Phytochemistry, 1980, Vol. 19, pp. 367–371. © Pergamon Press Ltd. Printed in England. 0031-9422/80/0301-0367 $02.00/0

AUXIN STRUCTURE/ACTIVITY RELATIONSHIPS: BENZOIC ACIDS AND PHENOLS

JENNIFER A. FARRIMOND*, MALCOLM C. ELLIOTT* and DENIS W. CLACK†

*School of Life Sciences, Leicester Polytechnic, Leicester, LE1 9BH; †School of Chemistry, University College, Cardiff, CF1 1XL, U.K.

(*Revised received* 13 *July* 1979)

Key Word Index—Auxins; structure/activity correlations; benzoic acids; phenols.

Abstract—The results of self-consistent field molecular orbital calculations on auxins of the benzoic acid and phenolic types are not inconsistent with the view that a positively charged site at about 0.5 nm from an acidic group could contribute to the reversible binding of an auxin to its receptor molecule.

INTRODUCTION

Zimmerman and Hitchcock reported in 1942 [1] that 2-bromo-3-nitrobenzoic acid promoted growth in auxin bioassays. Subsequently a wide range of benzoic acid derivatives were shown to have auxin activity [2–4]. More recently certain substituted phenols have been recognised as auxins [5, 6]. There are structural similarities between the phenolic and benzoic acid auxins. Thus, for auxin activity to be present in a benzoic acid derivative, at least one *ortho* position must be filled [2] while the active phenols are all di-*ortho*-substituted [6]. In both types of compound the introduction of a substituent in the 4 position leads to inactivity [2, 6, 7].

Veldstra [2, 3] considered that the introduction of *ortho* substituents in the benzoic acid series caused biological activation because of a steric effect on the conformation of the side chain resulting in the formation of a non-planar form of the molecule. Porter and Thimann [8, 9] concluded that the primary growth action exerted by auxins depends on the presence of a fractional positive charge on a planar lipophilic nucleus at *ca* 0.55 nm from the negative charge on an anionic group. Thimann [7, 9, 10] felt that the auxin activity of both the phenolic and benzoic acid auxins could be explained in terms of this theory although he noted [7] that the charge separation in the growth active phenols would be 0.43 nm and that in the benzoic acids 0.52 nm. In contrast Harper and Wain [6] suggested that the activity of the benzoic acid and phenolic auxins could be best explained by the modified 'two-point attachment' theory proposed by Cocordano *et al.* [11]. The latter workers reported molecular orbital calculations on several benzoic acids and concluded that the 4 and 5 positions in the growth-active molecules participate in a nucleophilic attachment reaction. Harper and Wain [6] suggested that the phenolic auxins react in a similar manner to the benzoic acids at the 4 and 5 positions. However, the type of calculation upon which the proposals of Cocordano and co-workers were based considered only pi electrons and has now largely been superseded by more sophisticated self-consistent field techniques which also consider all valence electrons.

We have previously [12–14] reported the use of self-consistent field molecular orbital (SCFMO) calculations to provide a quantitative assessment of conformation and electron density distribution, features which have figured prominently in theories of auxin structure/activity relationships [4, 8, 9, 15–22, 29]. Our SCFMO calculations did not support certain details of the Porter and Thimann [9] theory. In fact for both the natural auxin IAA and for 2,4-dichlorophenoxacetic acid the site regarded as carrying a positive charge proved to have a net negative charge. However, one unifying feature of the heterogeneous group of auxins studied [12, 13] was the presence of a fractional net positive charge at a position 0.5 nm from the carboxyl oxygens when the side chain is in the thermodynamically-favoured perpendicular conformation. In the case of the bicyclic auxins the positive site was located at a bridge carbon atom between the two rings. This precludes the idea that a nucleophilic substitution reaction at a positively-charged site on the nucleus [6, 11, 21] is a feature of the primary action of all auxin molecules [13]. It is considered, however, that the positively charged site could contribute to the reversible binding of an auxin to its receptor molecule [13].

The present paper describes the application of SCFMO calculation techniques to the benzioc acids and phenols, two classes of auxin molecule whose characteristics have had a considerable impact upon theories of auxin structure/activity relationships. The results have permitted a critical assessment of previous conclusions concerning the structural basis of the auxin activity of these molecules to be made, and represent an important addition to the range of auxins considered in our previous publications on this subject [12, 13].

RESULTS AND DISCUSSION

Molecular orbital calculations were carried out for 2,6-dichlorobenzoate which has slight to very high auxin activity depending on the test employed [4] and for 2,4-dichlorobenzoate which has no auxin activity [3]. For 2,6-dichlorobenzoate the conformation in which the carboxyl group was perpendicular to the ring plane was more stable than the planar conformation by 0.01 atomic units (26×10^3 J/mol). This substantiates Veldstra's conclusion [2, 3] that a non-planar conformation of the molecule is adopted by di-*ortho*-substituted benzoic acid derivatives. Calculations on the conformation of 2,4-dichlorobenzoate predicted that again the perpendicular conformation was more stable than the planar conformation, in this case by 0.006 atomic units (16×10^3 J/mol). This effect of a single *ortho* substitution in decreasing the stability of the conformation in which the oxygens are in the planar, resonance position is due to the close proximity of the carboxyl group to the *ortho* ring substituents. Clearly the inactivity of the 2,4-dichlorobenzoate cannot be explained by the existence of an energy barrier preventing the adoption of a conformation in which the side chain is perpendicular to the ring. Nevertheless the importance of the position of the second chlorine substitution in determining auxin activity in compounds of this type is highlighted by the results of assessments of relative activity in the pea curvature test [8] which indicate that 2-chlorobenzoate has 0.1% activity relative to IAA and 2,3-dichlorobenzoate has 1% activity whereas 2,5-dichlorobenzoate has 16% activity (activity in this test was calculated by dividing the concentration of IAA required to reduce outward curvature by 100°, by the concentration of the compound required to produce the same effect and multiplying the fraction obtained by 100).

According to the Thimann and Porter [8, 9] theory of auxin structure/activity relationships charge separation is crucial. For the benzoic acid series it was proposed that C_4 was the site of the fractional net positive charge [8]. In order to conform with the theory, the growth active benzoic acid derivatives should bear a fractional positive charge at this position which should be 0.55 nm from the carboxyl oxygens. The total net charge distribution on the atoms of 2,6-dichlorobenzoate and 2,4-dichlorobenzoate, together with a breakdown of sigma electron density and pi electron density, is shown in Fig. 1. In the case of unsubstituted, dissociated benzoic acid the carboxyl group oxygens are free to lie in the plane of the ring with resultant resonance stabilisation and pi electrons would be expected to be withdrawn from the C_4 (*para*) position resulting in a positive charge on this atom. The *ortho* substitutions which confer auxin activity lead to a favoured conformation in which the oxygens lie out of the ring plane, and hence this pi electron withdrawal effect would be expected to diminish. The molecular orbital calculations predict that C_4 would bear a negative charge in 2,6-dichlorobenzoate, the C_4 position being 0.502 nm from the carboxyl oxygens. The negative charge is due to an excess of pi electron density on C_4 (-0.06), although C_4 is slightly deficient in sigma electrons ($+0.04$). The C_4 position on 2,4-dichlorobenzoate bears a net positive charge of $+0.05$ units, as would be expected from the proximity of the electronegative chlorine which withdraws sigma electrons from C_4. Clearly the results of the SCFMO calculations do not support the Thimann and Porter theory with regard to the site of the fractional positive charge on the nucleus of auxins of the benzoic acid series [7]. Neither do they confirm the results of Cocordano *et al.* [11], which were based upon calculations of a modified free valence index using an extended Hückel approximation, and led them to deduce that carbon atoms 4 and 5 are involved in simultaneous nucleophilic fixation reactions [6]. The calculations do predict, however, that a positive charge exists at the equivalent carbons 3 and 5 in 2,6-dichlorobenzoate. These atoms are located 0.45 nm from the carboxyl oxygens as compared with a separation of *ca* 0.5 nm noted between the carboxyl oxygens and a net positive site (determined by SCFMO calculations) in other auxin series [12, 13]. However, it is noteworthy that net positive sites are also located at the 3 and 5 positions of 2,4-dichlorobenzoate so the inactivity of this molecule cannot be explained in terms of the modified charge separation hypothesis [13]. The fact that the substitution of an atom larger than fluorine at the C_4 position of benzoic acid derivatives is incompatible with auxin

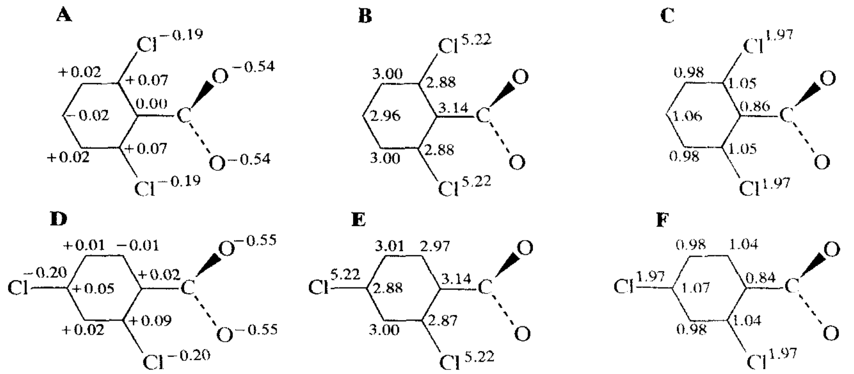

Fig. 1. Electron density distribution on the atoms of 2,6-dichlorobenzoate (A, B, C) and 2,4-dichlorobenzoate (D, E, F) molecules in the conformation with the carboxyl group perpendicular to the ring plane, total net charge (A, D), sigma electron density (B, E) and pi electron density (C, F) are shown. N.B.: for the carboxyl group perpendicular to the ring plane one cannot distinguish sigma and pi orbitals (and electron densities).

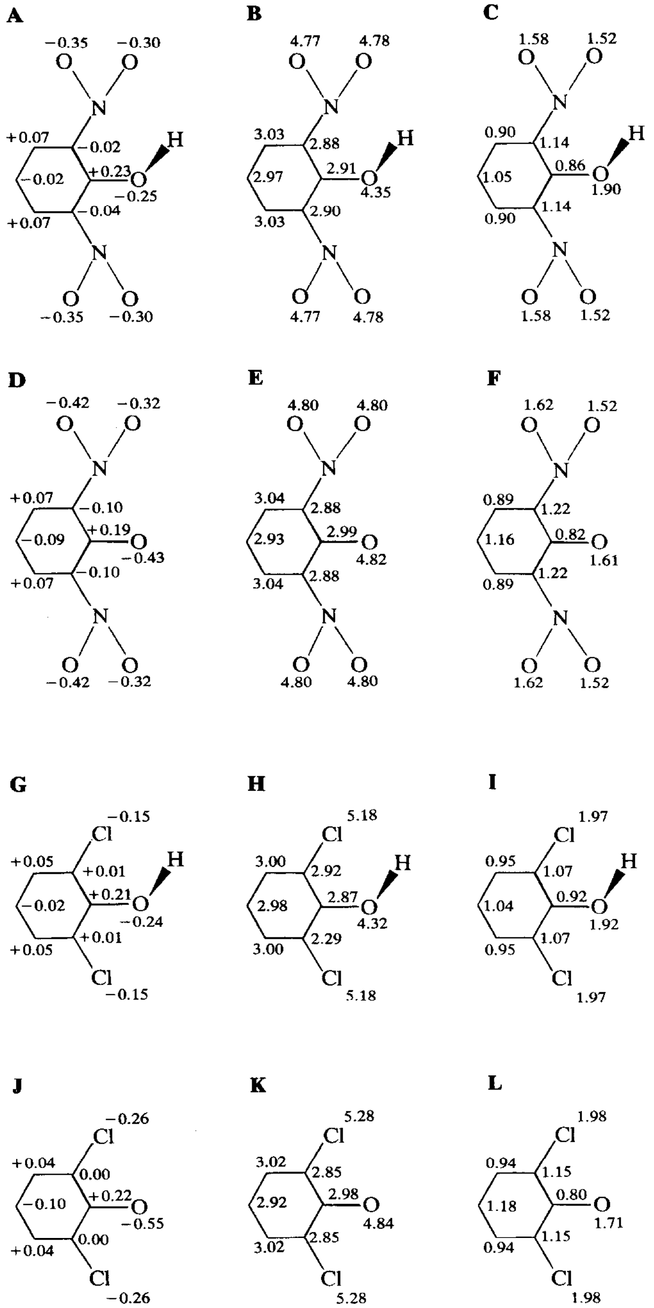

Fig. 2. Electron density distribution on the atoms of 2,6-dinitrophenol (A, B, C), 2,6-dinitrophenate (D, E, F), 2,6-dichlorophenol (G, H, I) and 2,6-dichlorophenate (J, K, L), total net charge (A, D, G, J), sigma electron density (B, E, H, K) and pi electron density (C, F, I, L) are shown.

activity [3] suggests that the auxin binding site may be such that unfavourable electronic or steric interactions, which prevent the effective binding of the auxin, may result when the C_4 position bears a substituent. Similar views have been expressed by Kaethner [20] and Katekar [29].

The activity of the phenolic auxins has been demonstrated by Harper and Wain [6] in the pea segment, pea curvature and tomato leaf epinasty tests. They are all di-*ortho*-substituted with chlorine, bromine, iodine or nitro moieties, all of which are electron-withdrawing substituents. Compounds containing at least one nitro substituent were found to be most active and Harper and Wain [6] noted that some of the nitrophenols studied were as active as 2,4-D in the pea tests. Thimann [7] reported that the activity of the di-*ortho* substituted phenols on pea stem segments averaged between 1 and 10% of that of IAA. The effect of electron-withdrawing substituents, especially in positions *ortho* and *para* to the hydroxyl group, is to increase the ionisation of phenols due to the stabilisation of the negative charge by both inductive and mesomeric effects. Harper and Wain [6] have shown that the growth regulating activity of disubstituted phenols increases with the acidity of the hydroxyl group. They also suggested that the possibility of intramolecular hydrogen bonding between the hydroxy hydrogen and an *ortho* nitro, chlorine or iodine group could explain the high activity of these compounds compared with di-cyano-substituted derivatives. However, auxin activity seems unlikely to be related to both the acidity of the hydroxyl group and its ability to hydrogen bond, since these two effects are mutually exclusive.

SCFMO calculations were carried out for 2,6-dinitrophenol and 2,6-dichlorophenol, in both the undissociated and dissociated forms; all showed a similar pattern of atomic charge distribution. The total net atomic charge distribution together with a breakdown of sigma electron density and pi electron density for the four structures are shown in Fig. 2. The calculations predict that in 2,6-dinitrophenol, 2,6-dinitrophenate, 2,6-dichlorophenol and 2,6-dichlorophenate C_4 is negatively charged due to an excess of pi electrons. The increased pi electron density in the ring due to the ionisation of the phenol results in the pi electron density on C_4 increasing from 1.05 in the undissociated 2,6-dinitrophenol to 1.16 in 2,6-dinitrophenate and from 1.04 in the undissociated 2,6-dichlorophenol to 1.18 in 2,6-dichlorophenate. These results concur with Harper and Wain's [6] prediction and are contrary to Thimann's [7] assumption that C_4 would be positively charged. Carbon atoms 3 and 5 are, however, deficient in pi electrons and the total net charge on each of these atoms is +0.07 in both 2,6-dinitrophenol and 2,6-dinitrophenate, +0.05 in 2,6-dichlorophenol and +0.04 in 2,6-dichlorophenate. These positive sites are at 0.367 nm from the phenolic oxygen, substantially less than the 0.45 nm noted above for the benzoic acids and 0.5 nm reported for various other auxins [12, 13].

The net charge on the oxygen atoms of the undissociated and anionic forms of 2,6-dinitrophenol are shown in Fig. 2. In each case the oxygen atoms of the nitro group bear substantial negative charges and in fact the charge on each oxygen of the nitro group of undissociated 2,6-dinitrophenol is greater than the negative charge on the oxygen of the hydroxyl group, although with a pK_1 of 4.47 the dissociated form would be expected to predominate at physiological pH's; whether dissociated or undissociated, the charge differences between the phenolic oxygen and the oxygen atoms of the nitro substituents are small. In the majority of instances, active auxins have had a terminal carboxyl group or a group easily converted into one [30]. However other acidic groups may impart activity and naphthyl-nitromethane, with a terminal nitro group, has auxin activity [3, 17]. *A priori* it seems that a nitro group (in which the oxygen atoms bear considerable delocalised electron density) could satisfy the requirements for binding at an auxin receptor despite competition from a phenolic oxygen if the charge separation distances on auxin and receptor match better. The fact that di-*ortho*-substituted phenols containing one *ortho* nitro group have relatively high activity [6] might therefore be explained by the fact that the nitro group could mimic the action of a carboxylic acid group at the auxin receptor site. In other words it is postulated that the greater auxin activity of the di-*ortho*-substituted phenols containing one *ortho* nitro group might be explained by the fact that the nitro group could act as the anion-like site in these molecules. The distance from the nitro group on C_2 to the positive charge on C_5 is 0.492 nm. This figure compares well with the 0.5 nm charge separation which was noted as a feature of other auxin series [12, 13] and in addition this conception would be in line with Veldstra's [2, 3] conclusion that auxins must have an anionic site capable of projecting out of the ring plane. The presence of a nitro group and a hydroxyl group in adjacent positions on the ring results in an area of electron density on the molecule which could be important to its auxin activity. If hydrogen bonding between one nitro group and the hydroxyl group were to occur [6], an electron-rich pseudo-ring would be formed and the resulting structure would bear certain stereochemical similarities to 1-naphthoic acid, which has auxin activity [23].

The auxin activity of 2,6-dichlorophenol is more difficult to explain in terms of structural similarities with other auxins since it does not contain an anion-like group* capable of projection out of the plane of the ring, the fact that dichlorine-substituted phenols are less active than nitrosubstituted derivatives [6] may be a reflection of the inherent planarity of the former molecules. In view of the weak acidity of 2,6-dichlorophenol (pK_a 7.11), the undissociated molecule would be expected to occur at physiological pH's. By analogy with the 2,6-dinitrophenol discussed earlier it is conceivable that a chlorine atom could function as the anion-like site in the molecule. The receptor site preference postulated for the 2,6-dinitrophenol could apply in this case also. The distance between an *ortho* chlorine atom and the positive charge on the ring position *para* to it is 0.476 nm. Jones and Watkinson [24] have reported that intramolecular hydrogen bonding can occur between an undissociated phenol and an *ortho* chlorine substituent. It is possible, therefore, that one *ortho* chlorine atom could function as the anion-like site on the molecule and the other chlorine atom engage in hydrogen bonding with the adjacent hydroxyl group as suggested by Harper and Wain [6].

The results of these calculations on benzoic acid and phenol derivatives are not inconsistent with the view that a positively charged site at *ca* 0.5 nm from an acidic group could contribute to the reversible binding of an auxin to its receptor molecule [12, 13] although the idea of an unfavourable interaction with the receptor site [3, 20, 29] would need to be invoked to explain the absence of auxin activity in 4-substituted benzoic acid and phenol derivatives [2, 6, 7].

EXPERIMENTAL

Calculations were carried out on an ICL 4-470 computer using a Fortran programme which is an extension of the CNDO 2 programme of Pople and Segal [25–28]. Atomic numbers, number of electrons and atomic co-ordinates are required as input data, the latter being derived from published X-ray crystallography analyses. A self-consistent

---

* The term anion-like is used throughout this paper to indicate an atom or group of atoms bearing a substantial fractional negative charge.

molecular wave function is derived from the computations. From this wave function a number of sub-molecular characteristics including orbital occupancies and hence atomic charges can be derived. The calculations may also be used to predict probable optimum geometric configurations of the molecules studied, by considering the total energy of various different conformations.

REFERENCES

1. Zimmerman, P. W. and Hitchcock, A. E. (1942) *Contrib. Boyce Thompson Inst.* **12,** 321.
2. Veldstra, H. (1952) *Rec. Trav. Chim.* **71,** 15.
3. Veldstra, H. (1956) *The Chemistry and Mode of Action of Plant Growth Substances* (Wain, R. L. and Wightman, F., eds.) pp. 117–133. Butterworth, London.
4. Jönsson, A. (1961) *Encyclopedia of Plant Physiology* (Ruhland, W., ed.) Vol. 14, pp. 959–1006. Springer, Berlin.
5. Wain, R. L. and Taylor, H. F. (1965) *Nature* (*London*) **207,** 167.
6. Harper, D. B. and Wain, R. L. (1969) *Ann. Appl. Biol.* **64,** 395.
7. Thimann, K. V. (1972) *Proc. Adv. Study Inst. Izmir* 1971 (Kaldeway, H. and Vardar, Y., eds.) pp. 155–170. Verlag Chemie, Weinheim.
8. Porter, W. L. (1962) Ph.D. Thesis, Harvard University, Cambridge, Ma, U.S.A.
9. Thimann K. V. (1963) *Annu. Rev. Plant. Physiol.* **14,** 1.
10. Thimann, K. V. (1969) *Physiology of Plant Growth and Development* (Wilkins, M. B., ed.). McGraw Hill, London.
11. Cocordano, M., Ricard, J. and Julg, A. (1966) *J. Theor. Biol.* **12,** 291.
12. Elliott, M. C., Farrimond, J. A., Hall, J. F. and Clack, D. W. (1977) *Plant Growth Regulators* (Kudrev, T., Ivanova, I. and Karanov, E., eds.)pp. 459–463. Publishing House of the Bulgarian Academy of Sciences.
13. Farrimond, J. A., Elliott, M. C. and Clack, D. W. (1978) *Nature* **274,** 401.
14. Farrimond, J. A. (1978) Ph.D. Thesis, Leicester Polytechnic.
15. Koepfli, J. B., Thimann, K. V. and Went, F. W. (1938) *J. Biol. Chem.* **122,** 763.
16. Veldstra, H. (1944a) *Enzymologia* **11,** 97.
17. Veldstra, H. (1944b) *Enzymologia* **11,** 137.
18. Veldstra, H. (1953) *Annu. Rev. Plant Physiol.* **4,** 151.
19. Jonsson, A. (1955) *Sven. Kem. Tidskr.* **67,** 166.
20. Kaethner, T. M. (1977) *Nature* **267,** 19.
21. Hansch, C. and Muir, R. M. (1950) *Plant Physiol.* **25,** 389.
22. Thimann, K. V. (1952) *Plant Physiol.* **27,** 392.
23. Veldstra, H. and van der Westeringh, C. (1951) *Rec. Trav. Chim. Pays-Bas* **70,** 1113.
24. Jones, D. A. K. and Watkinson, J. G. (1974) *J. Chem. Soc.* 2371.
25. Pople, J. A. and Segal, G. A. (1965) *J. Chem. Phys.* **43,** 5136.
26. Pople, J. A., Santry, D. P. and Segal, G. A. (1965) *J. Chem. Phys.* **43,** 5129.
27. Pople, J. A. and Segal, G. A. (1966) *J. Chem. Phys.* **44,** 3289.
28. Pople, J. A. and Beveridge, D. L. (1970) *Approximate Molecular Orbital Theory.* McGraw-Hill, New York.
29. Katekar, G. F. (1979) *Phytochemistry* **18,** 223.
30. Audus, L. J. (1972) *Plant Growth Substances.* Leonard Hill, London.