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PHYTOTROPINS: CONFORMATIONAL REQUIREMENTS FOR THE ABOLITION OF THE ROOT GEOTROPIC RESPONSE

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Key Word Index—Phytotropins; root geotropism; auxin transport inhibition; structure–activity; conformational requirements.

Abstract—Compounds of the phytotropin class have been assessed for possible conformational requirements with respect to their ability to affect the root geotropic response. It is shown that part of the molecule may need to adopt a planar configuration, and evidence is adduced which indicates that the remainder of the molecule may also have conformational requirements which need further definition. It is suggested that molecules which favour a conformation such that the aromatic ring which bears the carboxyl function is out of plane with the remainder of the molecule may have activity. Coplanarity of the carboxyl group with the ring to which it is attached is not a prerequisite for activity.

INTRODUCTION

It has previously been shown that a group of chemicals which were known to affect the root geotropic response in plants can be defined by a common set of chemical requirements [1,2]. These requirements are that active compounds should possess (Fig. 1): (1) a carboxylic acid function (or one which can become available by hydrolysis) which is attached to (2) an aromatic ring, which is connected at the *ortho* position to (3) a second aromatic ring. (4) The aromatic rings may be separated by a conjugated or planar system of atoms. (5) There is also a spatial requirement which can be expressed by saying that high activity will be reached when the distance between the centres of the two extreme aromatic rings is at least 0.73 nm.

Substituents on the non-carboxylated ring increase activity if they assist in the achievement of this molecular size, but otherwise appear to have little effect. It has also been shown that compounds which come within these rules appear to be auxin transport inhibitors [2, 3, 4] and the name 'phytotropins' has been proposed for this class of compound [5]. It is possible that this class may be of some importance, and further definition of the structure–activity correlation would seem desirable. As was noted when the rules were formulated [1,2] the requirement for conjugation or planarity (Rule 4) was not well defined, and it can be seen from the rules that conformational aspects were not considered. An analysis of possible planarity and conformational requirements was therefore undertaken to determine aspects which might be amenable to further definition.

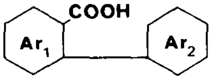


Fig. 1. Chemical requirements for root antigeotropic activity.

RESULTS AND DISCUSSION

An examination of molecular models shows that active members of the class can adopt the general conformation **1** (Fig. 2). While some active compounds can adopt alternative conformations, including differing tautomeric forms as in the aryl benzoic acids **3** and the propanediones **6** (Fig. 3), there are others (e.g. the fluoresceins **5**) which can only exist in the form shown.

The general formulation **1** has two degrees of freedom. Firstly, rotation is possible about the Ar_1-carboxyl bond, and secondly, there can be rotation about the Ar_1—Ar_2 bond. Extreme forms of the possible conformations can thus be represented by the structures **1a**–**1d**. The preferred conformation for compounds to be active with respect to the geotropic response would therefore lie within these structures.

Requirement for planarity

A requirement for planarity (or conjugation) has previously been shown [1]. In compounds with high activity the Ar_1 portion of the molecule is an aromatic ring and therefore this portion of the molecule must be planar. The Ar_2 portion of the molecule can contain atoms which are not part of aromatic rings and non-planar conformations of this region are possible for many active molecules. Although the 2-phenylbenzoic acids are not known to be phytotropins, they nevertheless can fulfil the rules for activity, and they are now found to be active with respect to the root geotropic response (Table 1). These compounds must of necessity be planar in the Ar_2 region. A planar structure would also appear to be required for high activity in the aryl phthalamic acids **4** (Fig. 3 and Table 2). The stable conformation of an anilide group would be expected to be planar due to conjugation (**a**, Fig. 4). It can be seen from Table 2 that monochloro-substituted phenyl phthalamic acids **4a** to **4c** have essentially the same activity regardless of the position of substitution. Similarly, the disubstituted compounds all show enhanced activity but for the 2,6-derivative. This latter compound is two to three orders of magnitude less active than all the other disubstituted analogues, cf. compounds **4d** to **4g** (Table 2). The significant activity of all the chloro-substituted derivatives but for the 2,6-compound indicate that the reason for the lack of activity in the latter is due to steric, rather than electronic effects. A similar pattern has been observed with the dimethyl analogues with 2,6-dimethyl phthalamic acid (**4i**) being inactive at 10^{-3} M [6]. By analogy, it is suggested that the inactivity of the 2,6-dimethyl compound is also due to steric reasons. It is suggested that the explanation of these observations is that 2,6-disubstitution prevents the anilide portion of the molecule from becoming planar due to steric hindrance (**b**, Fig. 4). The bulkier bromine atom, when placed in the 2-position, consistently gives rise to 10-fold lower activity as compared with its 3- and 4-substituted analogues: cf. **4j** to **4l** (Table 2). Further, other types of compound which are highly active with respect to the root geotropic response also exist in conformations in which the Ar_2 portion must either be planar (the fluoresceins **5**) or can easily become planar as with the propanediones (**6**) [7] and heterocyclic derivatives such as **7** [8]. It is concluded that for high activity with respect

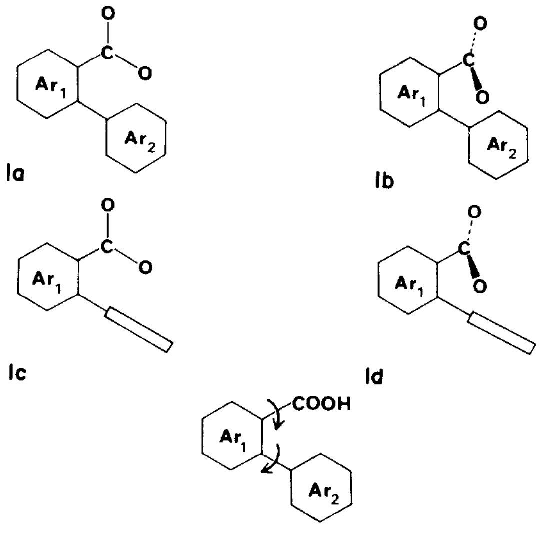
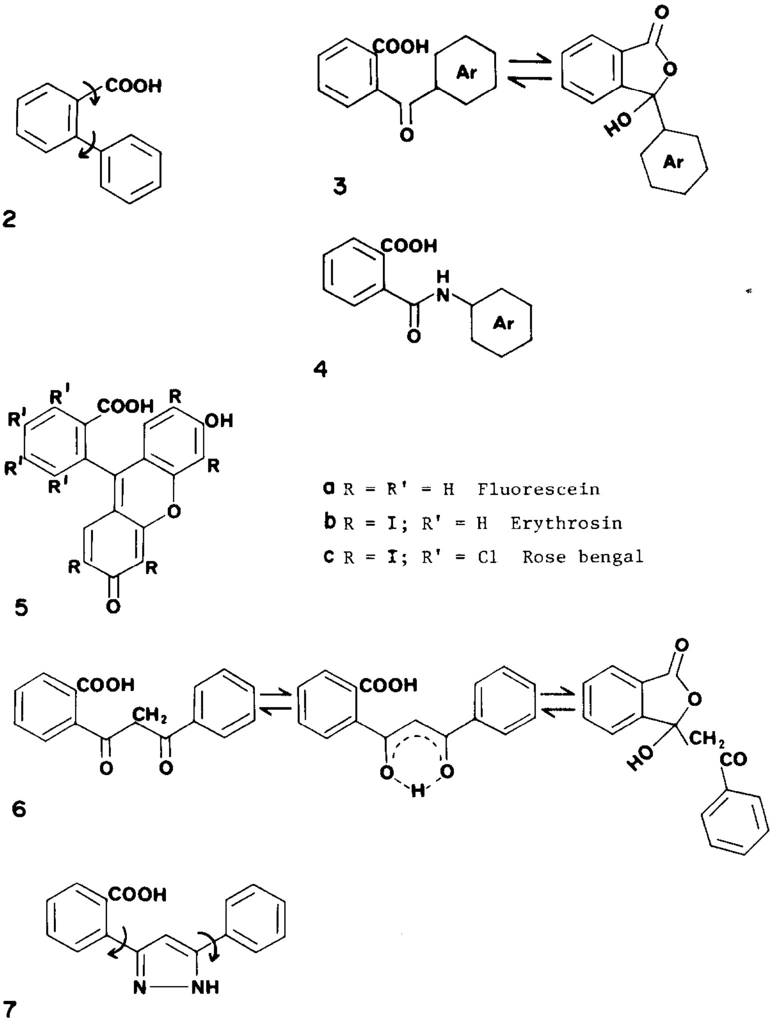


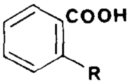
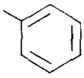
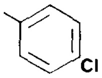
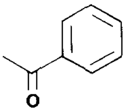
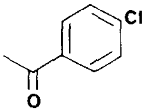
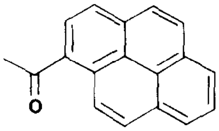
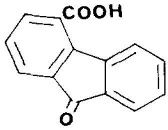
Fig. 2. Possible conformations of active molecules.



a R = R' = H Fluorescein
b R = I; R' = H Erythrosin
c R = I; R' = Cl Rose bengal

Fig. 3. Chemical structures.

Table 1. Root antigeotropic activity of aryl and aroyl benzoic acids

Compound		Structure 	Activity (M*)
2	R =		3×10^{-5}
2a	R =		3×10^{-6}
3	R =		10^{-4}
3a	R =		3×10^{-6}
3b	R =		10^{-8}
3c			10^{-3}

*Minimum concentration causing complete loss of geotropic response.

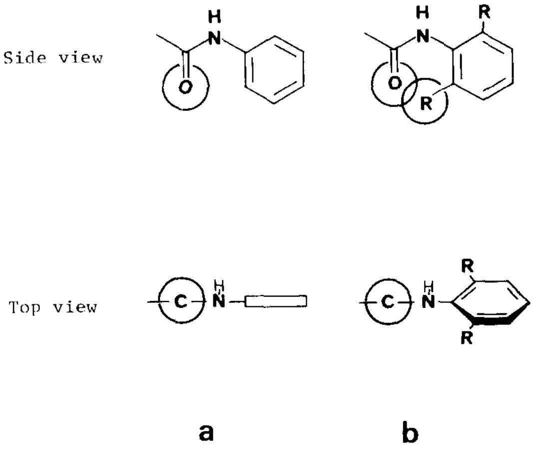


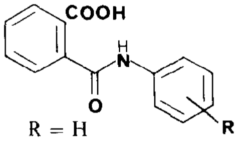
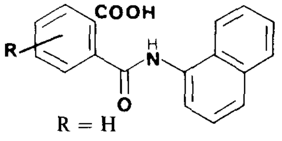
Fig. 4. Conformations of aryl phthalamic acids.

to the geotropic response the favoured conformation is that in which the whole of Ar_2 region is planar.

Conformation of the $Ar_1—Ar_2$ *grouping*

9-Fluorenone-4-carboxylic acid (**3c**) is an analogue of the 2-phenylbenzoic acids and is almost inactive with respect to the geotropic response, while 2-phenylbenzoic acid (**2**) has significant activity (Table 1). The inactivity of the fluorenone would not seem to be due to the electronic effect of the ketone group on the aromatic ring in the Ar_2 area because the ketone-containing benzoyl benzoic acids **3** and **3a** are both active (Table 1), and it has been

Table 2. Root antigeotropic activity of aryl phthalamic acids

		Activity (M*)		
Compound	Structure		Ref. [6]	Ref. [4]
4	 R = H	10^{-5}*	10^{-4}	10^{-4}
4a	R = 2-Chloro	10^{-4}	3×10^{-5}	
4b	R = 3-Chloro	10^{-4}	3×10^{-5}	
4c	R = 4-Chloro	10^{-4}	10^{-4}	
4d	R = 2,3-Dichloro	10^{-6}	10^{-6}	
4e	R = 2,4-Dichloro	10^{-5}	3×10^{-5}	
4f	R = 2,5-Dichloro	10^{-5}	3×10^{-5}	
4g	R = 2,6-Dichloro	10^{-3}	10^{-3}	
4h	R = 2,3-Dimethyl	10^{-6}		
4i	R = 2,6-Dimethyl	10^{-3}		
4j	R = 2-Bromo	10^{-4}		
4k	R = 3-Bromo	10^{-5}		
4l	R = 4-Bromo	10^{-5}		
4m	 R = H	10^{-6}	10^{-6}	10^{-6}
4n	R = 2-Nitro			10^{-6}
4p	R = 3-Nitro			10^{-6}

*Defined in Table 1.

concluded previously that electronic effects of substituents in this area appear to be of minor significance [1]. Electronic effects on the Ar_1 ring by the ketone group would also seem unlikely to be able to account for inactivity, because activity is not destroyed by substituents which differ widely in their electronic effects. For example, activity is not destroyed either by halogens cf. rose bengal (**5c**) with erythrosin (**5b**) (Table 3) or by a nitro group cf. **4m**, **4n** and **4o** (Table 2).

It has been suggested that phytotropin molecules may achieve their effects by interaction with a receptor site [5]. Another possibility, therefore, is that the ketone group may impinge on an area of steric obstruction when the molecule attempts to interact with that receptor site. A steric effect of this nature remains possible, but it would seem unlikely because halogen substitution in an analogous position on the Ar_1 ring in the fluoresceins reduces but does not destroy activity. Again **5b** and **5c** can be compared. It is suggested, therefore, that the reason for inactivity may be a conformational one, and may be found within the conformations shown in Fig. 2. Differences in conformation of the carboxyl group would seem an unsatisfactory explanation of differences in activity here, because it can be argued that if **2** and **3c** adopt the same (coplanar) conformation with respect to the $Ar_1—Ar_2$ grouping, there would be little difference between the preferred conformations of the carboxyl groups between the molecules. With respect to differences in the $Ar_1—Ar_2$ conformations, it can be seen that the fluorenone must adopt a fixed coplanar configuration and it is essentially inactive. It may be, therefore, that coplanar conformations as in **1a** and **1b** give rise to inactive compounds. On the other hand, all the active phytotropins known do not have a fixed configuration in this region, and all have a carboxyl group adjacent to the $Ar_1—Ar_2$ junction which would tend to favour an out-of-plane conformation. In the case of rose bengal, there is a chlorine and a carboxyl on either side of the junction, which would be expected to favour even more the out-of-plane conformation. It should be noted, however, that the compounds assessed, of their nature, only possess moderate activity at best ($\sim 10^{-5}$ M) so that inferences drawn must be treated with caution. With this qualification, it can be concluded that coplanarity between the $Ar_1—Ar_2$ groupings is not a prerequisite for phytotropin activity, and the possibility is raised that coplanar conformations as in **1a** and **1b** may be unfavourable. It is suggested, therefore, that there may be a conformational requirement in this region, but the influence of coplanarity on activity can only be assessed when molecules of fixed conformation and having the potential for high activity become available.

Conformation of the carboxyl group

By analogy with arguments put forward with respect to the auxin activity of *ortho*-substituted benzoic acids [1, 9–11] the requirement of *ortho*-substitution with respect to the Ar_1 ring renders it likely that the most favoured conformation of the carboxyl is one which is out of plane with the Ar_1 ring. While this may be the most stable conformation, it cannot be assumed that it must also be the interacting conformation [12]. In the case of the auxins, however, it was found that the less favourable conformation gave rise to lower activity [11]. To the extent that it can be assumed that the strength of interaction of the benzoic acid moiety of these antigeotropic compounds is of the same order of magnitude as the interaction of the benzoic acids of synthetic auxin molecules with their receptor site, then it would seem that an unfavourable conformation of the carboxyl group in phytotropin molecules may give rise to reduced activity. In the present case, however, compounds with very large *ortho* substituents can retain activity. For example, the fluoresceins (Table 3) have comparable activity to their less hindered benzoylbenzoic acid analogues. Di-*ortho* substitution also does not destroy activity; see **4n** and **5c**. Compounds of such types would be expected to favour highly the out-of-plane conformation of the carboxyl group. On this basis, we suggest that coplanarity of the Ar_1 and carboxyl groups is not required for activity and it may also be that the most favoured conformation is also the interacting conformation. It is therefore tentatively concluded that phytotropin molecules which highly favour conformations corresponding to **1d** (Fig. 1) can be active with respect to abolishing the root geotropic response. Further definition of the conformational parameters would seem to require the design and testing of appropriate model compounds.

Table 3. Root antigeotropic activity of fluoresceins

Compound	Name	Activity (M*)
5a	Fluorescein	10^{-4}(3)
5b	Erythrosin	10^{-6}
5c	Rose bengal	10^{-5}

*Defined in Table 1.

Conclusions

The structural rules previously proposed to define compounds with root geotropic activity have been shown to be capable of further refinement. It is concluded that the portion of the molecule attached to the aryl carboxylic acid must be planar or capable of becoming so. It is suggested that where the carboxylic acid favours the out-of-plane conformation with respect to the aromatic ring to which it is attached, and this aromatic ring is out of plane with the rest of the molecule, such compounds can be active. Since the rules which define compounds with root geotropic activity are similar to those which define the phytotropin class of auxin transport inhibitors, it is at least possible that there may also be conformational requirements for general phytotropin activity.

EXPERIMENTAL

The geotropic assay was carried out on cress (*Lepidium sativum*) roots, and has been previously described [1]. 2-Phenylbenzoic acid, 9-fluorenone-4-carboxylic acid, 2-benzoylbenzoic acid and, 2-(*p*-chlorobenzoyl)benzoic acid were obtained from the Aldrich Chemical Co. The arylphthalamic acids were prepared by standard methods [6]. 2(4-Chlorophenyl)benzoic acid was prepared by the method of ref. [13].

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