ, B., MAGNUS, V., COHEN, J. D., 1996: Structural studies on monofluorinated derivatives of the phytohormone indole-3-acetic acid (auxin). Acta Crystallogr. B52, 651–661.

ANTOLIĆ, S., SALOPEK, B., KOJIĆ-PRODIĆ, B., MAGNUS, V., COHEN, J. D., 1999: Structural characterization and auxin properties of dichlorinated indole-3--acetic acids. Plant Growth Regul. 27, 21–31.

BURES, M.G., BLACK-SCHAEFER, C., GARDNER, G., 1991: The discovery of novel auxin transport inhibitors by molecular modelling and three-dimensional pattern analysis. J. Computer-Aided Mol. Design 5, 323–334.

BOHM, H.–J., KLEBE, G., KUBINYI, H. (eds.), 1996: Wirkstoffdesign. Spektrum, Akademischer Verlag, Heidelberg.

CHANDRASEKHAR, K., RAGHUNATHAN, S., 1982: A reinvestigation of the structure of (3-indolyl)acetic acid. Acta Crystallogr. B38, 2534–2535.

EDGERTON, M.D., TROPSHA, A., JONES, A.M., 1994: Modelling the auxin-binding site of auxin-binding protein 1 of maize. Phytochemistry 35, 1111–1123.

FISCHER, E., 1894: Einfluss der Configuration auf die Wirkung der Enzyme. Ber. Dtsch. Chem. Ges. 27, 2985–2993.

GSCHWEND, D.A., GOOD, A.C., KUNTZ, J.D., 1996: Molecular docking towards drug discovery. J. Mol. Recognition 9, 175–186.

HATANO, T., KATAYAMA, M., MARUMO, S., 1987: 5,6-Dichloroindole-3-acetic acid as a potent auxin: its synthesis and biological activity. Experientia 43, 1237–1239.

HATANO, T., KATO, Y., KATAYAMA, M., MARUMO, S., 1989: A new antiauxin, α-(5,7-dichloroindole-3-)isobutyric acid: its chemical synthesis and biological activity. Experientia 45, 400–402.

JONSSON, Å, 1961: Chemical structure and growth activity of auxins and antiauxins. In: RUHLAND, W. (ed.), Encyclopedia of Plant Physiology, vol. 14, 960–1006. Springer-Verlag, Berlin.

KAETHNER, J.M., 1977: Conformational change theory for auxin structure-activity relationship. Nature 267, 19–23.

KARLE, I.L., BRITTS, K., GUM, P., 1964: Crystal and molecular structure of 3--indolylacetic acid. Acta Crystallogr. 17, 496–499.

KATEKAR, G.F., 1979: Auxins: on the nature of the receptor site and molecular requirements for auxin activity. Phytochemistry 18, 223–233.

KOJIĆ-PRODIĆ, B., NIGOVIĆ, B., TOMIĆ, S., ILIĆ, N., MAGNUS, V., KONJEVIĆ, R., GIBA, Z., DUAX, W.L., 1991: Structural studies on 5-(*n*-alkyl)-substituted derivatives of the plant hormone, indol-3-ylacetic acid. Acta Crystallogr. B47, 1010–1019.

KOSHLAND, D., JR., 1994: The key-lock theory and the induced fit theory. Angew. Chem. Int. Ed. Engl. 33, 2375–2378.

KUBINYI, H. (ed.), 1993: 3D QSAR in Drug Design. ESCOM, Leiden.

MCRAE, D.H., BONNER, J., 1952: Diortho substituted phenoxyacetic acids as antiauxins. Plant Physiol. 27, 834–838.

NAPIER, R.M., 1995: Towards an understanding of ABP1. J. Exp. Botany 46, 1787–1795.

NAPIER, R.M., VENIS, M.A., 1995: Auxin action and auxin-binding proteins. New Phytol. 129, 167–201.

NIGOVIĆ, B., ANTOLIĆ, S., KOJIĆ-PRODIĆ, B., KIRALJ, R., MAGNUS, V., SALOPEK--SONDI, B., 1999: Correlation of structural and physico-chemical parameters with the bioactivity of alkylated derivatives of indole-3-acetic acid, a phytohormone (auxin). Acta Crystallogr. B55, in press.

NIGOVIĆ, B., KOJIĆ-PRODIĆ, B., ANTOLIĆ, S., TOMIĆ, S., PUNTAREC, V., COHEN, J.D., 1996: Structural studies on monohalogenated derivatives of the phytohormone indole-3-acetic acid (auxin). Acta Crystallogr. B52, 332–343.

RAKHAMINOVA, A. B., KHAVKIN, E. E., YAGUZHINSKY, L. S., 1978: Postroenie modeli retseptora auksinov. Biokhimiya 43, 806–823.

RAMEK, M., TOMIĆ, S., 1998 a: RHF Conformational analysis of the auxin phytohormones *n*-ethyl-indole-3-acetic acid (*n*=4,5,6). Int. J. Quantum Chem.-Quantum Biology Symposium 70, 1169–1175.

RAMEK, M., TOMIĆ, S., 1998 b: *Ab initio* RHF investigation of mono- and dichlorinated indole-3-acetic acid (IAA) phytohormones. J. Mol. Struct. (Theochem.) 454, 167–173.

RAMEK, M., TOMIĆ, S., KOJIĆ-PRODIĆ, B., 1995: Systematic *ab initio* SCF conformational analysis of indol-3-ylacetic acid phytohormone (auxin): comparison with experimental and molecular mechanics calculations. Int. J. Quantum Chem. 22, 75–81.

RAMEK, M., TOMIĆ, S., KOJIĆ-PRODIĆ, B., 1996: Comparative *ab initio* SCF conformational study of 4-chloro-indole-3-acetic acid and indole-3-acetic acid phytohormones (auxins). Int. J. Quantum Chem. 23, 3–9.

RAY, P.M., DOHRMANN, U., HERTEL, R., 1977: Specificity of auxin-binding sites on maize coleoptile membranes as possible receptor sites for auxin action. Plant Physiol. 60, 585–591.

RUBERY, P.H., 1987: Auxin transport. In: DAVIES, P.J. (ed.), Plant hormones and their role in plant growth and development, 341–362. Martinus Nijhoff Publishers, Dordrecht.

SCHNEIDER, E.A., WIGHTMAN, F., 1978: Auxins. In: LETHAM, D.S., GOODWIN, T.W., HIGGINS, T.J.V. (eds.), Phytohormones and related compounds – a comprehensive treatise, vol. 1, 29–105. Elsevier, Amsterdam.

THIMANN, K.V., 1977: Hormone action in the whole life of plants. The University of Massachusetts Press, Amherst.

TOMIĆ, S., GABDOULLINE, R.R., KOJIĆ-PRODIĆ, B., WADE, R., 1998 a: Classification of auxin plant hormones by interaction property similarity indices. J. Computer-Aided Mol. Design 12, 63–79.

TOMIĆ, S., RAMEK, M., KOJIĆ-PRODIĆ, B., 1998 b: Combined *ab initio* SCF and molecular mechanics studies of propionic and isobutyric acids and their indole derivatives related to the phytohormone auxin (indole-3-acetic acid). Croat. Chem. Acta 71, 511–525.

TUOMINEN, H., PUECH, L., FINK, S., SUNDBERG, B., 1997: A radial concentration gradient of indole-3-acetic acid is related to secondary xylem development in hybrid aspen. Plant Physiol. 115, 577–585.

UGGLA, C., MELLEROWICZ, E.J., SUNDBERG, B., 1998: Indole-3-acetic acid controls cambial growth in Scots pine by positional signaling. Plant Physiol. 117, 113–121.

VENIS, M.A., NAPIER, R.M., 1995: Auxin receptors and auxin binding proteins. Crit. Rev. Plant Sci. 14, 27–47.