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MOLECULAR REQUIREMENTS FOR AUXIN ACTION—I.
HALOGENATED INDOLES AND INDOLEACETIC ACID

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Abstract—In order to obtain evidence for or against the concept that the biological activity of the indole auxins depends upon the fractional positive charge of the —NH group and its distance from the carboxyl group, two new indole compounds and their methyl and ethyl esters have been prepared. In these the intensity of the charge on the —NH would be predicted to be increased, while the auxin activity according to other theories should be decreased. Of these compounds, 2-chloro-indole-3-acetic acid has about 350 per cent of the activity of indoleacetic acid; the 2-bromo acid has 160 per cent activity, and the activities of the esters are even higher. Thus the activities of the 2-halogenated derivatives contrast strongly with that of the 2-methyl derivative, which has only 12 per cent of the activity of indoleacetic acid.

In a larger series of indole compounds with substituents on the benzene ring, the frequency of the i.r. solvent-bonded —NH stretching band in acetonitrile has been found to vary linearly with the Hammett sigma value for the substituent. The 2-halogenated derivatives agree perfectly with this behavior. Since σ measures the change in the tendency of an anion or proton to associate reversibly when a substituent is introduced, it is deduced that the —NH stretch frequency is a measure of the relative positive charge on the —NH group (other factors being equal). In standard auxin tests with the same compounds, using curvature of slit pea stems, it is shown that the log of the growth activity is also a roughly linear function of the solvent-bonded —NH stretch frequency and hence of σ, providing that gross steric effects and lipophilic properties of the compounds are not too different. The data are therefore all consistent with the theory stated above, and it is suggested that analogous considerations may hold for the biological activity of the indolealkylamines of the tryptamine and serotonin series.

INTRODUCTION

CONSIDERATION of the structure of a large number of synthetic auxins has led to the suggestion that the primary growth action exerted by indole auxins depends, among other requirements, upon the presence of a fractional positive charge on the —NH group at a favorable distance from the carboxyl.[1,2] The evidence for this conclusion is derived from a large number of facts. Prominent among these are the low activities, relative to that of indole-3-acetic acid (IAA,‡ taken as 100 per cent), of indole-2-acetic acid (3 per cent) and indole-4-acetic acid (2 per cent);[3] in these compounds the distances between the —NH and the carboxyl group are decreased and increased respectively. Similar though less striking disparities between the activities of the 3- and 2-isomers have been observed for the analogous benzofuran- and benzothiopheneacetic acids.[4] There are comparable effects also in a quite different type of

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‡ Abbreviations used in this paper: IAA, indole-3-acetic acid; 2,4-D, 2,4-dichlorophenoxyacetic acid. Unless otherwise defined, auxin activities are expressed as percentage of that of IAA, using the curvature of slit etiolated Alaska pea stems, measured as described in Ref. 30.

[1] W. L. PORTER and K. V. THIMANN, *Plant Physiol.* 36, suppl., xxxix (1961); *Proc. IXth Int. Botan. Congr.* II, 305 (1959).

[2] K. V. THIMANN, in *Plant Growth Regulation*, p. 444, Iowa State University Press, Ames, Iowa (1961).

[3] K. V. THIMANN, *Plant Physiol.* 33, 311 (1958).

[4] F. W. WENT and K. V. THIMANN, *Phytohormones*, p. 137, Macmillan Co., New York (1937); E. M. CROOK, W. DAVIES and N. E. SMITH, *Nature* 139, 154 (1937).

biological activity of indole derivatives, namely, the pharmacological action of the isomers of the indolealkylamines, serotonin and tryptamine.[5]

In respect of the auxins, supporting evidence is given by the total inactivity for indole-3-carboxylic acid and the low activity in most tests of indole-3-propionic acid:[6] these again indicate the importance of the distance between the carboxyl group and some nuclear moiety. Even more pronounced differences of the same type occur as a function of side-chain length in the indolealkylamines.[5]

A major source of evidence for the critical role of a fractional positive charge, and for its location, derives from the effects of ring substitution, especially chlorination, on the auxin activity of several quite different series of compounds. These effects are highly dependent on position; for example, in the phenoxyacetic acid series, the 3,5-dichloro acid is inactive in all tests, the 2,6-dichloro acid has low activity in some tests but not in all, while the 2,4-dichloro acid has about the highest activity of any auxin known. Such great sensitivity to position can most readily be interpreted in terms of the induction of a fractional positive charge at a position a favorable distance away from the carboxyl group.

Most of these comparisons have been made with the relatively simple members of several aromatic series, and little systematic consideration has been given to the indole auxins, which biologically are the most important. The present paper therefore extends the study of the effects of chlorination to the indole auxins.

IAA may be chlorinated at any ring position except the —NH group to give an auxin having greater activity than the parent. Some of the evidence for this statement will be supplied below. Methylation, on the other hand, is relatively ineffective in increasing auxin activity, and our tests show that methylation of the —NH group of IAA reduces the activity to 13 per cent of that of IAA. Since similar methylation drastically reduces the activity of the indolealkylamines, it may be deduced that the parameters of the —NH group play a critical role in both these types of biological activity. Of course the —NH group is not required for auxin activity, as is evident from the high activity of purely benzenoid compounds like naphthalene-1-acetic acid, 2,4-D, 2,3,6-trichlorobenzoic acid, etc. Rather, the —NH group appears to have some property in common with certain —CH groups of aromatic rings (or even with the oxygen atom of benzofurans), which controls biological activity and whose power is a function of the distance between it and the carboxyl group. This property is believed to be the possession of a fractional positive charge. The existence of such charges is now well recognized,[7–9] and its value in indole is agreed as being approximately 25 per cent of a full charge. The —NH group of indole is similar in this respect as well as in planarity to those of amides and urethanes. In the present work an attempt has been made to determine the charge on the NH group and to correlate it with biological activity. This has been done by careful determinations of the exact wavelength of maximum absorption of the solvent-bonded —NH stretching vibration in the i.r. at around 2·9 μ.

It was shown some years ago that 2-halogenation of lysergic acid diethylamide, which contains the tryptamine skeleton, markedly increases its pharmacological activity as a serotonin antagonist.[10] Such halogenation should have a pronounced effect on the net

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[5] V. Erspamer, *Prog. Drug Research* 3, 161 (1961).

[6] K. V. Thimann and J. Bonner, *Physiol. Rev.* **18**, 524 (1938).

[7] A. Albert, *Heterocyclic Chemistry*, p. 134, Essential Books, Fairlawn, N.J. (1959).

[8] C. K. Ingold, *Structure and Mechanism in Organic Chemistry*, p. 175, Cornell Univ. Press, Ithaca (1953).

[9] A. R. Katritzky and J. M. Lagowski, *Heterocyclic Chemistry*, p. 159, Methuen, London (1960).

[10] F. Troxler and A. Hoffmann, *Helv. Chim. Acta* **40**, 2160 (1957).

charge of the —NH, and in the pyridine series, indeed, this effect has been measured; the pK_a of pyridine is 5·15, of 2-chloropyridine 0·7.[11] It was deduced that increasing the charge on the —NH of IAA might correspondingly increase its growth-promoting activity. Experiments were therefore directed towards halogenating IAA in the 2-position.

Recently, N-bromophthalimide and N-bromosuccinimide have been used to halogenate both skatole and indole-3-propionic acid in the 2-position, though with low yields for the latter,[12, 13] and the 2-bromoindolepropionic acid was found extremely sensitive to acid hydrolysis, yielding oxindoles. So far as they have been studied, oxindole derivatives are devoid of auxin activity. It was concluded that halogenation of both the ethyl and methyl esters of IAA in dry CCl_4, and avoidance of exposure to acid, would probably improve the yield. This was found to be the case, the corresponding 2-chloro- and 2-bromo- esters being obtained in 20–50 per cent yield after chromatography. Alkaline hydrolysis of either ester, rapid precipitation at pH 4 with 0·1 N HCl, and extraction at room temperature yielded the corresponding acids. Although the 2-halogeno group is, as expected, somewhat acid-labile, yielding in moist air compounds with an oxindole-like u.v. spectrum, yet in dilute aqueous solutions buffered at pH 5·5, i.e. under plant growth conditions, no u.v. spectral change could be detected after 10 days. Our tests require 24 hr.

The results reported below show that 2-halogenation greatly enhances the growth activity of IAA on the curvature of slit pea stems, 2-chloroindoleacetic acid having 350 per cent and 2-bromoindoleacetic acid 160 per cent of the activity of the parent compound. The esters are even more active. These are the first 2-substituted indoleacetic acids to have greater activity than the parent. In contrast, the 2-methyl compound has only 12 per cent of the activity of IAA, although the 2-chloro- and 2-methyl-derivatives must be nearly isosteric and about equally lipophilic (cf. e.g. Ref. 14).

Furthermore, since Hoffman, Fox and Bullock[15] have prepared a series of benzene-ring substituted derivatives of IAA, including halogen and methyl group substituents, some of which have greater activity than the parent compound, the characteristics and growth activity of these acids have been compared with those of the six 2-substituted substances prepared as described above.

RESULTS AND DISCUSSION

(*A*) *Infrared Spectra of 2-Halogenated Indoleacetic Acids and Esters*

These derivatives are quite unstable to hydrolysis, even in the dark under dry air or nitrogen, the resulting compounds showing an oxindole spectrum in the u.v.[12, 13] Since oxindole-3-acetic acid is quite inactive in the pea-stem curvature test any such hydrolysis would only diminish the apparent activity. However, in dilute solution, buffered at pH 5·5, the 2-halogenated esters and acids are stable under the conditions of growth tests, as revealed by identical u.v. and i.r. spectra before and 10 days after growth.

Comparison of Figs. 1A and 1B with Fig. 1C shows that 2-halogenation produces clearly detectable changes. The halogenated esters have a free —NH stretch band in CS_2 at 2·82 μ, and an intermolecular H-bonded (presumably carbonyl) —NH stretch band at 2·92 μ, while

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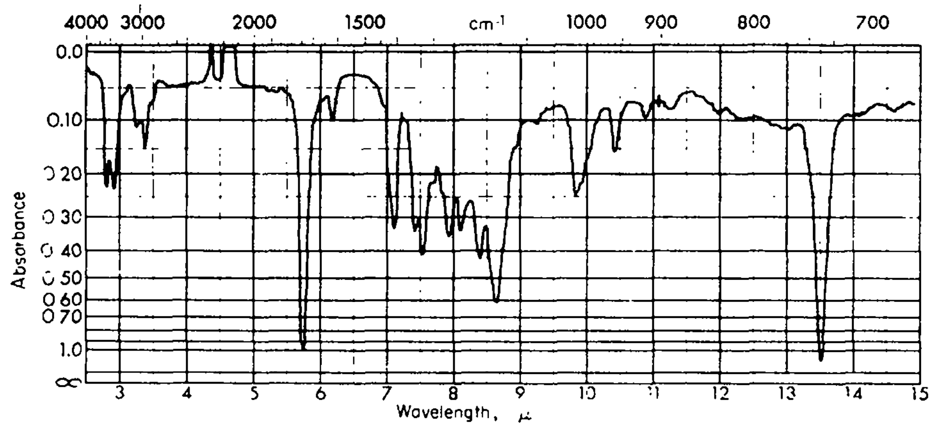
[11] E. H. Rodd, *Chemistry of Carbon Compounds*, IVA, p. 533, Elsevier, New York (1956).

[12] W. G. Lawson, A. Patchornik and B. Witkop, *J. Am. Chem. Soc.* **82**, 5919 (1960).

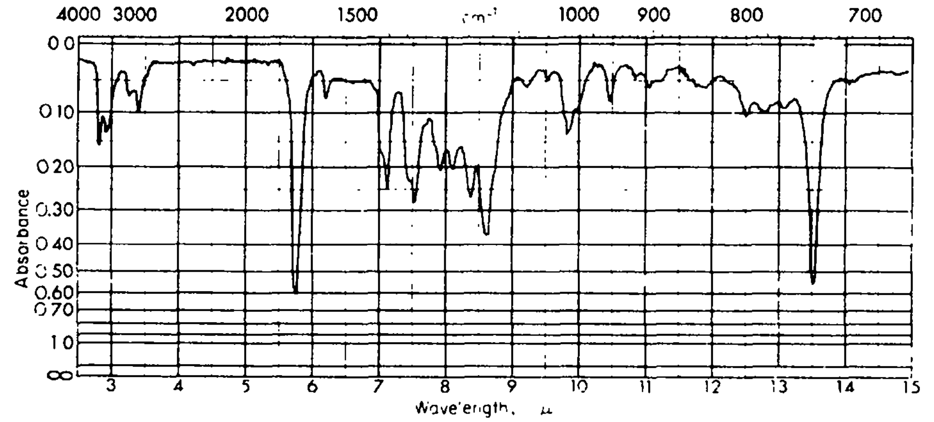
[13] R. L. Hinman, Private communication, Dec. 1960.

[14] C. Hansch, R. M. Muir, T. Fujita, P. Maloney, F. Geiger and M. Streich, *J. Am. Chem. Soc.* **85**, 2817 (1963).

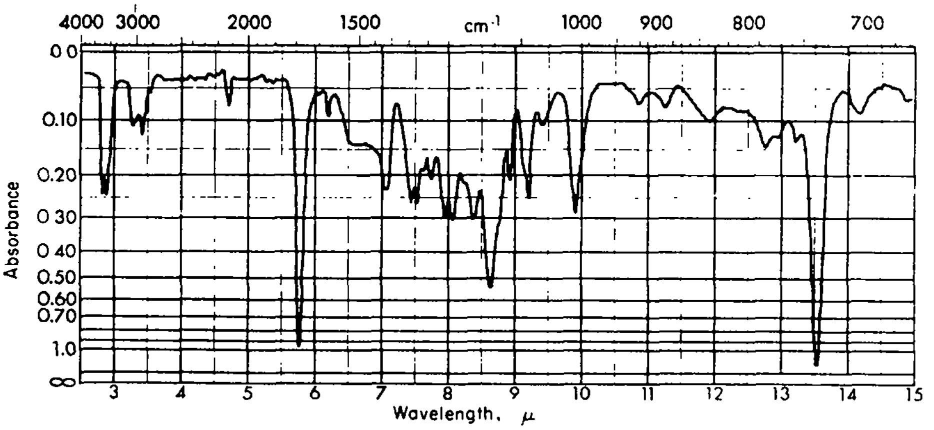
[15] O. L. Hoffman, S. W. Fox and M. W. Bullock, *J. Biol. Chem.* **196**, 437 (1952).



1A

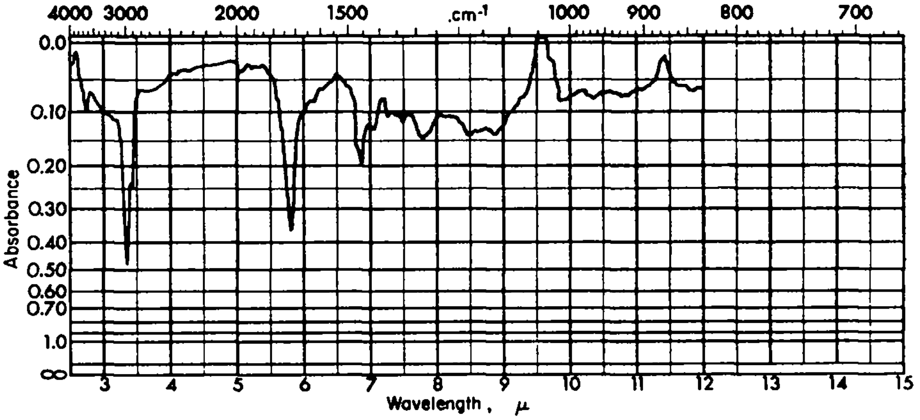


1B



1C

FIG. 1(A). INFRARED SPECTRUM OF METHYL 3-(2-CHLOROINDOLE) ACETATE (IN CS_2). (B) INFRARED SPECTRUM OF METHYL 3-(2-BROMOINDOLE) ACETATE (IN CS_2). (C) INFRARED SPECTRUM OF METHYL-3-INDOLYLACETATE (IN CS_2). (D) INFRARED SPECTRUM OF 2-CHLOROINDOLEACETIC ACID (IN $CHCl_3$).



1D

the free —NH stretch band in the unchlorinated ester (Fig. 1C) is at 2·81 μ, and the bonded at 2·88 μ. The most diagnostic change in the i.r. was in the 12–14 μ region. Here, the unchlorinated ester has a band at 13·22 μ for the —CH out-of-plane bending of the 2-proton and another at 13·55 μ for the benzene protons. Similarly, skatole shows two out-of-plane bending bands at 13·21 and 13·57 μ, while 2,3-dimethylindole has only one band at 13·61 μ. The 2-chlorinated ester has the benzene band unchanged, indicating no benzene ring substitution, but the 2-proton band is missing. This is clear evidence for the substitution of the 2-proton (cf. Ref. 16). It is notable that 2-halogenated derivatives of LSD prepared by the same procedure[12] give no Ehrlich reaction, which is specific for an unsubstituted 2-position.

(*B*) *The i.r. Frequency of the —NH Stretch Band*

The effect of 2-halogenation in clearly lowering the —NH stretch frequency of indole was studied in all the compounds, using a specially devised low scanning speed technique which permitted determination of the absorption peaks with great precision (see Experimental; cf. Refs. 17–24). It was found that the free —NH stretch wavelength in CCl_4 at high dilution (0·001 M), namely 2·866 μ, is readily shifted to longer wavelengths in the bonding solvent acetonitrile. Such shifts may occur in concentrated CCl_4 solutions due to association between the —NH and either the π-electrons in indole or the carbonyl group of indole-3-acetic acid or ester, but their occurrence here in dilute solutions in acetonitrile is due to bonding to the lone pair-donating solvent. The shift is pronouncedly greater, the band more intense and its shape different for the acetonitrile bonding, although the H-bonded band is relatively narrow and quite sharp at the tip. The extent of intermolecular H-bonding is dependent on concentration and temperature, and at appropriate intermediate concentrations in CCl_4 both free and bonded bands could be observed, changing in relative intensity with change of

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[16] E. Leete and L. Marion, *Can. J. Chem.* **31**, 778 (1953).

[17] D. G. O'Sullivan and P. W. Sadler, *J. Chem. Soc.* **876** (1959).

[18] L. W. Reeves, E. A. Allan and K. O. Strømme, *Can. J. Chem.* **38**, 1249 (1960).

[19] E. A. Allan and L. W. Reeves, *J. Phys. Chem.* **66**, 613 (1962).

[20] C. L. Ingraham, J. Corse, G. F. Bailey and F. Stitt, *J. Am. Chem. Soc.* **74**, 2297 (1952).

[21] N. Fuson, M. L. Josien, R. L. Powell and E. Utterback, *J. Chem. Phys.* **20**, 145 (1952).

[22] R. A. Russell and H. W. Thompson, *J. Chem. Soc.* **483** (1955).

[23] J. D. S. Goulden, *Spectrochim. Acta* **6**, 129 (1954).

[24] J. R. Merrill, *J. Phys. Chem.* **65**, 2023 (1961).

concentration. The free band was seldom seen in acetonitrile, however. Table 1 shows free, bonded and associated —NH stretch value for four compounds, determined as described above. In order to facilitate comparison with the literature, the values are given here in μ.

Indole has its free stretch line at the lowest wavelength of any secondary amine or amide.[25] This indicates, according to Richards,[26] very extensive sp_2 hybridization Superimposed on this is the effect of electron withdrawal by substituents or by H-bonding, which for indoles or amides lengthens the —NH bond and therefore increases the wavelength (i.e. lowers the frequency) for a given level of hybridization. Anilines, however, are less extensively hybridized at the nitrogen, as is shown by their known non-planarity, and the much reduced wavelength shift with H-bonding shown in Table 1. Comparable frequency changes in the —NH stretch as a result of substitution have been observed in this laboratory in the near i.r. The chemical shift in the nuclear magnetic resonance spectrum for the NH proton also shows similar changes as a result of substitution.

The bonded —NH line cannot be confused with that due to solvent-bonded —OH of the carboxylic acid (if such a bonded line exists), since in pairs of compounds like indole and indole-3-acetic acid the two —NH lines appear at similar (though not identical) wavelengths.

TABLE 1. FREE, ASSOCIATED AND BONDED —NH STRETCH WAVELENGTHS

Compound	Stretch wavelength μ			
	Free (CCl_4)	Associated (CCl_4)	Bonded (CH_3CN)	Difference due to solvent bonding (mμ)
Indole	2·866*		2·945	79
Methyl-3-indoleacetate	2·866	2·934	2·950	84
Acetanilide	2·902†		2·977	75
N-methylaniline	2·905		2·929	24

* Values for indole in the literature are 2·864[21] and 2·865.[22]

† Cf. 2·903 given in Ref. 22.

The typical broad carboxylic acid —OH stretch is almost exclusively found in the 3·1–4·0 μ region, corresponding to the dimer, while none of the wavelengths measured here was higher than 3·005 μ. Indeed, the bonded —NH frequencies measured in substituted indoles are very similar to those in analogously substituted IAA's, in which it is slightly shifted, by a nearly constant amount, to longer wavelengths.

As an example of the lack of ambiguity of the method, the solvent-bonded —NH stretch line measured for indole-3-acetaldehyde in acetonitrile was found to lie at about 2·994 μ, while the line due to intermolecular bonding between —NH and the carbonyl group of the aldehyde, either in alcohol-free chloroform or in the solid, appears as a very broad absorption from 3·2–3·5 μ.[17, 27] This partially zwitterionic compound does not show a typical indole u.v. spectrum and is relatively insoluble in ether, in contrast to benzaldehyde;[27] nevertheless it has about the same level of solvent-bonded i.r. stretch in acetonitrile as does 2-chloroindoleacetic ester.

The frequencies of the —NH stretch band for all the compounds tested are shown in

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[25] L. J. BELLAMY, *The Infrared Spectra of Complex Molecules*, John Wiley, New York (1958).

[26] R. E. RICHARDS, *Trans. Faraday Soc.* **44**, 40 (1948).

[27] R. T. ATCHESON, *An Introduction to the Study of Heterocyclic Compounds*, p. 134, Interscience, New York (1960).

Table 2. Frequency is expressed in wave numbers in column 2, and as change from the reference compound, indole or IAA, in columns 3 and 4. To obtain the total Hammett sigma constant where two substituents are present, their constants have been added (cf. Ref. 28). For substituents of unknown σ, predicted apparent or effective sigma constants have been derived from the —NH stretch frequency by use of Figs. 2 and 3. For substituents at position 3 on the indole ring, the known *para* σ constant was used, with the higher value for conjugating substituents.[29]

TABLE 2. AUXIN ACTIVITY, HAMMETT SIGMA CONSTANT,[29] AND INFRA-RED STRETCH FREQUENCY OF H-BONDED —NH GROUPS IN SUBSTITUTED IAA'S AND INDOLES

Compound	Frequency in wave numbers per cm in CH_3CN			Hammett sigma constant	Auxin activity as % of IAA	Log of auxin activity (relative to IAA = 1)
		$\Delta\nu$ from:				
		Indole	IAA			
Indole	3395·1	00·0	+16·5	0·00		
3-COOH-Indole	3325·6	−69·5		+0·73*		
3-CHO-Indole	3340·0	−55·1		+1·13*		
3-Aza-Indole	3343·4	−51·7		+0·81*		
7-Aza-Indole	3367·0	−28·1		+0·81		
3-CH_2COOH Indole (= IAA)	3378·6	−16·5	00·0	(+0·24)	100	0·00
2-CH_3-Indole	3388·7	− 6·4		(+0·10)		
5-CH_3-Indole	3403·7	+ 8·6		−0·17		
3-CH_3-Indole	3404·8	+ 9·7		−0·17*		
IAA	3378·6		00·0	0·00†	100	0·00
Methyl-3-Indoleacetate	3390·0	− 5·1	+11·4	(+0·07)	130	+0·11
Methyl 3-(2-chloroindole) acetate	3328·9				440	+0·64
Methyl 3-(2-bromoindole) acetate	3332·1				330	+0·52
Ethyl 3-(2-chloroindole) acetate	not determined				400	+0·60
Ethyl 3-(2-bromoindole) acetate	not determined				250	+0·40
2-Cl-IAA	3317·5‡		−60·7	(+0·90)	350	+0·54
2-Br-IAA	3321·2‡		−57·2	(+0·85)	160	+0·20
5,7-Di-Cl-IAA	3327·8		−50·6	+0·72§	15	−0·82
7-Cl-IAA	3341·1		−37·3	+0·49	200	+0·30
7-Aza-IAA	3350·1‖		−28·3	+0·81	100	0·00
2-CH_3-IAA	3371·6		− 6·8	(+0·07)	12	−0·92
4-Cl-IAA	3349·0		−29·6	+0·37	700	+0·85
6-Cl-IAA	3354·9		−23·7	+0·37	500	+0·70
5-Cl-IAA	3359·6		−19·0	+0·23	260	+0·42
5-F-IAA	3368·6		−10·0	+0·06	120	+0·10
5-CH_3-IAA	3384·3		+ 5·7	−0·17	60	−0·22
5-OH-IAA	3409·0		+30·4	−0·36	4	−1·40
N-CH_3-IAA	not expected				13	−0·89

( )—Sigma constant predicted from plot.

* Sigma for *para* position in benzene.

† Sigma constants for substituted IAA's are given with reference to unsubstituted IAA as zero, as in Fig. 3.

‡ Acid values derived from those of the esters.

§ Two sigma constants added.

‖ 7-Aza-IAN used for spectrum.

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[28] P. C. LAUTERBUR, *J. Am. Chem. Soc.* **83**, 1846 (1961).

[29] H. H. JAFFÉ, *Chem. Rev.* **53**, 191 (1953).

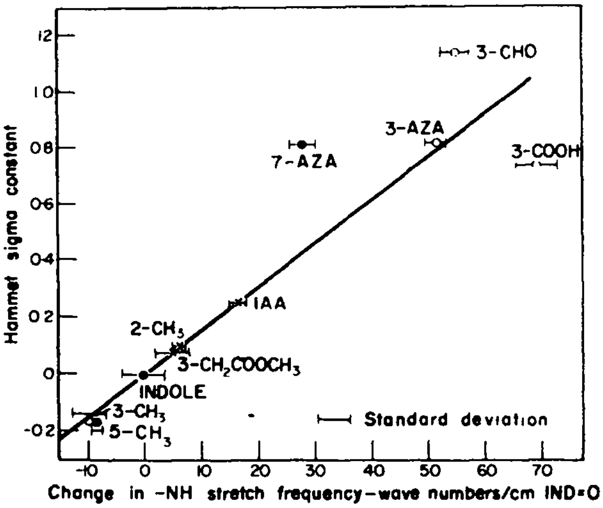

FIG. 2. RELATION OF H-BONDED I.R. STRETCH FREQUENCY TO HAMMETT SIGMA CONSTANT IN SUBSTITUTED INDOLES.

Bonding solvent—acetonitrile. 10 wave numbers = 0·155 sigma units, ●—Benzene ring substituent, · —3-pyrrole substituent—sigma value used is that for *para*-substituted anilines. × —sigma constant prediction from this plot.

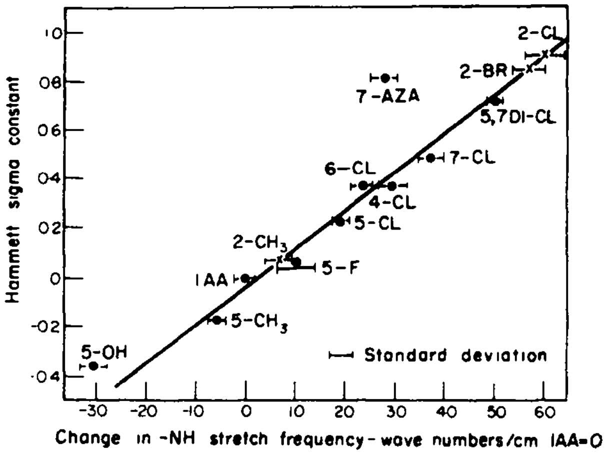

FIG. 3. RELATION OF H-BONDED I.R. STRETCH FREQUENCY TO HAMMETT SIGMA CONSTANT IN SUBSTITUTED INDOLE-3-ACETIC ACIDS.

Bonding solvent—acetonitrile. ●—Benzene ring substituent; constants added for disubstitution; ×—sigma constant prediction from this plot.

(*C*) *The —NH Stretch Band as a Measure of Charge on the Nitrogen Atom*

The net charge on a reaction center on a substituted aromatic ring is considered to be measured by the Hammett sigma constant for that substituent with respect to the reaction center. Thus, for a proton containing reaction center of relatively constant hybridization and mechanism of ionization, and for a constant donor of lone pairs or negative charges, the following should vary nearly linearly with net charge on the atom holding the proton, and hence with Hammett sigma constant for that substituent: (1) the free and bonded i.r. stretch frequencies; (2) the pK_a of the group holding the proton; (3) the tendency to hydrogen bonding as measured by other indices.

The extent to which the —NH stretching frequency correlates with the Hammett sigma constant for different substituents was therefore explored. A plot of stretch frequency versus sigma should theoretically be non-linear, because of the quadratic dependence of frequency on force constant,[26] but the range of the data is so small in relation to the magnitude of the frequencies that the deviation would be undetectable.

The relation between —NH stretch frequency and sigma is plotted graphically in Fig. 2 for substituted indoles and in Fig. 3 for substituted IAA's. Standard deviations are shown as a line for the frequencies, and compounds for which effective sigma constants were derived are shown as crosses, since they do not constitute a support of the correlation. The range of the data for substituted IAA's is about 70 cm^{-1} and that for substituted indoles about the same. This is at least twice the range of the data in other comparable studies. The precision is similar to that reported by other workers, even with fluoride optics.[20-22]

It is evident that the bonded stretch frequency correlates very well with sigma. Hence this frequency can be used, within limits, as a measure of the net charge on the —NH groups.

The evidence for very strong electron withdrawal by the 2-halogens is not unexpected, since it will be recalled that the pK_a of 2-chloropyridine is as low as 0·7. The shift may be enhanced by a contribution from the strong chlorine field effect. The acetic acid or ester side-chain is also electronegative in effect, with an apparent sigma for the acid of 0·24, i.e. its contribution is about equal to that of *para*-substitution with chlorine.

(*D*) *Halogenation, the —NH Stretch Frequency, and Auxin Activity*

All the compounds were tested at several dilutions for auxin activity using the standard pea curvature test.[30, 31]

Four examples of the determination of biological activity are shown in Fig. 4. Curves for all other compounds tested were very similar in shape, though the sharpness of the decrease at high concentrations, due to toxicity, varied notably. From each curve the relative activity was deduced as a percentage of the concentration of IAA over the concentration of the compound, required to reduce the outward curvature (130° in water controls) by 100° (Table 2). Duplicate series of tests, run on different days, are shown.

Since Figs. 2 and 3 have shown that the —NH stretch frequency is indeed a measure of net charge on the —NH group, it was next compared with the auxin activity of the various compounds. In Fig. 5 the change in stretch frequency caused by substitution on IAA is accordingly plotted against the log of the relative growth activity. The order of relative growth activities of these acids is similar to that observed by Hoffman *et al.*,[15] though the values are much more quantitative than their approximate scoring.

It can be seen that for substituents on the benzene ring *meta* or *para* to the —NH group, when the lipophilic property[14] and size of substituent are roughly the same, the relationship is linear. For substituents in the 2- or 7-positions, there is a line with similar slope but displaced about 1 log unit to lower growth activity, indicating that reversible association at the —NH group may be subject to steric hindrance, or hindrance due to the electrostatic field of the substituent. In confirmation of this idea the activities of all three 2,7- disubstituted

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[30] K. V. Thinmann and C. L. Schneider, *Am. J. Botany* **25**, 627 (1938).
[31] K. V. Thimann and C. L. Schneider, *Am. J. Botany* **26**, 328 (1939).

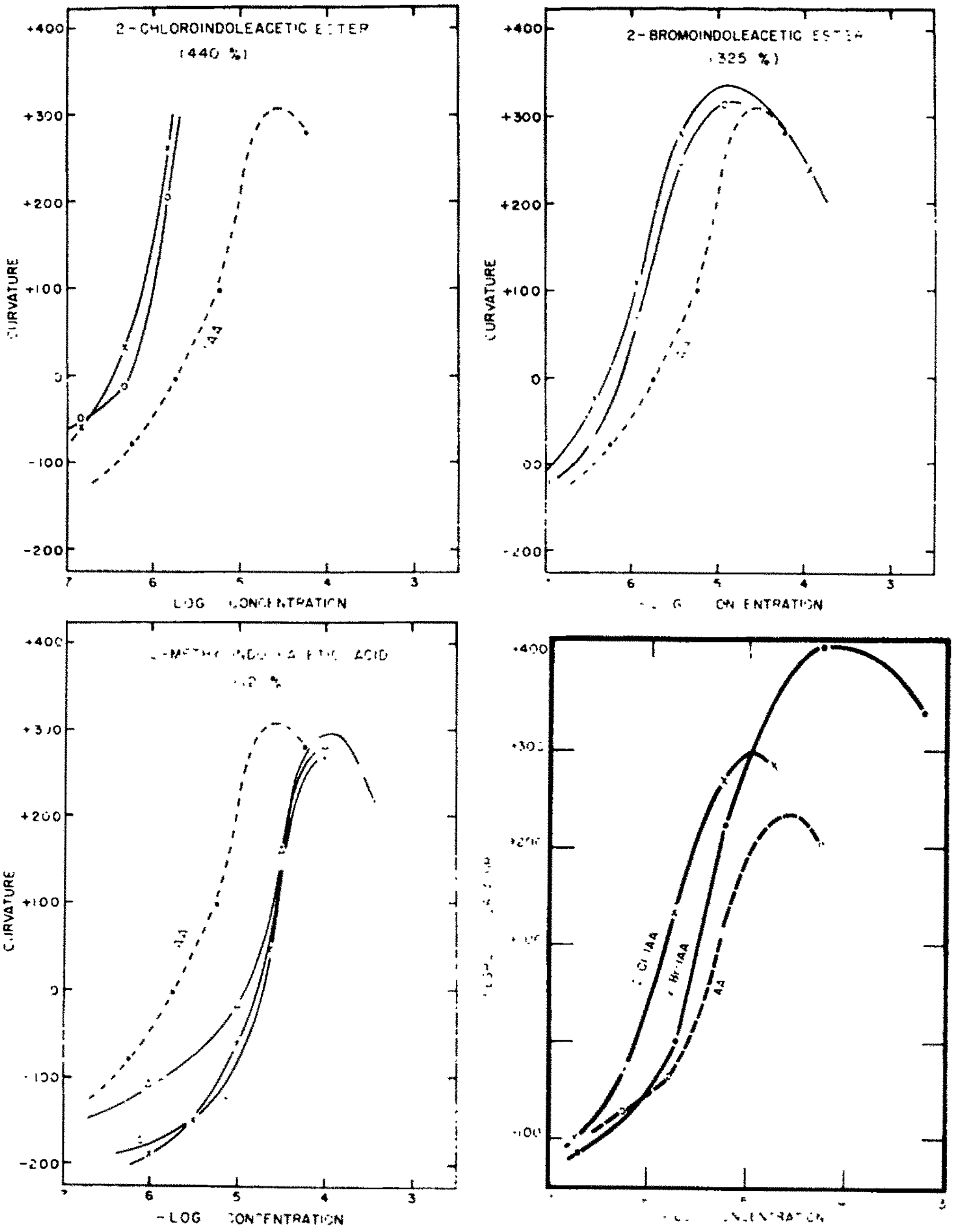

FIG. 4. RELATION OF GROWTH ACTIVITY IN PEA CURVATURE TO LOG MOLAR CONCENTRATION FOR SUBSTITUTED INDOLE-3-ACETIC ACIDS AND ESTERS.

IAA's reported are well below 1 per cent. These are 2-methyl-7-chloro-IAA, 2-methyl-5,7-dichloro-IAA and 2-methyl-4,7-dichloro-IAA.[32] The inclusion of the 5-OH group in the plot is dubious, since it has pronouncedly lower lipophilic activity, as deduced by Hansch *et al.*[14] from the octanol–water partition coefficients of hydroxyphenoxyacetic acids.

Of special importance are the following comparative activities of IAA derivatives. 2-Methyl-IAA has 12 per cent, while 2-chloro-IAA has 350 per cent; and 5-methyl-IAA has 60 per cent while 5-chloro-IAA has 260 per cent that of IAA itself. Since the methyl and chloro groups are similar in lipophilic activity[14] and are nearly isosteric, the effect of the change in electronegativity on auxin activity is clear cut. The methyl group in the more remote position does not deactivate so strongly. Methylation in the 2-position also has a strong deactivating effect in the indole ethylamines; thus in serotonin 2-methylation reduces the activity by 30 times.[5] Furthermore, contrasts just as strong between methyl- and chloro-activation occur also in the benzenoid auxins, 4-methyl-phenoxyacetic acid having 1·6 per cent while 4-chlorophenoxyacetic acid has 160 per cent activity in the pea test.

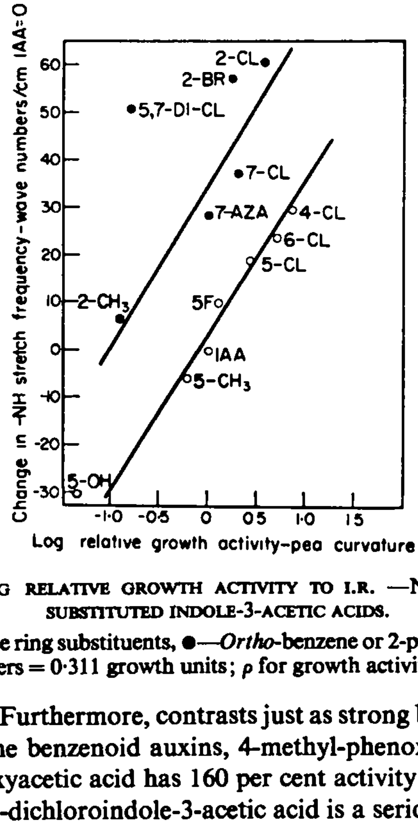

FIG. 5. RELATION OF LOG RELATIVE GROWTH ACTIVITY TO I.R. —NH STRETCH FREQUENCY IN SUBSTITUTED INDOLE-3-ACETIC ACIDS.

○—*Meta-* or *para-* benzene ring substituents, ●—*Ortho*-benzene or 2-pyrrole substituents. 10 wave numbers = 0·311 growth units; ρ for growth activity = 2·0.

The low activity of 5,7-dichloroindole-3-acetic acid is a serious deviation from the data. Since it would appear that the aggregate effect of chlorination at these two remote sites on the —NH charge density is less than that for the 2-chloro group (Table 2), it is difficult to rationalize it as due to an optimum property of the —NH charge. Also, considering the high activity of 2,3,4,6-tetrachlorophenylacetic acid (140 per cent of that of IAA), it would appear that excess lipophilic property induced by more than one Cl atom, beyond an optimum for the auxin as a whole, could hardly have the deactivating influence advocated by Hansch *et al.*[14] and earlier by Veldstra.[33] If this compound has indeed the structure assigned to it[15]

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[32] C. Hansch and R. M. Muir, *Plant Physiol.* **25**, 389 (1950).

[33] H. Veldstra, *Ann. Rev. Plant Physiol.* **4**, 151 (1953).

it deserves further study; also helpful would be tests of such auxins as 4,6-dichloroindole-3-acetic acid, a compound which has not yet become available.

It might be suggested that the increased auxin activity produced by 2-halogenation in indoleacetic acid is due to effects other than electron withdrawal from the —NH group, such as: (1) reduction of the tendency of indoles to oxidative degradation or attack by electrophilic species, or (2) enhanced lipophilic properties. If these changes were determining, the nearly isosteric auxins benzofuran-3-acetic acid (15 per cent) and benzothiophene-3-acetic acid (3 per cent) should have greater activity than IAA, since at least in the latter case the nucleus is less prone to oxidation and electrophilic attack,[7] and both nuclei are more lipophilic[34] than that of IAA. They are, however, less growth active, to a degree roughly corresponding to the less strong shift of electrons away from the heteroatom, i.e. its weaker fractional positive charge.[35] Similarly the 2-methyl, dichlorinated derivatives mentioned above should have high activity, whereas these activities are extremely low.

Since nucleophilic substitutions are essentially unknown on the electron-excessive carbon atoms of the indole rings as well as on the nitrogen,[7] the suggestion of Hansch and Muir[36] that activity depends on nucleophilic substitution at the *ortho* positions on the aromatic rings of auxins can hardly hold for both the indole and the halogenated benzenoid auxins. Similar inconsistencies should prevail with respect to charge-transfer or redox processes, between π-excessive indoles and π-deficient halogenated benzene auxins. On the contrary, the known positive charge on the indole N in the ground state and its tendency for further polarization (as shown by the shift in the —NH stretch frequency with electron-withdrawing and donating substituents) show that it is the —NH group whose charge is the most affected by ring substitution. Consequently, when factors such as steric hindrance or lipophilic properties do not intervene, it is to changes in the —NH charge on the nitrogen that one must look for the explanation of the marked effects of substitution on auxin activity.

EXPERIMENTAL

(*A*) *Preparation of 2-Halogenated Esters*

Ethyl 3-(2-*chloroindolyl*) *acetate*. *N*-chlorosuccinimide (269 mg, ~ 2 mM) was added to a solution of 394 mg (~ 2 mM) of ethyl 3-indolylacetate in 80 ml of reagent grade CCl_4 (dried over $CaSO_4$). After stirring for 1 hr at room temperature, the *N*-chlorosuccinimide had disappeared from the bottom of the flask, and a flocculent precipitate of succinimide formed at the surface. The golden-brown solution was extracted twice with dilute $NaHCO_3$, twice with saturated NaCl, and filtered through a cone of anhydrous Na_2SO_4, which was washed twice with CCl_4. The dark-red solution was evaporated at reduced pressure to a brown oil, which was taken up in ether and chromatographed with ether on aluminum oxide (Woelm No. 1, neutral). The fastest-running compound was the 2-chlorinated ester, immediately followed by unreacted ester. The 2-chloro ester was obtained as a clear white oil, temporarily crystallizing as fine white needles. Recrystallization and accurate m.p. determination were precluded by slow hydrolysis in air or nitrogen. Yield was 99 mg (21 per cent).

The product, ethyl 3-(2-chloroindolyl)acetate, is soluble in ethanol, acetone, ether, chloroform, and CCl_4, and moderately soluble in methanol and CS_2, but difficultly soluble

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[34] H. L. BOOIJ, *Proc. Konınkl. Akad. Wetenschap. Amsterdam* **52**, 1100 (1949).

[35] J. M. HOLLAS, *Spectrochim. Acta* **19**, 753 (1963).

[36] C. HANSCH and R. M. MUIR, in *Plant Growth Regulation*, p. 431, Iowa State Univ. Press, Ames, Iowa (1961).

in water. The u.v. absorption λ_{max} 95% ethanol 273 mμ (ε 7800) 289 mμ (ε 5900), differs from that of the unchlorinated ester which has λ_{max} 95% ethanol 280 mμ, with minor peaks at 273·5 and 289·5 mμ. The i.r. spectrum is identical with that of the methyl esters discussed below.

(Found: C, 61·08; H, 5·25; N, 5·49; Cl, 15·09. Calc. for $C_{12}H_{12}O_2N_1Cl_1$: C, 60·70; H, 5·09; N, 5·90; Cl, 14·92%.)

Ethyl 3-(*2-bromoindolyl*) *acetate. N*-bromosuccinimide (357 mg, ~ 2 mM) was added to a solution of 406 mg (~ 2 mM) of ethyl-3-indolylacetate in 80 ml of dry CCl_4. The mixture was heated with stirring for 10 min at 65–70°. The solution turned violet immediately and flocculent succinimide floated to the surface, the *N*-bromosuccinimide disappearing from the bottom of the flask. Extraction and chromatography were in all respects similar to that for the 2-chloro derivative, the 2-bromo compound emerging from the column before the unreacted ester. Yield after re-chromatography was 257 mg (46 per cent) of white needles, m.p. 44·5–45·0°. (The unbrominated ester has m.p. 40·5–43·0°.) Solubility characteristics are similar to those of the 2-chloro ester; λ_{max} 95% ethanol 274 mμ (ε 8300) 282·5 mμ (ε 8600), 290 mμ (ε 7300).

(Found: C, 51·99; H, 4·46; N, 4·81. Calc. for $C_{12}H_{12}O_2N_1Br_1$: C, 51·10; H, 4·28; N, 4·96%.)

Methyl esters. Methyl 3-(2-chloroindolyl) acetate and methyl 3-(2-bromoindolyl) acetate were prepared in the same manner from methyl 3-indolylacetate. The u.v. spectra were indistinguishable from those of the ethyl esters, as were the —NH, carbonyl, and out-of-plane —CH bending bands in the i.r. The 2-chloro methyl ester was obtained initially as fine white needles (44 per cent), which, however, like the ethyl ester, hydrolyzed too quickly, even in dry air or nitrogen, to obtain an accurate melting point. The 2-bromo ester was obtained after two chromatography runs as white needles (43 per cent), m.p. 62·0–64·5°. The unhalogenated ester has m.p. 51·0–52·0°.

The i.r. spectrum in CS_2 of methyl 3-(2-chloroindole) acetate (Fig. 1A) shows a strong ester carbonyl stretch band at 5·74 μ, a free —NH stretch band at 2·82 μ, and an intermolecular H-bonded (presumably carbonyl) —NH stretch band at 2·92 μ. There is a benzene —CH out-of-plane bending band at 13·55 μ.

The i.r. spectrum of the 2-bromo ester is almost the same (Fig. 1B). The free —NH stretch band is at 2·82 μ, the intermolecular H-bonded —NH stretch band at 2·92 μ, the ester carbonyl stretch band at 5·75 μ, and the unaltered benzene —CH out-of-plane bending band at 13·52 μ. The 13·22 μ —CH band for the 2-proton of indole is missing, as expected. The i.r. spectrum of methyl-3-indolylacetate in the same solvent is given in Fig. 1C for comparison.

(*B*) *Preparation of 2-Halogenated Indoleacetic Acids*

2-*Chloroindolylacetic acid.* To 56 mg (1 mM) of KOH in 1 ml of 50 per cent methanol–water was added 48 mg (0·2 mM) of ethyl 3-(2-chloroindolyl) acetate and the solution heated at 100° for 1 hr. The hydrolysate was acidified to pH 4 with 0·1 N HCl and extracted twice with ether. (Extraction into $NaHCO_3$ solution was not used, to avoid too much contact with acid on reacidification.) The ether extract was washed with saturated NaCl and dried by filtration through anhydrous Na_2SO_4. The solvent was removed at reduced pressure and room temperature with a drying tube to exclude moisture. Eleven mg (27 per cent) of radiating clusters of white needles, m.p. 83·0–85·5°, with a sweet aromatic smell, were obtained. The crystals yellow quickly in air. The u.v. spectrum gave λ_{max} 95% ethanol 273 mμ and 289 mμ, identical with the ester. The i.r. spectrum (Fig. 1D) has a broad hydroxyl absorption in the 3–4 μ region, and an acid carbonyl stretch band at 5·84 μ.

2-Bromoindolylacetic acid. To 60 mg ($\sim$ 1 mM) of KOH in 1 ml of 50% methanol–water was added 43 mg ($\sim$ 0·15 mM) of ethyl 3-(2-bromoindolyl) acetate and the solution heated 1 hr at 100°. Acidification and extraction as for the 2-chloro derivative gave 23 mg (60 per cent) of white radiating needles which begin to yellow on exposure to air. They have an almost sickeningly sweet aromatic odor, m.p. 85·0–87·0°. The u.v. spectrum is identical with that of the ester above 240 mμ and the i.r. spectrum shows the expected broad hydroxyl band and acid carbonyl at 5·84 μ.

Hydrolysis of the methyl esters produced acids identical in all physical characteristics to those obtained from the ethyl esters.

(C) *Measurement of Bonded i.r. —NH Stretch Frequencies in Indoles and IAA's*

In addition to the six compounds described above, fifteen substituted indoleacetic acids, ten from Dr. Sidney Fox and five available in this laboratory, were used for i.r. studies and growth tests. The spectral properties of an additional ten substituted indoles were studied in the i.r. All the compounds except the 2-chloro esters were solids, with melting points as reported in the literature;[15] these were sharp for each compound except 5,7-dichloro-IAA (194–199°). The very small sample of the latter precluded its recrystallization. The bonding solvent, acetonitrile, was of spectral grade and was not further purified before use, since a decomposition giving bands at 2·79 and 2·85 μ, tentatively ascribed to —NH and —OH groups arising from hydrolysis, slowly occurs under any conditions in the heat of the i.r. beam. Although these bands lengthen progressively, the frequency of the indole —NH bonded stretch does not change.

Acetonitrile was chosen as bonding solvent because: (*a*) it dissolves all the compounds, (*b*) it is a relatively good lone-pair donor, (*c*) it is relatively transparent in the region of bonded i.r. stretch frequencies, and (*d*) it has a low entropy change when hydrogen bonding to indole.[37] This reflects the small size and linear structure of the molecule, properties which in turn minimize any steric hindrance exerted by *ortho* substituents on the indole.

The measurements were made with a Perkin-Elmer Model 21 Infrared Recording Spectrophotometer, using an NaCl prism with a slit width varying from 18 to 20 mμ. The substances were made up in 0·5% solutions (i.e. 0·02–0·10 M) in acetonitrile and read either in a sodium chloride cell of 0·4 mm optical depth or an IR Trans cell of 0·5 mm depth. The temperature was 22° in an air-conditioned room. Scanning speed was 0·16 mμ/sec. The compounds at the ends of the range were available in large quantities and were checked in the near i.r., where absorptions similar to those described herein were found for the first overtone of the —NH stretch, although the amplitudes and hence the sharpness of the peaks were much lower.

Other measurements attaining similar precision with a similar instrument and NaCl optics have been reported;[17–19] also using lithium fluoride optics, giving somewhat greater precision,[20–22] and using a grating spectrophotometer.[23, 24]

The procedure used was as follows: At the very slow scanning speed the position of maximum absorption of each peak was first determined, then the dial reading in μ was recorded for each intersection of the pen with a line of absorbance near the peak, e.g. 0·42, 0·43. The mean of the descending and ascending values for each intersection was taken, and the two agreeing values closest to the tip were selected. The means seldom differed by more than 1 mμ.

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[37] H. DUNKEN and H. FRITZSCHE, *Z. Chem.* **2**, 379 (1962).

For calibration, a reading of similar precision was made (in the same way) of the line of water vapor absorption in air at 2·673 μ, which was used as reference.

Each solution was measured between three and eight times by the same procedure, until reproducibility of 1–3 mμ was achieved. The mean of the observations was taken, and a standard deviation computed.

On ten of the substituted indole-3-acetic acids, measurements were repeated after 3–4 months. For eight of these no mean differed from the previous mean by more than 5 mμ and the average shift was only 1 to 2 mμ. For the other two compounds, eight new measurements were made.

The mean standard deviation for all measurements used in graphs and charts was 1·7 mμ and the greatest standard deviation 3·0 mμ. After the means and standard deviations had been computed, they were converted to wave numbers for comparability with other values cited for i.r. and for use in correlation plots with sigma constants and log growth activities.

(*D*) *Plant Growth Measurements*

The compounds mentioned above were tested two to five times at six dilutions in the now standard pea curvature test method as described by Thimann and Schneider.[30] In this test the water controls curve outwards about 130°, and potent compounds at optimum concentrations can produce inward curvatures up to 600° or so. The curvature is approximately proportional to the log of the concentration over a range of two or three $\log_{10}$ units. To compare as many compounds as possible a low level of activity is used as the reference point. In this work relative growth activity is expressed in per cent of IAA activity as:

$$100 \times \frac{\text{Concentration of IAA}}{\text{Concentration of compound}}$$

required to reduce outward curvature by 100°. This unit was determined to be the most practical in early studies[31] and has been found applicable in much subsequent work (e.g. Ref. 3).

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