

STERIMOL and Its Role in Drug Research

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QSAR, STERIMOL Parameters, Regression Equations, Potosystem II Inhibitors, Herbicidal Activity

From the early beginnings of research in synthetic drugs, researchers were interested to know the effects of chemical structure on the pharmacological effects. As the first systematic studies, the work of Meyer (1899) and Overton (1901) on the narcotic effects of a series of chemicals is regarded, which resulted in their lipoid theory (see: Lipnick, 1986, 1989). Among various procedures to calculate the relationship between chemical structure and biological activity, abbreviated as QSAR, the 30 years old Hansch-Fujita approach has been most widely and effectively used up to now, in medicinal chemistry as well as in the field of agrochemicals. In the meantime, many conceptual supplementations and improvements as well as mathematical refinements have been carried out, the most important one its extension to three dimensions.

The original form of the Hansch-Fujita equation (Hansch and Fujita, 1964) read

$$1/\log C = a \cdot \pi + b \cdot \pi^2 + c \cdot \sigma + d$$

where π is the partition coefficient and σ the Hammett constant. $1/\log C$ stands for the biological activity. The coefficients a , b , c , d are calculated by the multiple linear regression method. A smaller number of compounds from a congeneric series, that is with the same mode of action, with a reasonable spread of their biological activity is required. Rather early researchers from industry became interested in the new method. At that time, 30 years ago, the screening 1000 or 2000 compounds was sufficient to find a new commercial product. Today this number has increased by a

factor of ten. To cope with this, QSAR appeared promising. It soon became evident, that hydrophobic (π) and electronic (σ) parameters were not sufficient to describe relationship between structure and activity. In addition, descriptors of the steric properties of the compounds were required. Originally, Hansch had suggested to use the E_s constants by Taft for that purpose (Hansch, 1969), but soon it turned that they were of limited use only. Moreover, they were not available for many substituents since they had to be determined experimentally. After some other approaches to steric parameters from various authors, Dr. Arie Verloop and coworkers in 1976 developed a method to calculate steric parameters from the Van-der-Waals descriptors of the respective substituents. He called them STERIMOL-parameters (A. Verloop *et al.*, 1976). Up to 1993, numerous authors from industry as well as from universities and other institutions have applied them successfully in drug design, agrochemical research and ecotoxicology.

A symposium was organized in June 1994 by H. Timmerman at Amsterdam, to honour Verloop whose achievement had turned out to become very productive. Nine speakers (Schlatmann, Zeelen, Fujita, Draber, Govers, Tollenaere, Tipker, van Geerestein, Seydel) presented talks on diverse aspects of drug design. Of these, one dealt with possible environmental relevance (Govers) and one with photosynthesis inhibitors (Draber). Verloop's approach played an important part in the whole symposium, but to varying degree. Especially valuable proved the Sterimol parameters in QSAR investigations of photosynthesis inhibitors as is shown below. In addition to the studies which were reported at the Verloop symposium, some more recent results are presented here.

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Examples of QSAR for Photosynthesis Inhibitors

A large number of commercial herbicides inhibit photosynthesis, representing still about 30 % of the market, despite of an increasing number of compounds with other modes of action. Finding a new herbicide, at first more or less a question of trial and error and of course of good luck, became an increasingly difficult job. Thus the QSAR method was used to study the relationship between chemical structure and herbicidal activity.

QSAR helped to find new clues to useful compounds but it also played an important role in the elucidation of the mechanism of photosynthesis. The investigation of inhibitors played an important role in the detailed understanding of the target protein, now known as the D-1 polypeptide subunit of photosystem II (PS II). Quite early it was felt that the original Hansch-Fujita equation required supplementation by steric parameters to describe the relationships between structure and activity of photosynthesis inhibitors. For this purpose we found the Sterimol parameters (Verloop *et al.*, 1976) very useful, superior to many other approaches to steric descriptors.

A rather early example of QSAR on photosynthesis inhibitors dealt with the triazinones of Fig. 1 (Draber *et al.*, 1969). In the 13 compounds on which the calculation was based, R¹ was SMe, OMe and NHMe, and R² were diverse alkyl groups.

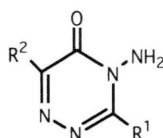


Fig. 1. 4-Amino-1,2,4-triazin-5-ones.

A simple squared regression equation without steric and electronic parameters was sufficient to describe the inhibitory activity.

$$pI_{50} = 4.608 + 1.959 \log P - 0.486(\log P)^2 \quad (1)$$

$$n = 13 \quad r = 0.951 \quad s = 0.288 \quad F = 47.4 \quad \log P_{\text{opt}} = 2.0$$

This led to the discovery of the herbicide metamitron (R¹ = CH₃, R² = Ph). If this compound is included, the equation reads

$$pI_{50} = 4.615 + 1.997 \log P - 0.504(\log P)^2 \quad (2)$$

$$n = 14 \quad r = 0.949 \quad s = 0.281 \quad F = 50.2 \quad \log P_{\text{opt}} = 2.0$$

In these equations, n is the number of compounds and r (correlation coefficient), s (standard deviation) and F (Fisher's F -test) are statistical criteria of the correlation. $\log P_{\text{opt}}$ is the calculated $\log P$ optimum.

A more complicated example on the same class of compounds, triazinones (R¹ = alkyl, R² = Ph) comprises Taft's E_s and the Verloop's Sterimol parameter L in the first to fourth power to account for an activity minimum at compounds with three carbon atoms in linear arrangement (Draber and Fedtke, 1979).

$$pI_{50} = -54.5 + 51.1L - 15.3L^2 + 1.97L^3 - 0.09 L^4 + 0.70 E_s \quad (3)$$

$$n = 11 \quad r = 0.986 \quad s = 0.14 \quad F = 37.7 \quad p = 0.0007$$

As an interpretation of this regression equation as early as 1979 – some time before Velthuys (Velthuys, 1981) stated convincingly that inhibitors act by competing with plastoquinone, PQ in Fig. 2, for the binding site – we drew a two-dimensional picture of the orientation of a triazinone inhibitor to an unknown target.

What we had termed “subreceptors” are, as we see it nowadays, hydrophobic interactions and hydrogen bonding between the various parts of the triazinone molecule and the amino acid residues of the D-1 protein. At that time we only had a rather vague idea of the binding site or, as we preferred to call it, the binding area. In 1981 (Pfister *et al.*, 1981) the “herbicide binding protein”, called the D-1 protein, was later identified as one of the reaction center peptides of photosystem II (PS II) (Trebst and Draber, 1986). This assumption could be supported by computer modelling of the D-1 protein with bound triazinones later on (Draber *et al.*, 1991) shown in Fig. 3.

Only the parts of helices IV and V that form the binding niche are displayed here. It should be noted that in stereo view the Serine-OH is indeed close to the triazinone carbonyl.

Fig. 4 shows a simplified reaction sequence of the photosynthetic electron flow together with some typical inhibitors that were useful in photosynthesis research.

In this figure, PQ symbolizes plastoquinone which is displaced by the herbicide DCMU (diuron) and many other photosynthesis inhibitors.

Another group of inhibitors of photosystem II are the substituted phenols. Trebst (1987) had sug-

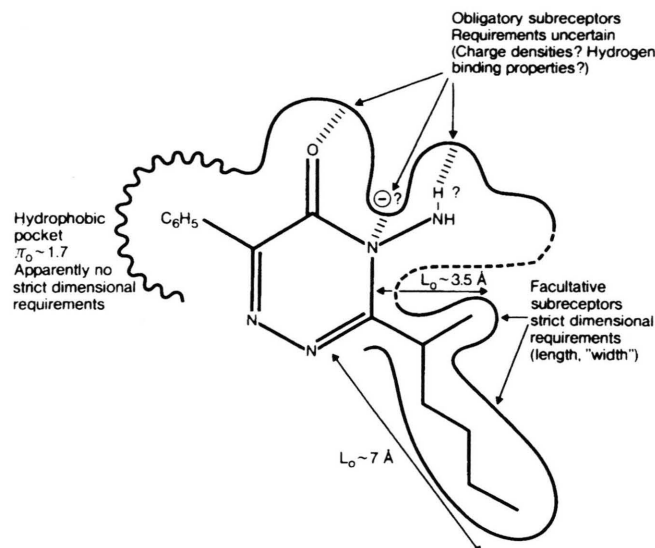


Fig. 2. Two-dimensional model of 3-alkyl-1,2,4-triazinones displaying steric, hydrophobic, and hydrogen bond interaction with the binding niche.

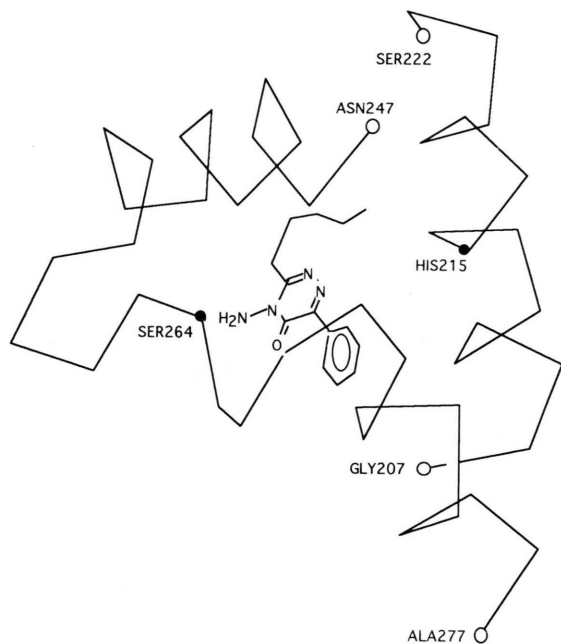


Fig. 3. 3-Pentyl-4-amino-6-phenyltriazinone in the binding niche of the D-1 protein.

gested a distinction between two inhibitor families that bind to the D-1 protein by displacing plasutoquinone. He called them the Serin₂₆₄- and the Histidin₂₁₅-family. They are characterized by the amino acids of the binding protein D-1 with which they interact preferentially. Though this distinction appeared to be a little arbitrary, it has proved

helpful in further research. Moreover, molecular modelling reveals that nitrophenols do not interact with Serin₂₆₄. Instead, their position is closer to Histidin₂₁₅ confirming Trebst's statement.

Again, our QSAR work dates back to 1978 when we presented a paper on the 4-nitrophenols (Trebst and Draber, 1979). Only Sterimol parameters were needed to describe the inhibitory activity of the compounds in Fig. 5a.

$$pI_{50} = -039 + 2.01B_{1-1} + 0.97B_{3-2} \quad (4)$$

$$n = 33 \quad F = 217 \quad r = 0.97 \quad s = 0.25 \quad p < 0.0001$$

The substituents R¹ were hydrogen, alkyl from C₁ to C₆ and phenyl, bromine and iodine, R² were hydrogen, the halogens and nitro. Two features of this equation are interesting. The first is that only Sterimol parameters were sufficient to result in an equation with excellent statistics, although we had tried many other kinds of molecule parameters and other descriptors like pK_a, R_m and the electronic field parameters *F* and *R* by Swain and Lup-ton (1968). The second is that in spite of the rather electron-withdrawing groups R¹ and R² and a pK_a range of 4.6 to 8.3, no electronic effects on pI₅₀ which ranged from 3.3 to 6.9, were required.

More recent work dealt with certain mutants of the green algae *Chlamydomonas reinhardtii* obtained by mutagenesis and screening under metribuzin pressure. These mutants exhibited tolerance against metribuzin by mutations in the *psba* gene

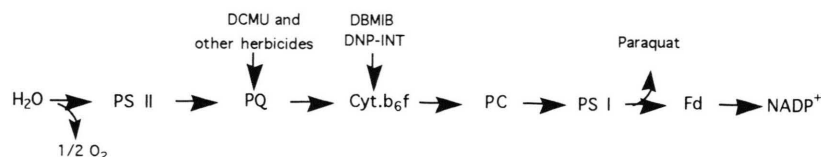


Fig. 4. Abbreviated reaction sequence of electron flow in photosynthesis. DCMU, 3,4-dichlorophenyl N,N'-dimethylurea; PQ, plastoquinone; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-benzoquinone; DNP-INT, dinitrophenylether of iodonitrothymol; PC, plastocyanin; Fd, ferredoxin.

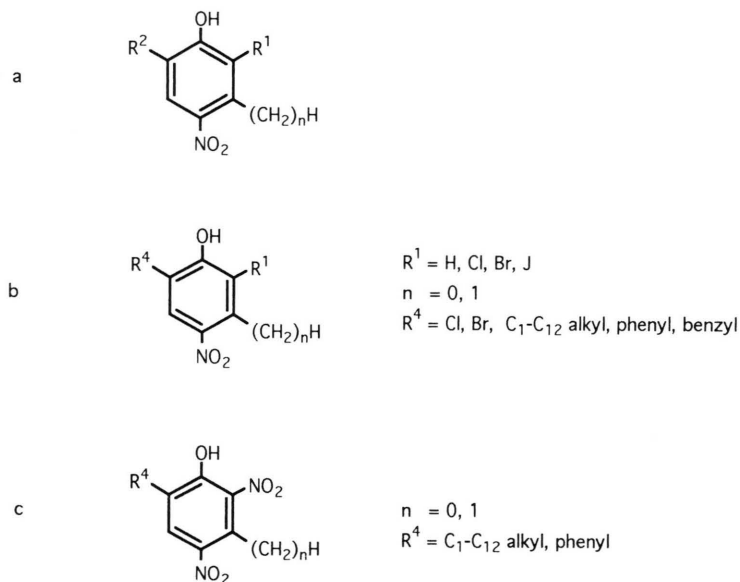


Fig. 5. 4-Nitrophenols (a), 2,6-substituted 4-nitrophenols (b), 6-substituted 2,4-dinitrophenols (c).

that encodes for the D-1 protein (Draber *et al.*, 1994). The substitutions in the amino acid sequences were determined and five mutants were chosen with only one amino acid change. These were Val219Ala, Ala251Val, Phe255Tyr, Ser264Ala, and Leu275Phe. The pI_{50} values of 27 2-halo-4-nitrophenols of Fig. 5b and 17 2,4-dinitrophenols of Fig. 5c were measured on wild-type and the mutants. QSAR analyses were carried out separately for the two groups and showed again the importance of the Sterimol parameter L. Apart from that, the partition coefficient and n were indispensable to obtain good statistics. Furthermore, our assumption was confirmed that the two families exhibited a very different inhibition pattern. Whereas the classical herbicides showed decreased activity in comparison with the Ser264Ala mutant as shown many times before, many nitrophenols became super-sensitive, the maximum of 1.9 pI_{50} units found with 2-bromo-4-nitro-6-methylphenol.

In contrast, the values with the Val219Ile mutant were decreased, the minimum of 1.7 pI_{50} units found with 2-bromo-4-nitro-6-benzylphenol. An example how a nitrophenol ($R^1 = \text{Br}$, $n = 0$, $R^4 = \text{benzyl}$) fits into the binding niche is shown in Fig. 6.

A next example where Sterimol parameters have proved useful are the 2-trifluoromethyl-4-hydroxyquinolines in Fig. 7a (Draber *et al.*, 1989). The activity range of the compounds is 4.1 to 7.9 pI_{50} units.

So it should be quite informative to look for a QSAR. The best equation is

$$pI_{50} = 3.81 + 1.12D_3 + 0.38B_5 + 0.67\sigma_6 \quad (5)$$

$$n = 17 \quad F = 21.1 \quad r^2 = 0.85 \quad s = 0.37$$

The most recent studies dealt with quinones and acridones of the structures shown in Fig. 7b.

As with the halonitro- and dinitrophenols, PS II inhibition was determined in wild-type and the

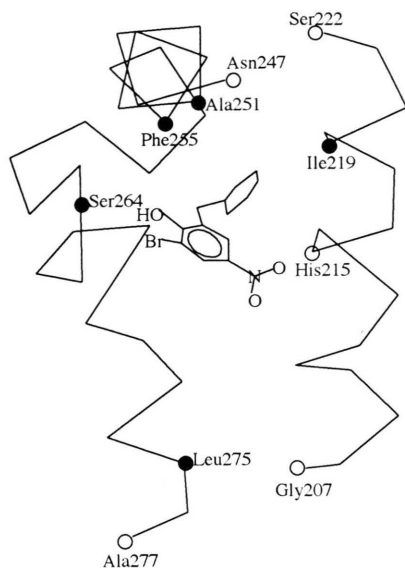


Fig. 6. 2-Bromo-4-nitro-6-benzylphenol in the binding niche of the D-1 protein.

same five mutants of the green algae *Chlamydomonas reinhardtii* (Draber *et al.*, 1995). pI_{50} values were available from wild-type and the mutants of 12 benzoquinones, 15 naphthoquinones and 11 acridones. The compounds were substituted by halogens, nitro, cyano, alkyl and alkoxy groups. Some typical equations are given below. QSAR equations for wild type and the Ser264Ala mutant were chosen, because especially this mutant shows a behaviour that strongly contrasts with the so-called classical inhibitors (Trebst, 1987) of the atrazine/metribuzin type: in most cases the inhibitory activ-

ity is enhanced instead of reduced in comparison to the wild-type.

The equations are shown below:

Benzoquinones

wild-type

$$pI_{50} = -5.60 + 2.23B_{12} + 3.08B_{13} + 0.20B_{53} + 0.33B_{56} \quad (6)$$

$$n = 12 \quad F = 35.7 \quad r^2 = 0.95 \quad s = 0.23$$

Ser₂₆₄Ala

$$pI_{50} = -1.09 + 4.21B_{12} - 2.14B_{52} + 1.63B_{13} + 0.49B_{56} \quad (7)$$

$$n = 12 \quad F = 18.7 \quad r^2 = 0.91 \quad s = 0.29$$

Naphthoquinones

wild-type

$$pI_{50} = +3.47 + 0.91B_{13} + 0.17B_{53} \quad (8)$$

$$n = 15 \quad F = 30.9 \quad r^2 = 0.84 \quad s = 0.24$$

Ser₂₆₄Ala

$$pI_{50} = +0.48 + 1.73B_{12} + 1.49B_{13} \quad (9)$$

$$n = 15 \quad F = 38.1 \quad r^2 = 0.86 \quad s = 0.36$$

Acridones

wild-type

$$pI_{50} = +1.00 + 0.29L_2 + 0.62L_4 - 0.72L_7 + 3.47B_{57} \quad (10)$$

$$n = 11 \quad F = 9.8 \quad r^2 = 0.87 \quad s = 0.29$$

Ser₂₆₄Ala

$$pI_{50} = +0.82 + 0.36B_{52} + 1.04L_4 - 0.70B_{54} + 0.41B_{55} + 0.49L_7 \quad (11)$$

$$n = 11 \quad F = 57.4 \quad r^2 = 0.98 \quad s = 0.13$$

It is clear from these examples that Sterimol descriptors lead to equations of good statistical quality, and it need almost not be emphasized that

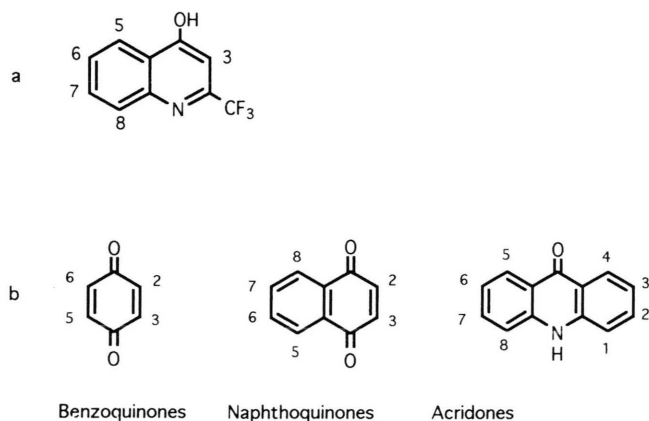


Fig. 7. 2-Trifluoromethyl-4-hydroxy-quinolines (a), 1,4-benzoquinones, 1,4-naphthoquinones, and acridones (b).

other parameters were inferior. What are the reasons for that preference, especially for the histidine family inhibitors?

Many QSAR calculations by numerous authors of "classical" inhibitors which belong to the serine family, have shown that lipophilic and electronic parameters are required for a proper description of their structure-activity relationship, suitable substitution range provided. In special cases Sterimol descriptors were applied successfully, but this is restricted to samples in which only lipophilic groups are changed. Some of them are mentioned here. In contrast to that, QSARs of inhibitors of the histidine family Sterimol and lipophilicity descriptors are sufficient, although the composition of the sample contained electron attracting as well as neutral substituents (Draber, 1992) as is shown above in many examples of 4-nitrophenols, 4-hydroxyquinolines, quinones and acridones. Of course, all these molecules carry polar groups as well, hydroxy or carbonyl, and nitro, which are

necessary for the orientation in the D-1 binding niche. But obviously the other substituents interact only sterically and lipophilically with the amino acid side chains of the binding niche. Thus, the distinction between the two inhibitor families (Trebst, 1987), as provisional it might be, has proved very useful in many respects.

Another fact deserves brief mentioning. Whereas inhibitors of the serine family inhibit the normally occurring rapid turnover of the D-1 protein (Mattoo *et al.*, 1981), histidine family inhibitors do not. This could also explain the fact that no powerful herbicides were found among these compounds.

These examples show that QSAR has contributed much to the knowledge of the binding niche in the photosynthesis inhibitor binding D-1 protein and provided hints to find new inhibitors. The QSAR equations would have presented a poor insight without the Sterimol parameters developed by Verloop.

- Draber W., Büchel K.H., Dickoré K., Trebst A. and Pistorius E. (1969) Structure-activity correlation of 1,2,4-triazinones, a new group of photosynthesis inhibitors. In: *Prog. in Photosynthesis Research* Vol. 3, (H. Metzner, ed.) Tübingen, pp. 1789–1795.
- Draber W. and Fedtke C. (1979), Herbicide interaction with plant biochemical systems. In: *Advances in Pesticide Chemistry, Part 3*, (H. Geißbühler, ed.) Pergamon, Oxford, pp. 475–486.
- Draber W., Pittel B. and Trebst A. (1989), Modeling of photosystem II inhibitors of the herbicide-binding protein. In: *Probing Bioactive Mechanisms*, (P.S. Magee, D.R. Henry, and J.H. Block, eds.) ACS Symposium Ser. 413, Washington D.C., pp. 215–228.
- Draber W., Hilp U., Schindler M. and Trebst A. (1994), Inhibition of photosynthesis by substituted 4-nitrophenols in wildtype and five mutants of *Chlamydomonas reinhardtii* thylakoids. In: *Natural and Engineered Pest Management Agents*, (P. Hedin, J.J. Menn and R.M. Hollingworth, eds.) ACS Symposium Ser. 551, Washington D.C., pp. 449–472.
- Draber W., Kluth J.F., Tietjen K. and Trebst A., (1991), Herbicides in photosynthesis research. *Angew. Chem., Int. Ed. Engl.* 30, 1621–1633.
- Draber W., (1992), Structure-activity studies of photosynthesis inhibitors. In: *Rational Approaches to Structure, Activity, and Ecotoxicology of Agrochemicals* (W. Draber and T. Fujita, eds.), CRC Press, Boca Raton, pp. 278–311.
- Draber W., Trebst A. and Oettmeier W. (1995), Structure activity relationships of quinone and acridone photosynthesis II inhibitors. In: *Classical and 3D QSAR in Agrochemistry and Toxicology*, (C. Hansch, ed.) ACS Symposium Ser., Washington D.C., in press.
- Hansch C. and Fujita T. (1964), π - σ - τ -Analysis. A method for the correlation of biological activity and chemical structure. *J. Am. Chem. Soc.* 86, 1616–1626.
- Hansch C. (1969), A quantitative approach to biochemical structure-activity relationships. *Acc. Chem. Res.* 2, 232.

- Lipnick R. L., (1989), H. H. Meyer and the lipoid theory of narcosis. *Trends Pharmacol. Sci.* **10**, 265–269.
- Lipnick R. L. (1986), C. E. Overton: narcosis studies and a contribution to general pharmacology. *Trends Pharmacol. Sci.* **7**, 161–164.
- Meyer H. (1899). *Zur Theorie der Alkohalnarkose*. Naunyn-Schmiedeberg's Arch. Exp. Pathol. Pharmacol. **42**, 109–118.
- Overton E. (1901), *Studien über Narkose, zugleich ein Beitrag zur Allgemeinen Pharmakologie*, Gustav Fischer, Jena.
- Pfister K., Steinback K.E., Gardner G. and Arntzen C.J. (1981), Photoaffinity labeling of an herbicide receptor protein in chloroplast membranes. *Proc. Natl. Acad. Sci. USA*, **78**, 981–985.
- Swain C.G., and Lupton E.C. (1968), Field and resonance components of substituent effects, *J. Am. Chem. Soc.* **90**, 615.
- Trebst A., and Draber W. (1979), Structure activity correlations of recent herbicides in photosynthetic reactions. In: *Advances in Pesticide Chemistry, Part 3*, (H. Geißbühler, ed.) Pergamon, Oxford, pp. 223–234.
- Trebst A. and Draber W. (1986), Inhibitors of photosystem II and the topology of the herbicide and QB binding polypeptide in the thylakoid membrane. *Photosynthesis Research* **10**, 381–293.
- Trebst A. (1987), The three-dimensional structure of the herbicide binding niche on the reaction center polypeptides of photosystem II. *Z. Naturforsch.* **42c**, 742–750.
- Velthuis B.R. (1981), Electron-dependent competition between plastoquinone in isolated chloroplasts. *FEBS Lett.* **126**, 277–288.
- Verloop A., Hoogenstraten W., and Tipker A. (1976), Development and application of new steric parameters in drug design. In: *Drug Design*, (E.J. Ariens, ed.) Academic Press, New York, Vol. **VII**, 165–207.