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AUXIN STRUCTURE/ACTIVITY RELATIONSHIPS: ARYLOXYACETIC ACIDS

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Abstract—The results of self-consistent field molecular orbital calculations on auxins of the aryloxyacetic acid type do not support the 'charge separation' hypothesis. Some molecules with a positive charge in the position required by the hypothesis are inactive while some active molecules possess no such charge. The proposal that there is a correlation between the magnitude of a fractional positive charge on the auxin nucleus and degree of biological activity has also been shown to be untenable.

INTRODUCTION

Thimann and Porter [1] hypothesized that the primary growth action exerted by auxins depends upon the presence of a fractional positive charge on a planar lipophylic nucleus at about 5.5 Å (0.55 nm) from a negative carboxyl oxygen. Thimann himself [2, 3] drew attention to some problems with the theory but nevertheless concluded [3] that the theory provided the best explanation for the biological activity of compounds of many different chemical types. Subsequently Kaethner [4], Rakhaminova *et al.* [5] and Katekar [6] discounted charge separation as a key feature of auxin molecular structure. Our self-consistent field molecular orbital (SCFMO) calculations for auxin molecules [7, 8], whilst not confirming the precise charge distribution required by the Thimann and Porter [1] theory, revealed that for molecules in the thermodynamically favoured conformation a net positively charged site at approximately 5 Å (0.5 nm) from the acidic group was a consistent feature. It was considered, therefore, that this positively-charged site could contribute to a reversible binding interaction between auxin and receptor. Subsequent calculations carried out on benzoic acid derivatives [9] revealed, however, that both the highly active 2,6-dichlorobenzoic acid and 2,4-dichlorobenzoic acid, which is inactive in auxin bioassays [10], possessed net positive sites at the equivalent carbon atoms 3 and 5 which were 0.45 nm (rather than 0.5 nm) from the carboxyl oxygens. This indicated that charge separation could be only one of the factors involved in determining auxin activity, if indeed it were important at all.

In order to establish more clearly the relevance of the location of electron-deficient sites on the ring structures of auxin molecules to their biological activity, it was clearly essential to increase the number of auxins studied.

Irvine [11] noted that the aryloxyacetic acid, naphth-2-yloxyacetic acid was an auxin. Subsequently Zimmerman and Hitchcock [12] reported that a large number of naphthoxy- and phenoxy-acetic acids were highly active in tests for auxins although phenoxyacetic acid itself had negligible activity. The most potent activating substituents were found to be the halogens chlorine and fluorine, although the substitution of small alkyl groups, such as methyl groups, into the ring of phenoxyacetic acid had a slight activating effect [13, 14]. The location of the substituents was clearly critical; thus 2,4-dichlorophenoxyacetic acid (2,4-D) was the most active compound of the phenoxyacetic acid series, while 3,5-dichlorophenoxyacetic acid (3,5-D) had zero activity [15–17]. In both the phenoxyacetic- and naphthoxyacetic-acid series, the activity was lost, or markedly reduced by the introduction of di-*ortho* substituents larger than fluorine [16, 18–20]. After consideration of the ways in which phenoxyacetic acid was activated by halogen substitutions, several workers concluded that if a molecule was to have auxin activity the ring structure must contain an electron-deficient position, or positions, at which reaction with a receptor was assumed to take place [21–25]. In the context of their charge separation theory, Thimann and Porter [24, 25] envisaged a reversible binding interaction between auxin and receptor, involving a positively charged site (C_6 for the phenoxyacetic acids) on the auxin nucleus 0.55 nm from the acidic group. In contrast, the 'two-point attachment' theory of Hansch and Muir [21] visualized an *ortho* position on the ring as a likely binding site, whereas Julg and Cocordano [22] suggested that the 3 and 6 positions were involved in simultaneous nucleophilic fixation reactions.

SCFMO calculations have been carried out for aryloxyacetic acids with a wide range of auxin activities. The results of these calculations, and a discussion of their implications with regard to theories of auxin structure/activity relationships are presented in this paper.

RESULTS AND DISCUSSION

Several proposals regarding the molecular requirements for auxin activity draw attention to the importance of conformation [4–6, 13, 26–28]. SCFMO calculations on conformation in the indolylacetic, arylacetic and

benzoic acid auxins [7–9, 29] are consistent with Veldstra's conclusion [10, 26, 27, 30] that the conformation in which the side chain lies in a plane perpendicular to the ring plane is the most favourable for auxin activity.

In the case of the aryloxyacetic acids, however, the side chain is free to rotate about the C_1—O phenoxy bond in addition to rotation about the O phenoxy–C methylene bond and the C methylene–C carboxyl bond. This means that the side chain has one additional degree of freedom not present in the arylacetic acids and indolylacetic acids. The C methylene atom of phenoxyacetic acid could conceivably lie in the plane, perpendicular to the plane or at any position out of the plane. The position of the oxygen–C methylene bond in the side chain of the aryloxyacetic acids will clearly have a profound effect on any stereochemical or charge–distance relationship between the anion and the ring and therefore consideration was given to the possible conformations which could be adopted by the active phenoxyacetic acid auxins, and 2,4-D in particular. Four of the possible conformations which could be adopted by 2,4-D are shown in Fig. 1. In conformations 1a and 1d, the C methylene atom is positioned in the ring plane, and in conformations 1b and 1c, the C methylene atom lies in a plane, defined by the O phenoxy, C methylene and C carboxyl atoms, which is perpendicular to the ring plane. Stereochemical considerations suggest that a conformation in which the C methylene atom lies in the plane, on the same side of the C_1–C_4 axis of the phenyl ring as a bulky *ortho* substituent such as chlorine or methyl would be relatively unstable due to interactions between the *ortho* substituent and the carboxyl oxygens (in the planar conformation) or the methylene hydrogens (in the perpendicular conformation). The conformation for 2,4-D in which the C methylene atom is in the plane of the ring and bent towards C_6 (shown in Fig. 1d) with the carboxyl group bent away from the *ortho* substituent seems possible, however.

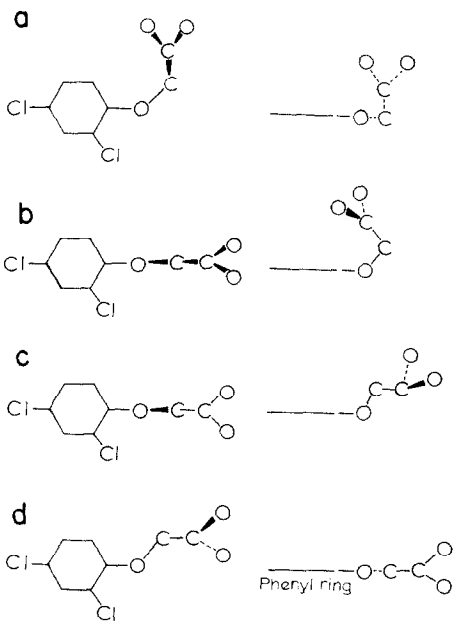

Fig. 1. Possible side chain conformations of 2,4-D (dissociated) viewed from above the plane of the ring (left) and side elevation (right).

Since rotation could also take place about the O phenoxy–C methylene bond, a variety of possible conformations of the acidic group relative to the ring plane could also exist. Two extremes of this rotation, for the conformation in which the methylene carbon lies furthest from the ring plane, are shown in Fig. 1 (b and c).

In both conformations 1c and 1d (and the equivalent conformation to 1d, with the methylene carbon atom in the plane, but towards carbon 2), the anion is bent away from the ring. Whilst these are possible conformations for 2,4-D and the aryloxyacetic acids, neither seems likely to be the required conformation for auxin activity, since the side chain of *trans*-cinnamic acid is fixed in an analogous position, so that it always remains bent away from the ring, and *trans*-cinnamic acid is inactive as an auxin. In the isomer *cis*-cinnamic acid, the acidic group is fixed so that it is bent towards the ring, and this molecule has auxin activity [26]. Conformations 1c and 1d were thus not considered.

When the C methylene atom of the 2,4-D side chain is positioned in the plane of the phenoxy ring, quite close stereochemical similarities are found to exist between 2,4-D, indol-3-yl-acetic acid (I-3-AA) and naphth-1-yl-acetic acid (N-1-AA) with regard to the position of the side chain relative to the 6-membered ring. In view of the results obtained from studies into the conformation of I-3-AA and N-1-AA [8, 29], when the C methylene atom of 2,4-D is lying in the ring plane the perpendicular conformation shown in Fig. 1a might be expected to prevail. Thus SCFMO calculations were carried out for 2,4-D in conformations 1a and 1b, as shown in Fig. 1. The calculations suggest that conformation 1a, in which the C methylene atom is in the plane of the ring, would be the more stable by 25×10^3 J/mol. In conformation 1a rotation of the carboxyl group around the carboxyl carbon–methylene carbon bond resulted in only a small change in energy. The fact that in conformation 1a the phenoxy oxygen atom has a π orbital suitable for conjugation with the ring π system thereby increasing resonance stabilization, is probably a contributory factor to the stability of this conformation, while the repulsion between the carboxyl oxygens and the ring π electrons relatively destabilizes conformation 1b.

In addition to fulfilling the criterion of a relatively low energy, this proposed conformation 1a for 2,4-D provides a basis for stereochemical comparisons between the diverse auxin series, many of which, such as the aryl- and indolylacetic acid series have less flexibility than the phenoxyacetic acid auxins in the possible side chain conformations [29]; stereochemical considerations suggest that the inactivity of the di-*ortho*-substituted aryloxyacetic acids is due to unfavourable interactions, preventing the adoption of this conformation. Accordingly, for the subsequent calculations reported for the aryloxyacetic acids, a conformation analogous to that shown for 2,4-D in Fig. 1a was adopted, with the C methylene atom lying in the ring plane and the O phenoxy–C methylene–C carboxyl plane perpendicular to the ring plane.

The total net atomic charge on the atoms of 2,4-D, together with the σ and π electron density distributions are given in Fig. 2. The calculations predict that in the case of 2,4-D the putative positive site of the Thimann and

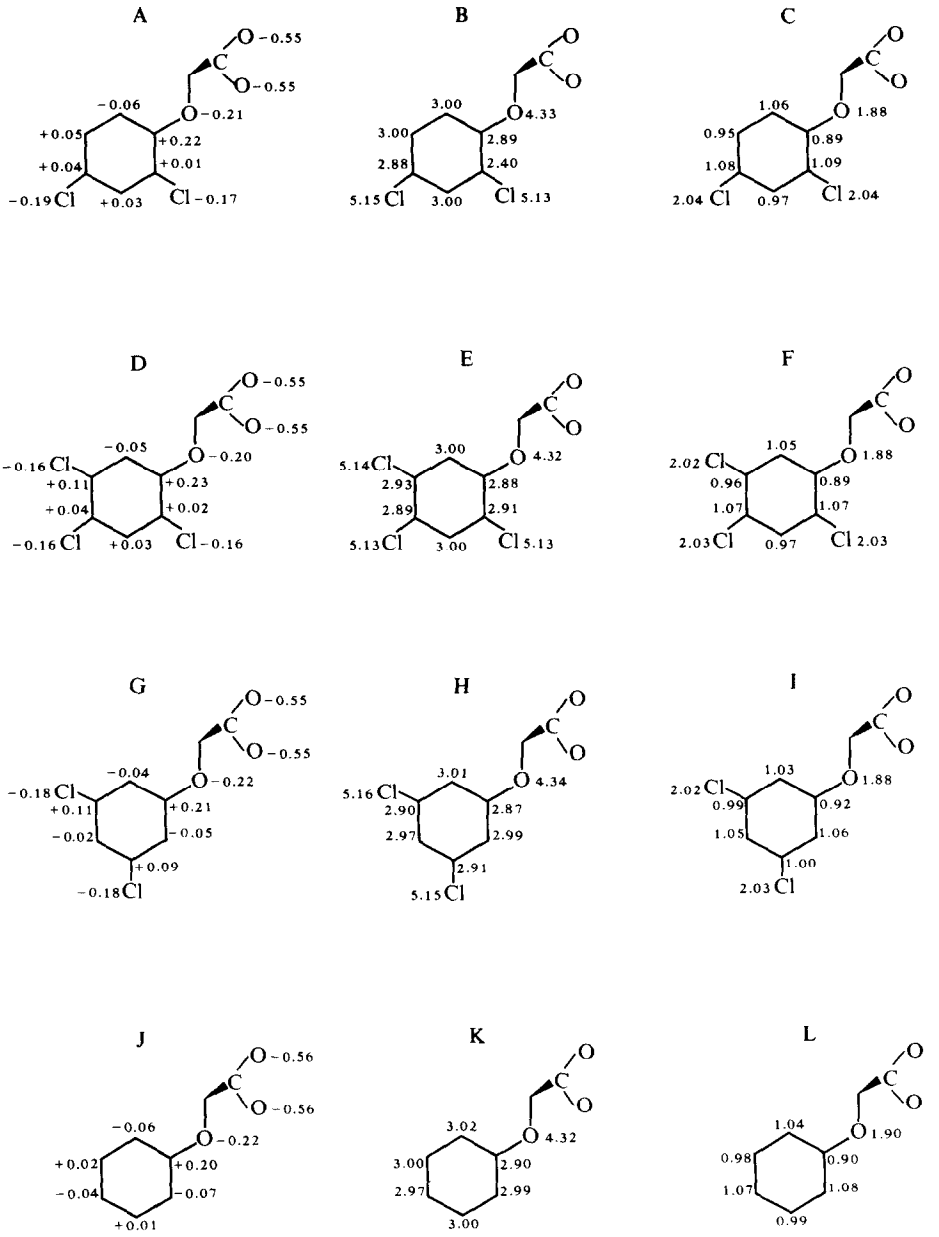

Fig. 2. Electron density distribution on the atoms of 2,4-dichlorophenoxyacetate (A, B, C), 2,4,5-trichlorophenoxyacetate (D, E, F), 3,5-dichlorophenoxyacetate (G, H, I) and phenoxyacetate (J, K, L). Total net atomic charge (A, D, G, J), σ electron density (B, E, H, K) and π electron density (C, F, I, L) are shown.

Porter theory, carbon 6, actually carries a net negative charge; although chlorine substitutions tend, overall, to be electron-withdrawing, when attached directly to an aromatic ring delocalization of the π electrons on chlorine into the ring can occur [31]. These data are also inconsistent with the proposal [22], based on calculations of a superdelocalizability index, that C_3 and C_6 are involved in a simultaneous nucleophilic fixation reaction.

It has previously been reported [7–9] that the existence of a net positive charge at about 0.5 nm from the acidic group proved to be a feature of the active auxins studied. The requirement for a net positive site at 0.5 nm from the acidic group was thus proposed to contribute to the auxin-binding interaction. Porter and Thimann [32] claimed that there was a correlation between the magnitude of the fractional positive charge featured in their theory and relative growth activity. It was clear from our data, however, that the magnitude of the net charge alone could not satisfactorily explain relative activity [8]. For example, the charge on the net positive site (C_8) of I-3-AA is +0.11, whereas the charge on the positive site (C_5) of 2,4-D is +0.05. The charge on 2,4-D is comparable, however, with a net charge of +0.04 on the positive site (C_{10}) of N-1-AA. A further inconsistency is revealed in the fact that in the case of I-3-AA the net positive charge on C_8 results from the inductive effect of nitrogen through the σ

bonds, there being no excess or deficiency of π electrons, whereas the net positive charge on C_5 of 2,4-D is due entirely to its deficiency of π electrons.

Additional calculations have been carried out for 3,5-D and phenoxyacetic acid, which are inactive as auxins, and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), which is highly active [15–17], to establish whether any feature of electron density distribution emerged which could satisfactorily explain relative biological activity. The total net atomic charge, plus the σ and π atomic electron densities of 3,5-D and phenoxyacetic acid, and 2,4-D and 2,4,5-T are given in Fig. 2. The effect of chlorine substitution was consistent with its known electronegativity, and the overall electron density on the ring atoms was decreased due to chlorine substitution. The electron-withdrawing inductive effect of chlorine on the adjacent carbon atom was counteracted to some extent in 2,4-D and 2,4,5-T by some delocalization of the π electrons on chlorine into the ring.

As shown in Fig. 2, a net positive charge is predicted at C_5, which is 0.505 nm from the acidic group in each case. Since phenoxyacetic acid and 3,5-D are inactive as auxins, it seems that the existence of a net positive charge at approximately 0.5 nm from the acidic group does not ensure auxin activity. C_6, the proposed positively charged site of the Thimann and Porter theory [1, 15, 25], was negatively charged in each case.

An explanation for the inactivity of 3,5-D might be sought in terms of a possible stereochemical effect due to chlorine substitution at the site of the positive charge. However, the high activity of 2,4,5-T militates against this possibility; carbon atoms 3 and 5 are not equivalent in 2,4,5-T due to the stereochemical effect of the *ortho*-chlorine substituent on the conformation of the side chain, and the small charge of +0.03 on C_3 is located at 0.596 nm from the acid group, and hence does not conform to a 0.5 nm charge separation.

A more plausible interpretation of the inactivity of the di-*meta*-substituted phenoxyacetic acids is that unfavourable interactions occur at the receptor site when both *meta* positions are occupied [29]. Unfavourable interactions with the 'floor' of a hypothetical auxin receptor site have been proposed by Kaethner [4] and implied by Rakhaminova *et al.* [5] and Katekar [6]. It seems possible that when only one *meta* position is occupied, the ring could orientate itself so that the unfavourable

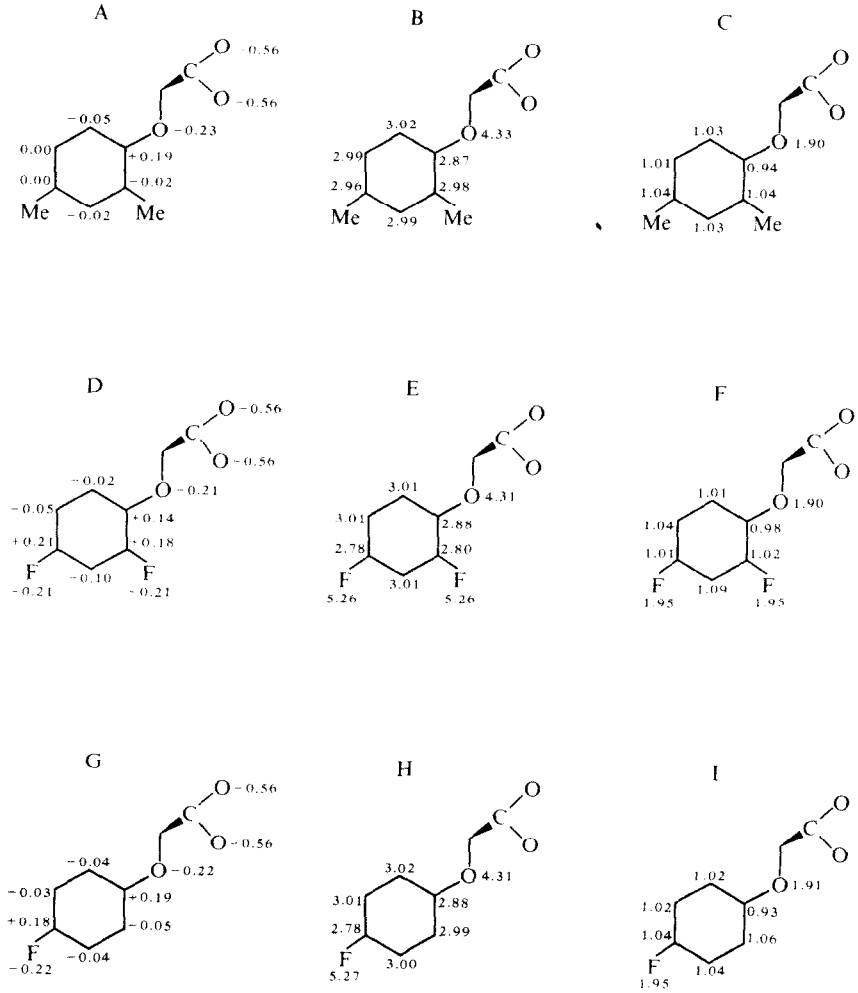

Fig. 3. Electron density distribution on the atoms of 2,4-dimethylphenoxyacetate (A, B, C), 2,4-difluorophenoxyacetate (D, E, F) and 4-fluorophenoxyacetate (G, H, I). Total net atomic charge (A, D, G), σ electron density (B, E, H) and π electron density (C, F, I) are shown.

interaction is avoided. The fact that 3-chlorophenoxyacetic acid and 2,5-dichlorophenoxyacetic acid are more active than 2,3-dichlorophenoxyacetic acid [13] seems likely to be a reflection of the fact that the 3 and 5 positions are not equivalent in the two latter compounds, and gives an indication of the position relative to the side chain of the proposed blocking effect at the receptor.

The range of calculations was further extended to include moderately active auxins with substituents other than chlorine: 2,4-dimethylphenoxyacetic acid, 2,4-difluorophenoxyacetic acid and 4-fluorophenoxyacetic acid, to establish the effect of these substituents on the predicted electron density. Fig. 3 shows the total net atomic charge, and σ and π atomic electron densities for these molecules.

None of these molecules contained the positive site at C_5 present in the other phenoxyacetic acid auxins studied. The only positive site on 2,4-dimethylphenoxyacetic acid was located at C_1 and is due to the electron-withdrawing effect of the adjacent oxygen atom; this is in accord with the observation that methyl substituents increase electron availability on an aromatic nucleus [31]. Fluorine is the most electronegative of the halogens, and this is reflected in the withdrawal of σ electrons away from the ring. This resulted in a substantial net positive charge on the carbon atoms to which the fluorine is attached. Whilst the positive charge on C_2, at 0.498 nm from the acidic group, could explain the activity of 2,4-difluorophenoxyacetic acid in terms of a 0.5 nm separation between a positive site on the nucleus and the acidic group, the only positive site (apart from C_1) for the active 4-fluorophenoxyacetic acid is at 0.598 nm from the acidic group.

These data indicate that the activating effect of methyl substitutions in phenoxyacetic acids cannot be explained in terms of the existence of a positive site on the nucleus. The electron-withdrawing effect of fluorine was localized at the substituted carbon atom in 4-fluoro- and 2,4-difluoro-phenoxyacetic acid, and no net positive charge existed on C_5, a positively-charged site in the other phenoxyacetic acid auxins studied.

The results of the SCFMO calculations for these phenoxyacetic acid auxins do not support the view that the relative auxin activities of this series and the activating effects of chlorine substituents can be explained in terms of the magnitude of a positive charge at a ring atom.

Calculations carried out on the naphthoxyacetic acids produced similar results. The charge distributions of naphth-2-yloxyacetic acid, which exhibits high auxin activity, and naphth-1-yloxyacetic acid, which has negligible auxin activity [26] are shown in Fig. 4. (The σ and π electron densities are also given.) Both molecules possess a net positively-charged site at 0.505 nm from the acidic group, hence the inactivity of naphth-1-yloxyacetic acid is not explicable in terms of the absence of such a positively-charged site. However, as depicted in Fig. 4, the stereochemical relationship between the nucleus and the side chain is different in the two molecules, and these stereochemical considerations seem likely to explain the inactivity of naphth-1-yloxyacetic acid (Farrimond, Elliott and Clack, in preparation).

These results on the electron density distribution on aryloxyacetic acids indicate that the net positively-charged site previously noted at approximately 0.5 nm from the acidic group in active auxin molecules [7, 8] is unlikely to be of primary importance in determining auxin activity: the data obtained from the calculations on the phenoxyacetic acids revealed not only that some molecules with a positive charge in the prescribed position are inactive, but also that molecules of moderate activity may possess no such charge. The proposal [32] that there is a correlation between the magnitude of a fractional positive charge on the auxin nucleus and biological activity has also been shown to be untenable.

Thus, the nature of the effect of specific substituents, particularly of chlorine, in increasing activity in the phenoxyacetic acid and other auxin series remains unclear. Veldstra's view [26, 33] that the lipophilic character of certain of these substituents could be the determining factor for relative auxin activity was not substantiated, since auxin activity does not increase with increasing lipophilic character, and molecules of similar lipid solubility have very different auxin activity [16, 27]. It seems probable that there is a specific interaction of the auxin nucleus with a receptor molecule. Although it is

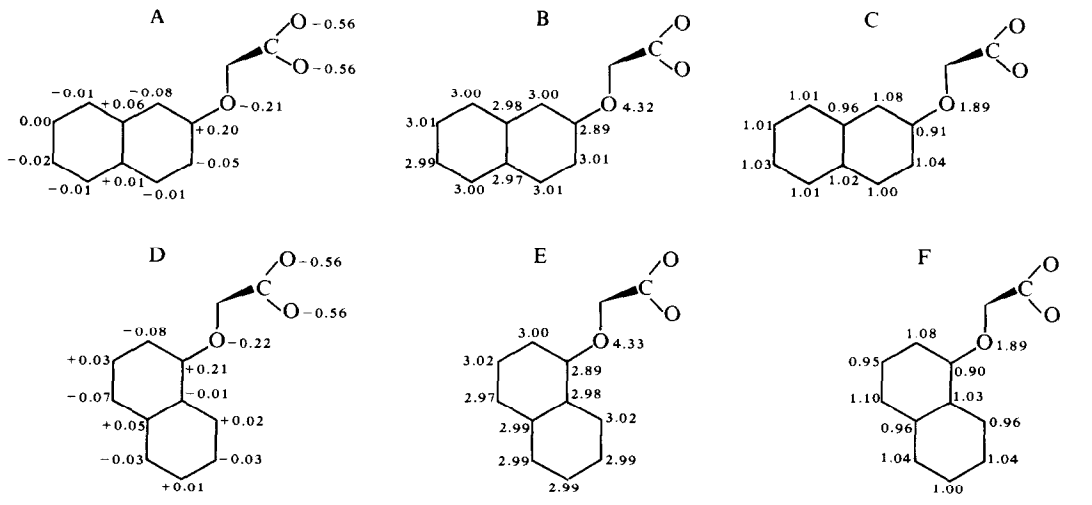

Fig. 4. Electron density distribution on the atoms of naphth-2-yloxyacetate (A, B, C) and naphth-1-yloxyacetate (D, E, F). Total net atomic charge (A, D), σ electron density (B, E) and π electron density (C, F) are shown.

now seen to be unlikely that the magnitude of a positive charge at one specific site [5,25] is important in this interaction, the electron density distribution on the nucleus [6] should not be disregarded when considering auxin structure/activity relationships. It is considered that auxin action depends upon a critical pattern of electron distribution on the nucleus of active auxins which matches appropriate areas on the receptor molecule (Farrimond, Elliott and Clack, in preparation).

EXPERIMENTAL

The calculation techniques have been described previously [9].

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