

Table 2

Copper complex	Stability constants of copper complexes based on measurements in the absence of a strong acid			
	$pK_a$	$pK_1$	$pK_2$	$pK_c$
Naphthalene acetic acid	5.76	5.1	4.5	9.6
2:4-Dimethylphenoxyacetic acid	4.54	4.6	4.2	8.8
2-Methyl-4-chlorophenoxyacetic acid	4.36	4.3	4.0	8.3
2:4-Dichlorophenoxyacetic acid	4.20	4.3	3.8	8.1

$pK_a$  is the equilibrium constant of the acid; temperature, 21° C. ( $pK_1$  and  $pK_2$ ) =  $pK_c$ , the chelate stability constant of the reaction:  $2A^- + Cu^{2+} \rightleftharpoons (A)_2Cu$ ; all determinations made in 50 per cent ethanol.

Table 3

Copper complex	Stability constants of copper complexes based on measurements made in the presence of $10^{-2} M$ nitric acid				Growth activity of acid (dose in $\mu gm.$ needed to give 50 per cent leaf repression)
	$pK_a$	$pK_1$	$pK_2$	$pK_c$	
Naphthalene- $\alpha$ -acetic acid	5.75*	5.2*	4.5*	9.7*	0.2 $\pm$ 0.1
2:4-Dimethylphenoxyacetic acid	4.53	4.6	3.9	8.5	28 $\pm$ 2
2-Methyl-4-chlorophenoxyacetic acid	4.39	4.4	3.8	8.2	0.15 $\pm$ 0.02
2:4-Dichlorophenoxyacetic acid	4.19	4.3	4.2	8.5	0.04 $\pm$ 0.005

\* Identical values obtained in independent experiments; temperature, 25° C.; all measurements in 50 per cent ethanol.

in calculating the  $pK_c$ . Similar disagreement with the recorded  $pK_c$  for indoleacetic acid-copper complex has been reported recently by Recaldin and Heath<sup>5</sup>, although it is not clear what conditions were used for their measurement.

Parallel with the measurements of the stability of the copper complexes, we have made quantitative estimates of the growth-regulating activity of each of these growth substances by the method of Brown and Weintraub<sup>6</sup>. In the last column of Table 3 are listed the quantities of each acid required to depress the growth of the first trifoliate leaf of a *Phaseolus* seedling by 50 per cent. The percentage repression in this test is linearly related to the logarithm of the dose, and the figures for 50 per cent repression are inversely related to the growth activity. It will be seen that 2:4-chlorophenoxyacetic acid is almost a thousand-fold more active than dimethylphenoxyacetic acid, though its ability to bind copper is not measurably different.

We therefore suggest that in spite of some evidence in the literature for a relation between metal-complexing capacity and plant-growth activity, the properties by which certain molecules are specifically able to act as growth regulators still remain a mystery.

Our thanks are due to the directors of Imperial Chemical Industries of Australia and New Zealand for permission to publish this communication, and to Mr. D. Fieldhouse, Mrs. M. Markus and Mrs. V. Zangari for technical assistance.

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<sup>1</sup> Cohen, D., Ginzburg, B.-Z., and Heitner-Wirguin, C., *Nature*, **181**, 686 (1958).

<sup>2</sup> Martin, R. L., and Whiteley, A., *J. Chem. Soc.*, 1394 (1958).

<sup>3</sup> Calvin, M., and Melchior, N. C., *J. Amer. Chem. Soc.*, **70**, 3270 (1948).

<sup>4</sup> Martell, A. E., and Calvin, M., "Chemistry of the Metal Chelate Compounds", 76 (Prentice Hall, Inc., N.J., 1956).

<sup>5</sup> Recaldin, D. A., and Heath, O. V. S., *Nature*, **182**, 539 (1958).

<sup>6</sup> Brown, J. W., and Weintraub, R. L., *Bot. Gaz.*, **111**, 448 (1950).

In putting forward the hypothesis that plant-growth substances might act as such through their metal complex-forming activities<sup>1,2</sup> we did not suggest that no other properties of the molecules concerned could modify their effects upon growth. We specifically raised the question of rate of penetration, and in view of the very various growth-promoting activities of the different agents tried (a variation by no means parallel with their metal-complexing capacities) we had in mind the possibilities that steric hindrance or toxicity might also be modifying the amount of growth promotion obtained.

One might well hesitate to accept that the 'growth-regulating activity' of 2:4-dichlorophenoxyacetic acid is almost a thousand times as great as that of dimethylphenoxyacetic acid on the basis of an assay test depending on the inhibition of growth, such as that used by Armarego *et al.* (preceding communication), but other work<sup>3</sup> suggests that their growth-promoting activities in the *Avena* cylinder test are in a similar ratio. Even if this is not, in fact, due to a difference in penetration, the binding of copper or some other metal may yet be concerned with the spatial configuration of the sites favouring on acid rather than the other.

The growth-promoting activity of ethylenediamine tetraacetate has been variously reported as similar to that of indoleacetic acid<sup>4</sup>, or as much less<sup>5,6</sup>, according to the techniques and plant material used; with the strain of wheat used by Fawcett *et al.*<sup>3</sup>, and using their technique, we have been able to reproduce their results. However, even if the growth-promoting activity of these two agents be accepted as of the same order of magnitude, the fact remains that their *in vitro* complexing activity differs vastly. It is, in fact, surprising that so powerful a chelating agent as ethylenediamine tetraacetic acid does not disrupt the chemical organization of the cell at many sites; the growth-promotion obtained may be the net result of stimulatory and inhibitory processes due to competition with various chelating agents in some such way as that suggested by Burström and Tullin<sup>7</sup>.

Evidence seems to be accumulating that known natural and synthetic growth substances can form complexes *in vitro*<sup>8,9</sup>, but more important than this is the question whether they do so in controlling growth in the living plant. Some of the evidence for this was very briefly reported in the communications cited<sup>1,2</sup> and the results of these and other experiments will be published in detail as soon as possible.

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<sup>1</sup> Heath, O. V. S., and Clark, J. E., *Nature*, **177**, 1118 (1956).

<sup>2</sup> Heath, O. V. S., and Clark, J. E., *Nature*, **178**, 600 (1956).

<sup>3</sup> Toothill, J., Wain, R. L., and Wightman, F., *Ann. App. Biol.*, **44**, 547 (1956).

<sup>4</sup> Weinstein, L. H., Meiss, A. N., Uhler, E. L., and Purvis, E. R., *Nature*, **178**, 1188 (1956).

<sup>5</sup> Bennet-Clark, T. A., in "The Chemistry and Mode of Action of Plant Growth Substances", 284 (Butterworth, London, 1956).

<sup>6</sup> Fawcett, C. H., Wain, R. L., and Wightman, D. F., *Nature*, **178**, 972 (1956).

<sup>7</sup> Burström, H., and Tullin, V., *Physiol. Plantarum*, **10**, 406 (1957).

<sup>8</sup> Cohen, D., Ginzburg, B.-Z., and Heitner-Wirguin, C., *Nature*, **181**, 686 (1958).

<sup>9</sup> Recaldin, D. A., and Heath, O. V. S., *Nature*, **182**, 539 (1958).