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STEREOSELECTIVITY AND AFFINITY IN MOLECULAR PHARMACOLOGY. III. STRUCTURAL ASPECTS IN THE MODE OF ACTION OF NATURAL AND SYNTHETIC AUXINS*

P.A. LEHMANN F.

Departamento de Farmacologia y Toxicologia, Centro de Investigacion y de Estudios Avanzados, Instituto Politecnico Nacional, A.P. 14-740, Mexico 14, D.F., (Mexico)

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SUMMARY

Analysis of available potency estimates for 35 pairs of enantiomeric arylcarboxylic acids with auxin activity (flax-root-growth inhibition test) revealed extensive correlations between the activity of the more potent and less potent isomers, as well as between the log of the ratio of potencies and the log potency of the more active isomer when structurally similar analogs are compared. 5 structural subgroups were discernible (n, eudismic-affinity quotient (EAQ), r^2); (1) arylpropionic acids (6, -0.36, 0.66); (2) 2-naphthoxy-carboxylic acids (6, +1.07, 0.99); (3) 1-naphthoxycarboxylic acids (3, +1.56, 0.96); (4) ortho-substituted phenoxycarboxylic acids (10, +0.97, 0.96) and (5) ortho-unsubstituted phenoxycarboxylic acids (10, +0.56, 0.70). For achiral lower homofogs such as auxin itself 3-indolyl-acetic acid (IAA), phenoxyacetic acid and 1-naphthoxyacetic acid, extrapolated potencies were found to agree well with experimental values.

On the basis of these observations an auxin receptor is postulated and binding arrangements are described which explain most of the experimental data available. A 3-point attachment when allowed is the only binding mode compatible with the reported data.

INTRODUCTION

In spite of the enormous amount of work [1] carried out on natural

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Abbreviations: Dis, distomer; EAC, eudismic-affinity correlation; EAQ, eudismic-affinity quotient; EI, eudismic index; Eu, eutomer; IAA, 3-indolyl-acetic acid.

TABLE I

AUXIN ACTIVITY (FLAX-ROOT-GROWTH INHIBITION) OF CARBOXYLIC ACIDS AR-X-CH(R)COOH

No.[a]	Ar	R	X	pC1 50%[b]		Eie	Ref.
				Eu[c]	Dis[d]		
Group A							
1	Phenyl	Me	—	5.52	4.15	1.37	6
*2**	4-Methyl-1-naphthyl	Me	—	6.00	5.40	0.60	6
*3**	2-Naphthyl	Me	—	6.10	5.30	0.80	6
*4**	1-Naphthyl	Me	—	7.00	6.82	0.18	6
5	[*d*]-Benzo-3-thenyl	Me	—	7.30	6.82	0.48	6
*6**	3-Indolyl	Me	—	8.00	7.70	0.30	6
GroupB							
Sub-group B-1							
7	1-Chloro-2-naphthyl	Me	0	4.92	4.21	0.71	6
8	2-Naphthyl	n-Bu	0	5.55	4.30	1.25	6
9	2-Naphthyl	Me	CH_2	5.59	4.46	1.13	6
10	2-Naphthyl	Me	S	6.74	4.30	2.44	6
11	2-Naphthyl	Et	0	7.00	4.14	2.86	6
12	2-Naphthyl	Me	0	7.70	4.09	3.61	6
Sub-group B-2							
13	1-Naphthyl	Me	0	4.85	4.64	0.21	6
14[1]	1-Naphthyl	Et	0	5.05	4.33	0.72	6
15g	1-Naphthyl	Me	CH_2	5.49	4.24	1.25	6
Sub-group B-3							
16	2,4,5,6-Tetrachlorophenyl	Me	0	4.70	4.60	0.10	7
17	2,4,6-Trichlorophenyl	Me	0	5.10	4.60	0.50	7
18	2,6-Dichlorophenyl	Me	0	5.60	5.40	0.20	7
*19**	2-Iodophenyl	Me	0	6.00	5.00	1.00	7
20	2,3-Dichlorophenyl	Me	0	6.50	5.00	1.50	7
21	2,5-Dichlorophenyl	Me	0	7.30	5.00	2.30	7
22	2,4-Dichlorophenyl	Me	0	7.48	4.89	2.59	6
23	2,4,5-Trichlorophenyl	Me	0	7.49	5.00	2.49	6
24	2,5-Dichlorophenyl	Et	0	7.55	4.85	2.70	6
25	2-Methyl-4-chlorophenyl	Me	0	7.68	4.74	2.94	6
Sub-group B-4							
26	Phenyl	n-Bu	0	~3.52	~3.52	0.00	6
27	3,5-Dichlorophenyl	Me	0	5.40	4.80	0.60	7
28	Phenyl	Me	NH	5.6p	3.52	2.14	6
29	Phenyl	Et	0	5.85	4.05	1.80	6
30	Phenyl	Me	0	5.89	3.96	1.93	6
31	4-Fluorophenyl	Me	0	6.00	4.00	2.00	7
32	4-Chlorophenyl	Me	0	6.50	4.40	2.10	7
33	4-Bromophenyl	Me	0	6.60	4.50	2.10	7
34	3-Iodophenyl	Me	0	7.40	5.10	2.30	7
35	3,4-Dichlorophenyl	Me	0	7.55	5.46	2.09	6

No.a	Ar	R	X	pC150% [b]		Ele	Ref.
				Eu[c]	Dis[d]		
Groupe							
36	Phenyl	H	O	3.40		(0.00)	7
37	1-Naphthyl	H	O	4.20		(0.00)	7
38	2-Naphthyl	H	O	7.10		(0.00)	7
39	3-Indolyl (IAA)	H	O	8.20		(0.00)	7

a An asterik after the number indicates that the eutomer has an absolute configuration opposite to that of the series as a whole (D).
b pCl 50% is the negative logarithm of the molar concentration of the substances which in the flax-root test reduces growth to 50% of that of the controls (equivalent to p).
c Eu, eutomer i.e. more potent isomer of a pair.
d Dis, distomer i.e. less potent isomer of a pair.
e EI, eudismic index i.e. log (activity Eu/activity Dis)= log Eu - log Dis.
f Eutomer potency calculated from the potencies of the distomer and of the DL-mixture considering them to be additive.
1 Eutomer potency taken as double that of the DL-mixture.

and synthetic plant growth regulators structurally related to IAA, the rationalization of their activity on the basis of their structure has been only partly successful. In particular, disagreement still exists [2] as to whether these substances interact with their putative receptor through a 2-point [3] or a 3-point [4] attachment.

Recently [5] we pointed out heretofore unrecognized correlations between the isomeric potency ratios and the biological potency of such auxins. In this paper these will be examined more closely in the hope of shedding light on the above-mentioned controversy.

Through the painstaking work of the Swedish workers in this field [2], there have been made available activity estimates for both isomers of no less than 35 pairs of enantiomeric aryl carboxylic acids in a variety of test systems (oat-coleoptile, wheat-root and flax-root) which have been listed conveniently by Jonsson [6] and Aberg [7]. In Table I are given those results for the flax-root inhibition test for which quantitative potency estimates (essentially pA_2) values are available for both isomers. The next-to-last column shows the difference between them.

We have shown [5] that in general stereoselectivity can be correlated with relative affinity regardless of absolute configuration. To avoid confusion in discussing this topic some new terms were introduced: eutomer (Eu) and distomer (Dis) designate the more and less potent isomer respectively; the ratio of their potencies is termed the eudismic ratio and its logarithm the eudismic index (EI). The rate of change of EI with change of log Eu is called the eudismic-affinity quotient (EAQ) and constitutes a quantitative measure of the stereoselectivity of the receptor or enzyme involved.

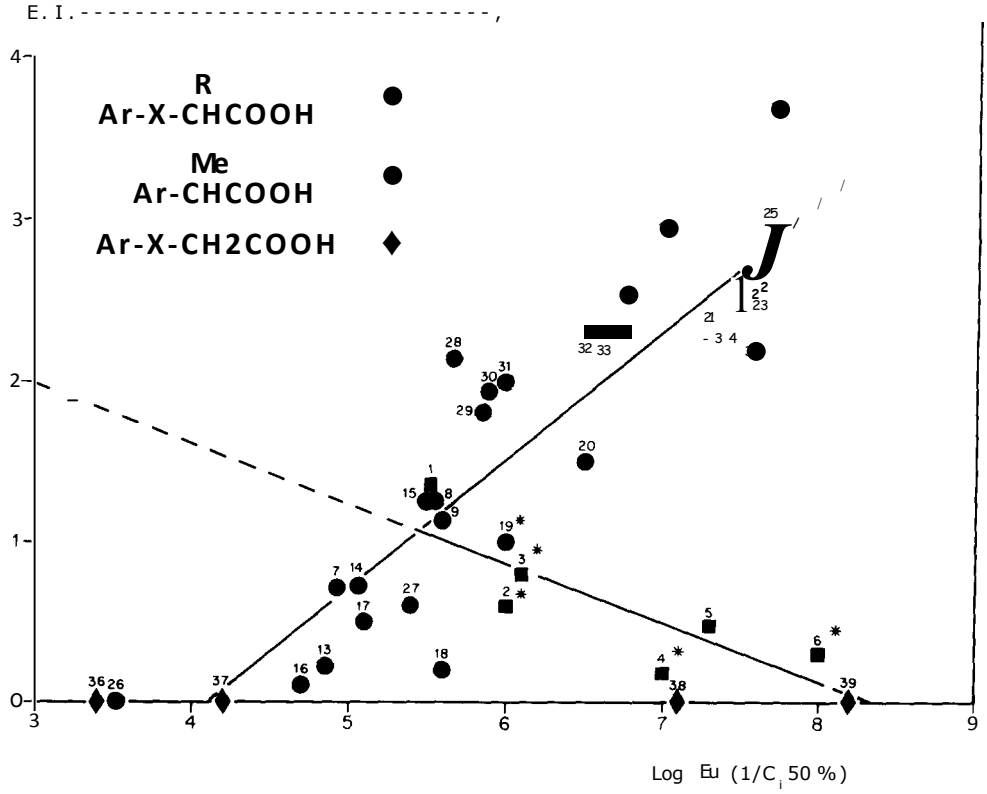

Fig. 1. EAC plot for the 35 chiral and 4 achiral auxins of Table I of general formula Ar-X-CH(R)COOH. Squares are for analogs with X missing (Group A), circles for X = 0, CH_2, NH or S (Group B) and diamonds for the achiral analogs. The lines shown are for EAC Nos. 3-2 and 3-3.

In Fig. 1 has been plotted for each enantiomeric pair of Table I, the EI (ordinate) against the log potency (pC1 50%) of the Eu (abscissa). The lines shown are least-square's estimates through the pertinent points; further analytical and statistical information concerning them can be found in Table II. The log Eu scale spans 4.5 log units (a 32 000-fold range of activity) and the EI scale 3.6 log units (4000-fold range).

Inspection of Fig. 1 reveals that the pairs fall naturally into 2 groups on the basis of their structure, i.e., depending on the presence or absence of a link between the aryl and acetate moieties. Those in which it is absent (Group A, a-arylpropionic acids, X missing, squares) fall about the line with a negative slope, whereas all the others (Group B, X = 0, CH_2, NH and S, circles) group about the line with positive slope. The statistical data for these regressions are given as EAC Nos. 3-2 and *3-3* in Table II.

If the aryloxyacids and their analogs (Group B) are broken down further into structural sub-groups, the correlations improve dramatically. Thus 6 2-naphthoxy analogs (Table I sub-group B-1, Fig. 2, EAC No. 3-4) show an

TABLE II

STATISTICAL DATA FOR VARIOUS EUDISMIC-AFFINITY CORRELATIONS AMONG ARYLCARBOXYLIC ACIDS WITH AUXIN-LIKE ACTIVITY

EAC.a No.	Group or sub-group	Line equation parameters[b]				Significance			
		EI_0	EAQ	log Eu_0	nc	s_1d	r^2e	*t* (P%)f	*F* (P%)g
3-1	All chiral pairs	-2.15 ± 0.78	+0.58 ± 0.12	3.71	35	0.772	0.400	<0.1	<1
3-2	Group A (1-6)	+2.98 ± 0.86	-0.36 ± 0.13	8.32	6	0.267	0.659	1 < P < 2	1 < P < 5
3-3	Group B (*7-35)*	-3.27 ± 0.48	+0.80 ± 0.08	4.10	29	0.447	0.798	<0.1	<1
3-4	Sub-group B-1 *(7-12)*	-4.71 ± 0.32	+1.07 ± 0.05	4.39	6	0.121	0.991	<0.1	<1
3-5	Sub-group B-2 *(13-15)*	-7.26 ± 1.64	+1.56 ± 0.32	4.66	3	0.148	0.960	10 < P < 20	>5
3-6	Sub-group B-3 *(16-25)*	-4.69 ± 0.49	+0.97 ± 0.07	4.69	10	0.246	0.956	<0.1	<1
3-7	Sub-group B-4 *(26-35)*	-1.71 ± 0.80	+0.57 ± 0.13	3.02	10	0.442	0.703	0.1 < P < 0.5	<1
3-8	Sub-group B'	-3.85 ± 0.45	+0.85 ± 0.07	4.51	14	0.303	0.921	<0.1	<1
3-9	Sub-group B''	-2.56 ± 0.71	+0.72 ± 0.11	3.57	15	0.448	0.758	<0.1	<1

a Eudismic-affinity correlation number.

b Line equation parameters: $EI = EI_0$ (±S.D.) + EAQ(±S.D.) log Eu; log $Eu_0 = -(EI_0/EAQ)$.

c n, number of pairs in the correlation.

d s_1, standard deviation of the regression.

e r^2, coefficient of determination; multiplied by 100 it gives the percent variance explained by the regression.

f The precentage probability according to Student's t-test that the absolute t-statistic is greater than the determined t-value on the assumption that there is no correlation.

g The percent probability according to the F-test that the regression does not significantly reduce the variance.

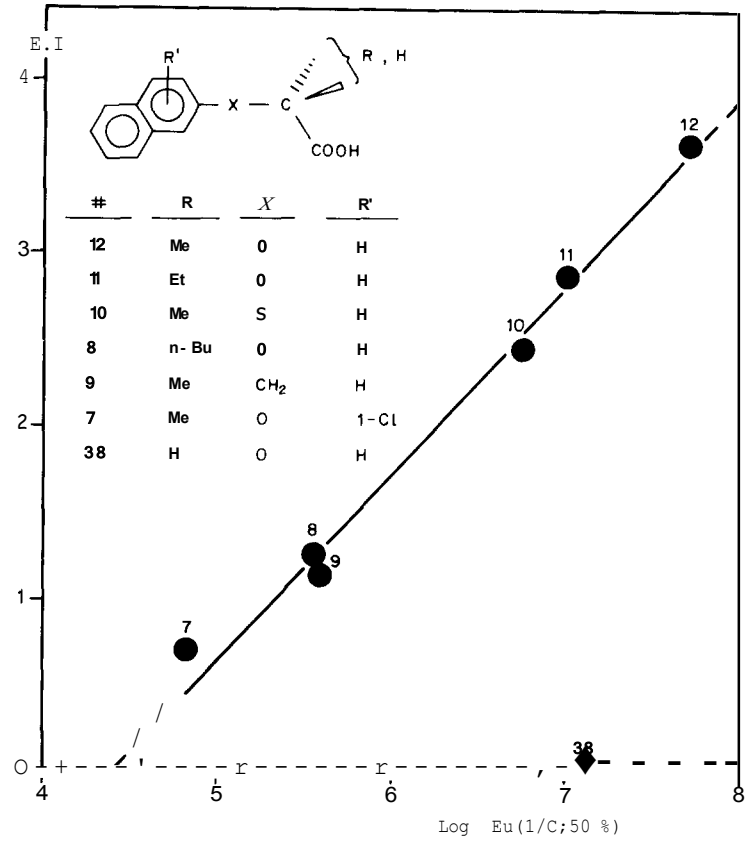

Fig. 2 EAC plot for 2-naphthyl analogs (Sub-group B-1, EAC No. 3-4).

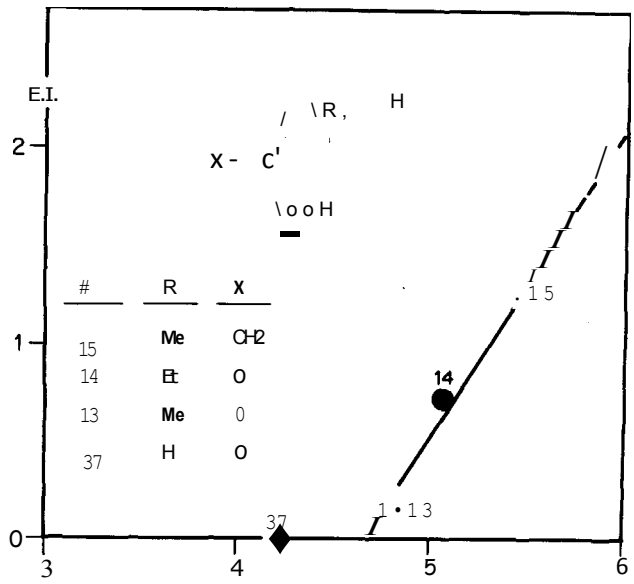

Fig. 3 EAC plot for 1-naphthyl analogs (Sub-group B-2, EAC No. 3-5).

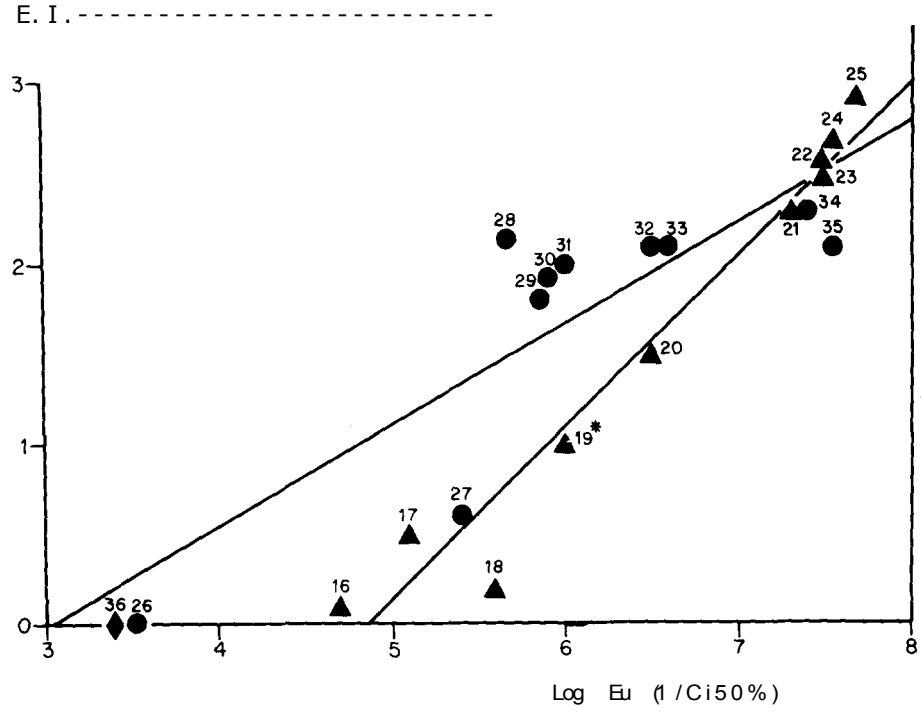

Fig. 4. EAC plots for o and o,o'-phenyl analogs (triangles, Sub-group B-3, EAC No. 3-6) and unsubstituted phenyl analogs (circles, Sub-group B-4, EAC No. 3-7).

excellent correlation. Also, 3 1-naphthoxy analogs (Table I sub-group B-2, Fig. 3, EAC No. 3-5) correlate very well, although the EAC is not significant because there are only 3 data points. When the remaining pairs are plotted separately (Fig. 4) a good correlation becomes evident for *o*- and o,o-disubstituted analogs (Table I sub-group B-3, triangles in Fig. 4, EAC No. 3-6), but it is not as good for the unsubstituted phenoxy analogs (Table I sub-group B-4, circles in Fig. 4, EAC No. 3-7). When the pairs of Group Bare simply divided into substituted (sub-group B') or unsubstituted (B") at the *ortho* positions, the correlations (EAC 3-8 and 3-9) are still significant.

Some further observations can be made from these plots:

(1) When all 35 enantiomeric pairs are considered together there is still an apparent correlation (EAC No. 3-1).

(2) The EAQ for the sub-groups in group Bis positive, whereas that for A is negative. 5 analogs (asterisked) correlate well although the Eu has an opposite absolute configuration to that of the series as a whole.

(3) Within certain homologous sub-series, e.g., *12, 11, 8* (Fig. 2) and *30, 29, 26* (Fig. 4) both affinity and stereoselectivity decrease as R *increases* from Me to Et ton-Bu.

(4) The natural plant growth hormone IAA (39) falls precisely at the point at which the regression line of group A intercepts the abscissa. Of the other achiral lower homologs, phenoxyacetic acid (36) and 1-naphthoxy-

acetic acid (37) fall close to the predicted point, whereas 2-naphthoxyacetic acid (*38*) does not.

DISCUSSION

The extremely high correlations observed between stereoselectivity and affinity for these auxins when grouped according to structural type must reflect what occurs at the molecular level. The following explanation of these observations is based on the assumption that the available data accurately estimate true affinities; differences in intrinsic activity, differential distribution and metabolism, as well as dualism of activity (concurrent auxin and antiauxin activity) are disregarded in this first approximation.

According to the Easson-Stedman model [8] and its extension in Part 2 of this series [5], homologous enantiomeric series would have an EAQ of 1: the distomers would all interact by the same 2 groups and have essentially the same potency; the eutomers interact with these two plus the third, whose relative contribution accounts for the progression along the log Eu and EI scales. In Table II it can be seen that only in 2 correlations (Nos. 3-4 and 3-6) are they close to 1, whereas others are smaller than 1 (No. 3-7), greater than 1 (No. 3-5) or negative (No. 3-2). These can be understood as follows.

A receptor is proposed (Fig. 5) in which 3 coplanar interacting areas radiate out from the center of the "active spot" (projection of the chiral center onto the receptor plane) at approx. 120° from each other. One interacts specifically with the carboxyl group (denoted site c) and has a net positive charge. The other 2 sites are both hydrophobic but slightly different from each other. The first (denoted ar) is rather narrow and has a steric block at the distal end; it is able to interact with aromatic systems when these are perpendicular to the receptor plane (in fact it may resemble a groove). The second (r) is similar to the first but longer. When seen from above the receptor surface, the sequence *c, r, ar* is clockwise.

We further assume a rigid receptor, 3 point interactions wherever possible (the high affinity constants observed cannot normally be explained otherwise), and negligible binding contribution by the hydrogen directed away from the receptor plane. The interactions of the structurally different auxins can then be visualized as follows.

IAA, aryloxyacetic and cinnamic acids

IAA (39), which shows the highest affinity of all analogs tested, must interact in a near optimal fashion with the receptor (Fig. 5A). Since normally the hydrophobic binding contribution by hydrogen is small, the optimal interaction must be due to a near perfect fit by both the indole nucleus (denoted Ar) and the carboxyl group and/or some charge-transfer type interaction peculiar to this kind of nucleus. When the indole nucleus is replaced by phenyl *(36),* activity drops considerably. High affinity can be regained with Ar = 2-naphthyl *(38,* Fig. 5B), but not with 1-naphthyl *(37,*

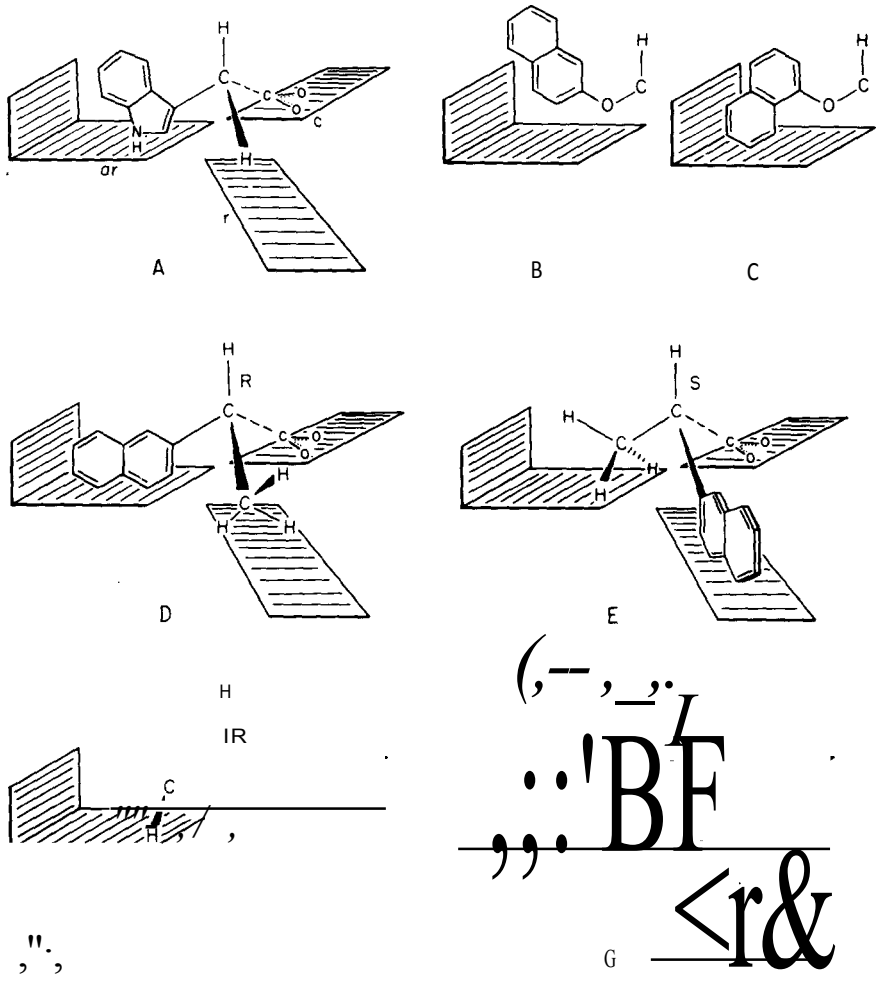

Fig. 5. Auxin receptor and its interaction with different auxins (see text).

Fig. 5C), either because of steric hindrance or because the outer ring does not fit the groove well and cannot interact. A similar disposition explains the activity of the *cis* and inactivity of the *trans-cinnamic* acids [1].

Arylpropionic acids

This group of 6 analogs (1-6) is striking in that both enantiomers show high affinities. From the corresponding data in Table I it can be seen that for the same sequence of change in Ar (1-6) the distomers increase more quickly in potency than the eutomers, which is reflected in a negative EAQ (EAC No. 3-2) for this group. 4 of the 6 analogs (asterisked) have the L-configuration which for these compounds corresponds to an R-chirality and occupy the receptor by binding COOH-c, Ar-ar, R-r (normal binding

mode Fig. 5D). The other 2 *(1* and *5)* must bind in an alternate binding mode, i.e., COOH-c, *Ar-r, R-ar* (Fig. 5E); when arranged by the first binding type the potency sequence 4.15, 6.00, 6.10, 7.00, 6.82, 8.00 reflects the relative affinity of Ar for site *ar*. The distomers of the 4 analogs which have S-chirality bind in the alternate mode.

The high activities of both series show that both *ar* and *r* must be more like grooves than planes; if they were flat they would have to be in planes angled to each other at awkward angles, unlikely in small areas of the size covered by these molecules.

Aryloxycarboxylic acids

The eutomers of this series (Group B) all (except for *19)* have D absolute configuration which (by coincidence) is also an R-chirality. The eutomers all bind in the alternate mode with all 3 groups contributing (Fig. 5F). Note that the steric block in site *ar* explains the decreases in activity noted in 2 homologous sub-series on going from Me to Et to *n-Bu*.

The distomers, on the other hand, bind in the normal mode (Fig. 5G) but because of the geometry induced in the molecule by X and the interactions of X itself, Ar contributes to the overall binding as follows.

In the 2-naphthoxy series it contributes nothing (for all 6 distomers log Dis is 4.25 ± 0.12). This explains the EAQ of 1. In the 1-naphthoxy series the outer ring does not fit properly because of the steric block; thus as the eutomers get better, the distomers get worse, resulting in an EAQ greater than 1.

In the phenoxy series (see Fig. 4) the same happens as with the 2-naphthoxy analogs: the eutomers interact by 3-point binding in the alternate mode (Fig. 5F) while the distomers do so by 2-point binding in the normal mode (EAQ = 1). This can be said with assurance for the *o-* and *o,o'*-substituted analogs (EAC No. 3-6), but not for the unsubstituted ones for which scatter is greater. This may be a reflection of a conformational difference (out-of-plane twisting of the ring) due to steric hindrance by substituents *ortho* to the ether link [9]: it is unimportant in the alternate binding mode of the eutomers, but is a further detriment to *Ar-ar* interaction in the distomers so that again here its contribution is nil and the average log Dis for all 10 distomers is 4.91 ± 0.22. The large scatter for the unsubstituted series makes difficult the establishing of the EAQ. If further work shows that it is 1, then its explanation is as before; it it is less than 1, it means that as potency increases in the eutomeric series, it also increases in the distomeric series, but more slowly. On a molecular level this can be explained by a positive, but small contribution to binding by the Dis in the normal mode, made possible by the greater conformational freedom present when *ortho* substituents are absent.

In summary, all known activity estimates of enantiomeric auxins in the flax root growth inhibition test can be rationalized on the basis of the proposed receptor and 1 of 2 alternate binding modes. A 3-point attachment obtains whenever the auxin stereochemistry permits such an interaction with

the receptor; otherwise a 2-point attachment must be invoked.

New data on additional enantiomeric pairs would be helpful in clarifying some further details of this aspect of auxin activity.

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REFERENCES

1 R.L. Wain and C.H. Fawcett, Chemical plant growth regulation, in F.C. Steward, (Ed.), Plant Physiology Vol. VA, Ch. 4, Academic Press, New York, 1969.
2 A. Fredga and B. A.berg, Annu. Rev. Plant Physiol., 16 (1965) 53.
3 C. Hansch, R.M. Muir, T. Fujita, P.P. Maloney, F. Geiger and M. Streich, J. Am. Chem. Soc., 85 (1963) 2817.
4 R.L. Wain and C.H. Fawcett, Chemical plant growth regulation, in F.C. Steward (Ed.), Plant Physiology vol. VA, Ch. 4, Academic Press, New York, 1969, pp. 255-260
5 P.A. Lehmann F., J.F. Rodrigues de Miranda, and E.J. Ariens, Prog. Drug Res. 20 (1976) 101.
6 A. Jonsson in W. Ruhland, (Ed.), Handbuch der Pflanzenphysiologie vol. 15, Springer--Verlag, Heidelberg, 1961, pp. 999-1001.
7 B. A.berg in Plant growth regulation, 4th International Conference, Yonkers, N.Y. 1961, pp. 219-232.
8 L.H. Easson and E. Stedman, Biochem. J., 27 (1933) 1257.
9 P.A. Lehmann F. and D.M. McEachern B., J. Mol. Struct., 7 (1971) 253.