

Measuring chemically-induced elongation of plant sections

PLANT HORMONES

By JAMES BONNER

Basic studies on plant hormones have resulted in the development of a new science — the science of the chemical control of plant growth

THROUGH BASIC WORK on the plant growth hormones, the concept has become available to us that particular substances may be applied to the plant to accomplish particular useful purposes—to make the leaves drop off, to make the fruit stay on, to induce flowering, to inhibit flowering, and even to kill undesired plants.

The different chemicals which are used for the supervision of these varied aspects of plant development are also without exception substances whose biological effectiveness is based upon structural similarity to one or another of the native plant growth hormones. Since a great many substances have been investigated or screened as to ability to evoke this or that plant growth response, we have today what is almost a pharmacology of plants.

It is, of course, well known that the growth of the plant depends upon the process of photosynthesis by which the carbon dioxide of the air is transformed into the manifold materials of which a plant is made. The growth of the plant is dependent, too, on the water and on the varied mineral constituents of the soil which are ordinarily taken up by the root and incorporated into plant materials.

As photosynthesis, mineral uptake, and the chemical transformations of metabolism proceed, the plant increases in size and in mass, and, in due course, proceeds from the vegetative state to a reproductive one. The total

increment of plant which occurs as a result of synthetic reactions must be apportioned between the several organs, the roots, leaves, stems, and, ultimately, the flowers and fruits.

It is the harmonious integration of the development of the several plant organs and tissues in space and in time which appears to be the primary function of the plant hormones. Each of these materials is produced in a particular organ in minute amounts, and is then transported to other organs where it brings about a specific effect on growth. It is through the growth hormones that the individual parts of the plant interact with one another and mutually regulate one another's growth.

That the growth of the root is dependent upon specific chemical substances which are normally supplied by the aerial organs can be shown simply and elegantly by the cultivation of the excised tips of roots. If we remove a tip a few mm. long from a growing root and place this in an appropriate nutrient solution the tip will grow into a whole root. If the nutrient solution has been correctly selected, the excised root will, in fact, grow in length as rapidly as it would have done had it remained attached to the intact plant.

We may now ask ourselves what chemical substances must be added to the nutrient solution used for the culture of excised roots in order to bring about continued

root growth. One knows *a priori* that an appropriate nutrient solution must contain a carbohydrate, which may be used by the root tissue as a respiratory substrate, and which may serve as a source of building blocks for the synthesis by the root of the many substances of which roots are made.

The nutrient solution must also contain the inorganic nutrients which plants require for their growth. A nutrient solution containing only mineral salts and sucrose, however, is inadequate for the cultivation of excised roots. An excised root tip of flax, for example, cultivated in such a nutrient, ceases its growth in a few days or a few transfers. In order to cause excised flax roots to grow at the normal rate, it is necessary to add a small amount of thiamine to the nutrient solution. All of the species of excised roots which have been studied up to the present time require thiamine and, in general, one or both of the vitamins pyridoxine and niacin, in order to continue growth as isolated roots.

It is possible by simple plant physiological experiments to show that these three vitamins of the B complex are produced in mature green leaves, primarily in the light. They are then transported from the leaf, through the stem to the root. Since roots cannot produce these vitamins, and since these materials are required for root growth, thiamine, pyridoxine, and niacin constitute root growth hormones.

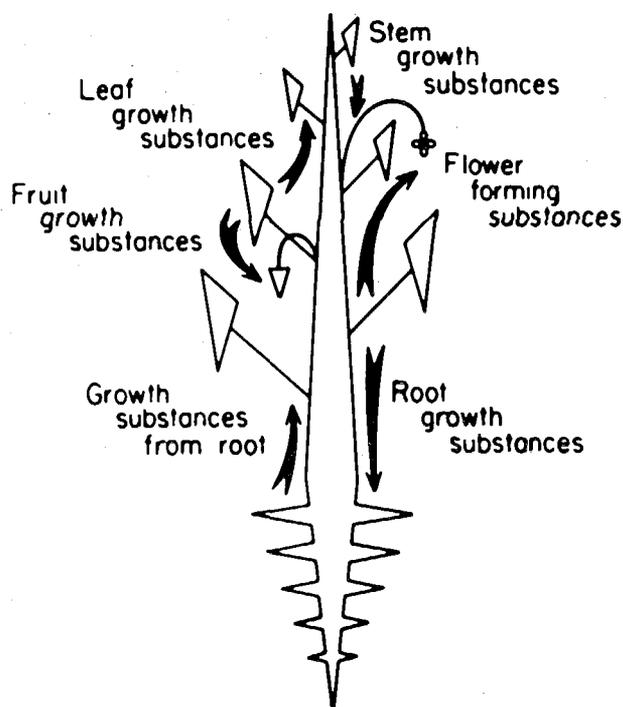
Other types of growth hormones

The same basic kind of experiment used for the demonstration of the presence in the plant of root growth hormones may be used for the demonstration of the existence of other types of growth hormones as well. Thus, we know of leaf growth hormones, stem growth hormones, fruit growth hormones, and hormones for the initiation of flowering.

We must then visualize the growing plant as containing many currents of transport of a variety of growth regulating substances. The mature leaves produce the vitamins of the B complex which are transported to and used in the regulation of root growth. The same leaves produce the leaf growth factors such as adenine, as well as the hormones which regulate flower initiation and reproductive development—hormones which are possibly protein in nature.

Still other factors produced in the roots are required for the growth of the aerial parts. And to this array of known or suspected correlational carriers we will doubtless add, in the future, further hormones whose existence is not even suspected today.

A major portion of our knowledge of plant growth substances has been derived from the study of one particular group of materials, known collectively as the auxins. This term is a physiological one, and the auxin concept is physiological rather than chemical. An auxin is a substance produced in the apical bud and young leaves of the plant and transported from this point to



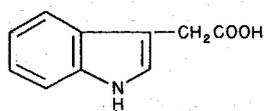
Within the plant are varied currents of hormone flow.

the growing region of the stem, where it is used in the support of stem elongation.

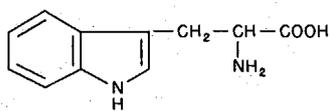
It can be simply demonstrated that special chemical substances have to do with the regulation of stem elongation. If we excise sections from the growing region of, say, a seedling oat plant, and place the sections in a nutrient solution containing only sugar and mineral salts, they grow but little. However, if we add a small amount of indole acetic acid (IAA) to the nutrient solution, the rate of growth of the sections is greatly increased, and may in fact achieve or surpass the rate normally attained in the plant.

That IAA is a naturally occurring plant material has been shown by isolation from a variety of plant products. It is produced in the apical bud and young leaves and transported downward through the stem and thus satisfies the requirements of the auxin concept. IAA is in fact the best known and perhaps the most important of the stem growth regulating substances produced by and used by the plant.

Although the relation of IAA, and of auxins generally, to plant growth was first appreciated in relation to stem elongation, we know today that the functions of this hormone are actually manifold. IAA appears to act as a master hormone, inciting cells or tissues to activities of the most varied kinds. Among the terminal manifestations of auxin-induced activity are growth in length (stems), suppression of growth in length (roots), induction of cell division in the cambium, in callus and in tumors, inhibition of leaf and fruit fall, production of adventitious roots, and finally the production or nonproduction of other and more specific hormones which are in turn responsible for further responses, such as the initiation of reproductive activity.



Indole-3-acetic acid



Tryptophan

Indole acetic acid is related to—and derived from—the amino acid tryptophan.

It is precisely because of the wealth of different responses which can be brought about by appropriate auxin application that plant hormone therapy has assumed such agricultural significance today and this therapy is in turn largely based on our knowledge of auxin physiology.

Auxins: native and synthetic

IAA, as is shown above, consists of an indole nucleus to which an acetate side chain is appended at position 3. It is thus related in structure to the amino acid tryptophan, and is in fact synthesized in the plant from tryptophan by a series of reactions mediated by an enzyme system which is found in apical buds and other centers which possess the ability to generate the hormone.

Approximately 15 years ago it became known through the work of Haagen-Smit and Went that IAA is not alone in its ability to regulate the stem growth of plants. Certain related chemical substances, not necessarily naturally occurring, were found to be able to duplicate the effects of IAA in causing the elongation of excised sections of stems of oat coleoptiles. Naphthalene acetic acid is not a naturally occurring plant material. It is a chemical substance which is able to simulate the effects of a plant hormone, although it is not itself a plant hormone. In the years since 1935 a very great number of compounds have been tested for their ability to replace IAA in the induction of plant growth responses. So much study has been put into this matter that at present our knowledge of the relation of chemical structure to biological activity among the auxins is one of the best documented studies of this nature. Let us therefore see what new insight into auxin physiology these intensive chemical studies have given us.

The conclusions which we draw from the study of the activity or non-activity of a great many different substances may be summarized in the empirical rules that a compound, in order to manifest auxin activity, must possess the following minimum qualifications: (1) A ring system containing at least one double bond; (2) A side chain ending in a carboxyl group; (3) Ability to assume a certain configuration in which the carboxyl group is suitably arranged relative to the ring system.

It has recently become possible to define more closely the role of the cyclic unsaturated nucleus in relation to auxin activity and from this to learn more concerning the biochemistry of auxin action.

Muir, Hansch and Gallup (1949) of Pomona College have shown that a compound, to be active as an auxin, must possess in its cyclic nucleus a substitutable hydrogen or other group of a minimum critical reactivity. This reactive position is in general either of the two positions ortho to the carboxyl group containing side-chain. This is shown by the example below. The ortho reactivity of phenoxyacetic acid is increased by halogen substitution in the 2 and 4 positions and 2,4-D is active as an auxin. Blocking of both ortho positions renders the molecule inactive.

It appears, then, that a molecule, to be an auxin, must possess not only a carboxyl group but also a cyclic nucleus with an ortho group of some critical reactivity. These two functional groups must further be capable of assuming some suitably spatial relationship with one another. All of our present knowledge of structure and activity among the auxins appears to be qualitatively encompassed by this generalization.

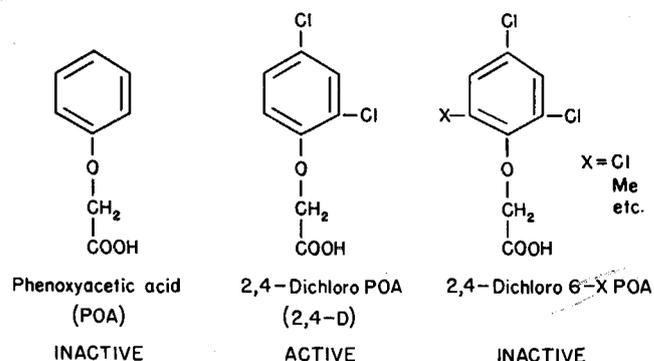
Two-point attachment

The fact that auxins act at low concentrations suggests at once that they may perform their work in promoting growth by acting as prosthetic groups of an enzyme or enzymes; that in short they may be bound to protein in the plant.

It has long been known that auxin is in fact bound to protein within the plant and that auxin thus bound may be again released by proteolytic hydrolysis. This suggests that the carboxyl group of the auxin molecule may be involved in the binding, perhaps through the formation of peptide-like linkages. That the ortho group is also involved in chemical reaction within the plant, and that this is also related to binding, is indicated by both chemical and kinetic studies.

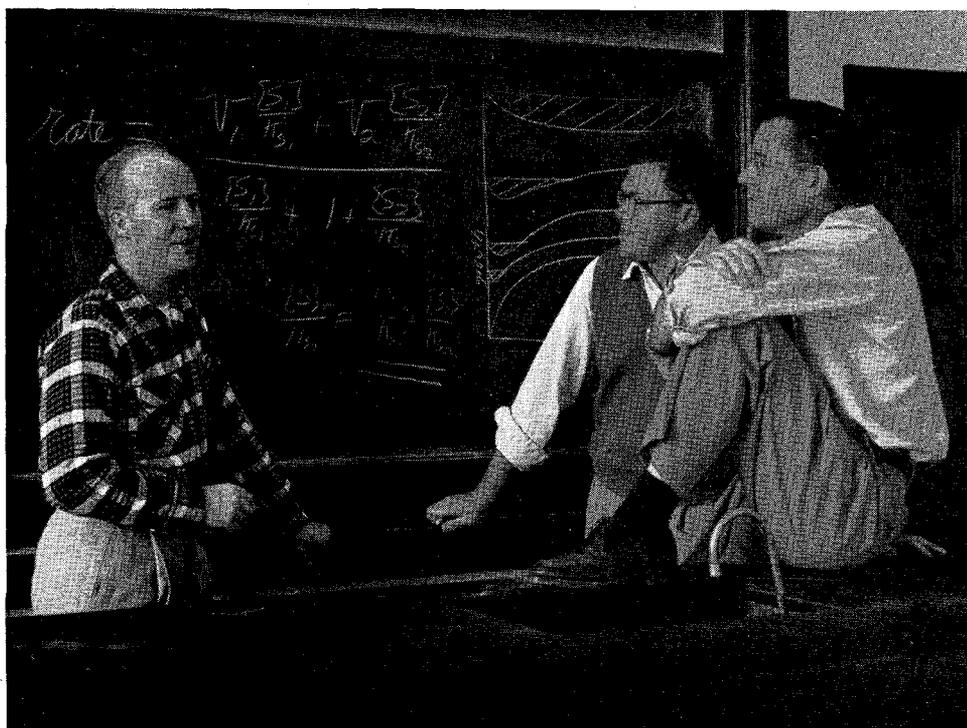
We may suggest, therefore, as a tentative working hypothesis, not only that the auxin molecule possesses two reactive functional groups, but that the molecule, in carrying out its growth-promoting task, reacts with and binds to two suitable receptor sites within the plant.

This concept—the two-point attachment concept—has served as a fruitful basis for, and is strongly supported



Growth substance activity can either be increased or decreased by the proper ring substitution.

D. Harold McRae, Robert J. Foster, and James Bonner—Caltech co-workers on the chemical control of plant growth. The equation they're discussing here describes plant growth rate for cases in which two growth substances are simultaneously applied.

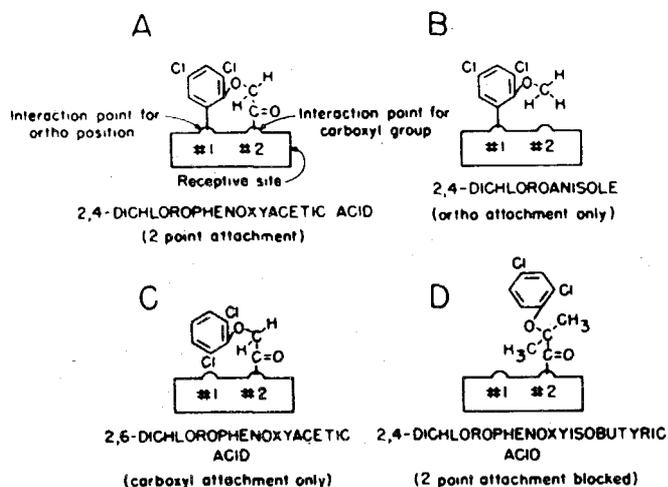


by, further experimentation, particularly in the way of auxin antagonists.

Inhibition of growth

The extensive studies of D. H. McRae have shown not only that an active auxin is normally bound to the plant receptor entity through its two reactive groups, but that in addition a substance capable of combining with but one site of the receptor entity and incapable of consummating two-point attachment is thereby an antiauxin—an inhibitor of auxin action. Examples of antiauxins and the way in which they act to block receptor molecules are shown below.

The effect of auxins in promoting plant growth, like



Growth substances are active by consummating two-point attachment. Molecules which can make only single-point attachment are growth inhibitors.

the effects of so many biologically active substances, is a twofold one. Although auxins promote plant growth over a wide range of relatively low concentrations (10^{-8} - 10^{-5} M), at still higher concentrations the same substances become inhibitory. Thus, if we plot growth rate against concentration of added auxin we find that the hyperbolic relationship of growth rate to auxin concentration obtains up to a concentration of roughly 10^{-5} M. At this concentration, growth rate passes through a maximum and dies away to 0 as the concentration is still further increased.

That auxins in high and unphysiological concentrations elicit growth inhibitions of this type has been known for many years. This effect now assumes new interest and importance, since it can be shown that such a dual growth response is a natural and indeed an inescapable consequence of the two-point attachment by which the auxin molecule is bound to the receptor entity within the plant.

Auxin-induced growth inhibition appears to be nothing more than the kinetic expression of two-point attachment. Let us consider the sequence of events which leads to the formation of the active auxin-receptor complex. A molecule of auxin, IAA for example, approaches the receptor entity and combines with it through one of its two functional groups. After a suitable period of twisting it adjusts itself in such a manner as to be able to consummate its second point of attachment through its second functional group.

Now let us consider the sequence of events at higher auxin concentrations. At some sufficiently high concentration the probability will become appreciable that two molecules will simultaneously approach the receptor

entity and will simultaneously combine with it. In this instance, one auxin molecule will necessarily become attached through its carboxyl group while the other must attach through its reactive ortho position. Each molecule will therefore prevent the other from consummating the two-point attachment which, as we have seen, is essential to the manifestation of auxin activity. The bimolecular auxin-receptor complex is therefore inactive in promoting growth, and to the extent that such bimolecular auxin-receptor complex formation occurs, growth rate will be depressed, as is summarized in the chart at the right.

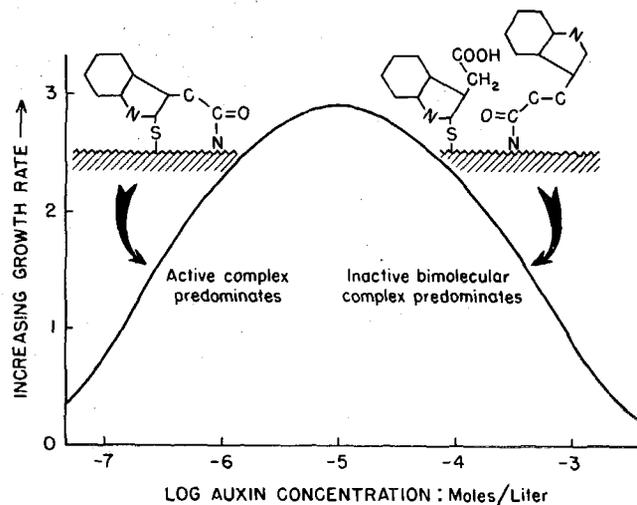
Conclusion

It is a matter for some intellectual satisfaction that the structural demands for auxin activity can be resolved into three simple and inclusive requirements; namely, (a) a requirement for a carboxyl group, (b) a requirement for a critically reactive ortho group, and (c) a requirement for a specific spatial relationship between these two. These three requirements must, as we have seen, all have their basis in the nature of the binding of the auxin molecule to its receptor entity within the plant. In any case we are now in a position to predict with some assurance the activity or inactivity of further compounds. In any future search for new materials with auxin activity, the past approach of empirical and indiscriminate screening might well be replaced by an approach based on thoughtful consideration of the structures involved.

Of more importance to physiology, however, is the insight which the two-point attachment concept has given us into the structural requirements for antiauxin activity. An antiauxin is, it appears, merely a prospective auxin in which one of the three above requirements is not fulfilled. Through applications of this knowledge a number of highly effective antiauxins have already been made available and it is already apparent that these materials may fill a host of agricultural needs. A hormone is by its very nature normally contained in the living creature, and the kinds of responses which may be elicited in this creature by the application of further hormones are necessarily limited. The antiauxins now offer us the opportunity to practice a chemotherapy of plants which is the obverse of that which we practice with 2,4-D.

Finally, the two-point attachment concept of auxin action has provided us with new insight into the nature of the deleterious effects of high auxin concentrations. This, in turn, has illuminated the question of why it is that a synthetic growth substance such as 2,4-D, which behaves as an auxin at low concentrations, is nevertheless so extremely deleterious as to be herbicidal at higher concentrations.

IAA itself cannot ordinarily be used to kill normal green plants, since most such plant tissues are equipped with an enzymatic system for the removal of IAA in excess of a concentration which is well below those which



The inhibition of plant growth by auxin at high concentrations is due to bimolecular complex formation.

give complete growth suppression. This safety mechanism does not work with such non-native materials as 2,4-D, which are then free to accumulate in plant tissues in concentrations which appropriately reflect those applied. It is characteristic of the 2,4-D concentrations which are used for herbicidal purposes that they are far into the range at which bimolecular complex formation must be expected to predominate. Herbicidal activity of an auxin may therefore be nothing more than an extreme expression and ultimate consequence of the two-point attachment principle.

We have seen that, in the plant, communication of information as between the separate and varied organs and tissues is mediated by a complex system of appropriate chemical materials. Integration, control and synchronization of the plant's activities are established and maintained by a whole series of hormones, each of which bears from its point of origin to other receptor points an appropriate signal and evokes appropriate responses. Only when we view the plant as a whole can we perceive the wonderful and complete autotrophism of these organisms, their ability to transform such simple materials as carbon dioxide, water, light energy, and a few mineral elements into the complex array of organic substances which constitute living matter. The individual organs and tissues of the plant are appreciably less autotrophic than is the whole, and are each dependent upon other organs and tissues for particular and specific chemical substances. Thus, in a sense, the plant makes use of slight variations in its autotrophism to assure the harmonious development of its several parts.

The fact that plant growth and development is so firmly based on hormonal relationships gives us a corresponding opportunity to control and modify the course of plant development by the artificial application of chemical substances which are either identical with, synthetic substitutes for, or antagonists of the native hormones.