THE NATURE OF TIBA ACTION

R. M. Irving

August—1968
Technical Bulletin
T-128

CONTENTS

The Nature of TIBA Action

R. M. Irving

Department of Horticulture

This bulletin was designed as a review and analysis of the literature reported on the use of 2,3,5-triiodobenzoic acid (TIBA) and its influence on the plant growth and development. The purpose is to provide a brief comparative survey of the various effects and physiological details of action of this compound. No effort has been made to cover the voluminous literature in a comprehensive manner, but rather to provide a survey of the scope and diversity as well as a description of present knowledge on the mode of action of this material.

The influence of TIBA in various plant processes has been recognized for more than a decade. Its actions on plant growth and their subsequent manifestations are very striking in many cases. As yet no comprehensive explanation of its effects has emerged. Various proposals have been offered, but these generally have been in reply to singular responses to TIBA influences and, hence certain discrepancies occur both in relating its effect and in explaining its mechanism(s) of action. It is the nature of these inconsistencies and similarities that constitutes the enigma of TIBA action and suggests the need for an analysis of the literature on this subect.

Effects of TIBA

Since 1942, when Zimmerman and Hitchcock first found TIBA to be effective as a plant growth regulator, the number of investigations involving TIBA has become quite substantial. Although it was originally believed that TIBA might be a true flowering hormone (what growth regulating compound hasn't?) and could induce the floral response without a prior photoinduction period, it is now accepted that TIBA can initiate early flowering only in a properly induced normal flowering cycle (Galston, 1947; Esteves-de-Sousa, 1950, 1953).

The morphological aberrations induced by TIBA have also been studied in great detail. Gorter (1949) showed that the characteristics of TIBA-treated tomatoes included a reduction of leaf area, darker color, epinasty of leaves, reduced internodal growth, and inhibition of apical dominance. In addition, she and other workers (Wardlaw, 1953; Osborne and Wain, 1950; Bedesam, 1958) observed a highly unique abnormality from TIBA treatment known as ring fasciation. This phenomenon is an extremely rare characteristic, which is an inherited recessive trait in some

plants, that produces an enlargement of the stem tissue and/or cupping of leaves as if several were fused.

TIBA has been shown to produce alterations of sex expression (Wittwer and Hillyer, 1954), reverse geotropism of root growth (Roberts, 1959; Audus and Thresh, 1956), inhibit xylem differentiation (Roberts, 1960), and bring about chemical vernalization of Alaska peas (Leopold and Guernsey, 1954). Other effects include inhibition of root and root hair elongation (Aberg, 1953; Gorter, 1949), synergistic promotion of growth with IAA (Thimann and Bonner, 1948), and stimulation of rooting in *Coleus* cuttings.

It is generally agreed that TIBA action results from its influence on auxin. However, there is some disagreement about *how* TIBA influences the auxin molecule and whether or not its effects are brought about by anti-auxin action.

Strictly speaking, the term anti-auxin should be reserved for those compounds which compete with auxin for some specific reaction center in the growing cell. The extent to which auxin action is suppressed will then depend on the relative amounts of auxin and anti-auxin competing, according to the laws established for substrate-inhibitor interaction in competitive enzyme systems. True anti-auxins would then be auxin homologues that have little or no auxin activity and show competitive antagonism to auxin action, a classification drawn up mainly on the basis of the two-point attachment theory of auxin activity (McRae and Bonner, 1953).

The Anti-Auxin Properties of TIBA

How well does TIBA fit these requirements? Table 1. gives an indication of many of the responses which appear to be mediated by lowered auxin activity produced by TIBA anti-auxin action. The resulting increase in flowering has been rather generally interpreted as arising from a lowering of auxin levels following treatment, an explanation derived to some extent from the assumption that since auxin levels may inhibit flowering, flower initiation must depend upon a retardation of auxin in the plant (Lang, 1952).

Bonner and Thurlow (1949) demonstrated the inhibitory effect of auxin on photoperiodic induction of flowering in *Xanthium*. When TIBA was added to the solutions in which the cocklebur was growing, the auxin inhibition was overcome, and the rate of floral development was increased. Moreover, Bonner (1949) found that although long days caused *Xanthium* to remain vegetative, applications of TIBA brought about the initiation of flower buds, although they did not mature into fruits. Similarly, Resende and Viana (1952) found that *Kalanchoe blossfeldiana*

which had initiated flower primordia could be returned to the vegetative state by applications of IAA.

Since the presence of apical dominance has been shown to result from high auxin concentrations (Thimann and Skoog, 1933), the loss of apical dominance by TIBA treatment is likely produced by lowered auxin levels in the plant. Similarly, Leopold (1949) indicated that tillering in barley can be increased by TIBA or any method—physical or chemical—which destroys apical dominance. The early flowering effect can also frequently be duplicated mechanically by severing the apical bud with subsequent production of much lateral branching. In whatever manner TIBA causes early flowering in many plants, the phenomenon is accompanied by a loss of apical dominance and it appears reasonable that this results from lowered auxin levels.

The production of ring fasciation is also regarded as an effect on growth as a result of direct interaction of TIBA with indole-acetic acid

Table 1. Examples of TIBA effects which appear to be of an anti-auxin nature

Effect	*Plant*	*Author*
Enhancement of flowering	Soybeans	Fisher and Loomis (1954)
	Red kidney bean	Whiting and Murray (1948)
	Lima bean	Dedolph (1959)
	Kalanchoe	Esteves-de-Sousa (1953)
Loss of apical dominance	Tomato	Gorter (1951)
	Hops	Seeley and Wain (1955)
	Hemp	Heslop-Harrison (1957)
	Barley	Leopold (1949)
Epinasty	Red kidney bean	Whiting and Murray (1948)
	Tomato	Gorter (1951)
Shortening of internodes	Red kidney bean	Snyder (1949)
	Tomato	Gorter (1951)
Abscission of terminal buds	Beans	Waintraub (1952)
	Tomato	Gorter (1951)
Ring fasciation	Tomato	Gorter (1951)
	Tomato	Osborne and Wain (1950)
	Tomato	Wardlaw (1953)
	Tomato	Bedesam (1958)
Alteration of sex expression	Cucumber	Wittwer and Hillyer (1954)
Increase in tillering	Rice	Misra and Sahu (1957,1959)
	Corn	Gausman and Dugger (1954)
	Barley	Leopold (1949)
Reversal of geotropism	Rice	Roberts (1959)
	Sunflower	Audus and Thresh (1956)
Inhibition of xylem diff.	Coleus	Roberts (1960)

(IAA). The nature of the alteration appears to be the critical issue (Heslop-Harrison and Heslop-Harrison, 1957) and will be discussed later in more detail. Additional evidence of the antagonism of these substances is evident from the fact that the formation of ring fasciations by TIBA in *Linum* may be nullified by IAA (Dostal, 1963). Further, Dostal reports that the suppression of petiole growth in *Pisum* seedlings brought about by TIBA can be reversed by IAA.

It is also reasonable to surmise that differential sensitivity of male and female apices to TIBA is connected with a difference in their auxin metabolism at the time of flowering, for there is evidence of unlike native auxin in the two sexes in the early stages of flowering, the male plants being lower than the female (Heslop-Harrison and Heslop-Harrison, 1957).

In essence, these results indicate that marked reduction in free IAA content caused by TIBA applications at non-toxic levels would explain the physiological activities of the compound. It would account for its general inhibition of extension growth in internodes, its action favoring abscission, its suppression of apical dominance, its promotion of maleness, and, if one can accept the postulate that flowering is associated with low auxin levels, its stimulation of flowering.

The Non Anti-auxin Properties of TIBA

The case in favor of TIBA as an anti-auxin is not clearcut, however. The results that fail to relate to the anti-auxin nature of TIBA are summarized in Table 2. Undoubtedly quantitative flower production can be increased by TIBA and other benzoic derivatives, but the question that arises is, do these compounds act as anti-auxins? One must acknowledge that there is a considerable amount of indirect evidence that TIBA is an antagonist of auxin, yet there is not positive, direct evidence available to verify this position.

Table 2. Examples of TIBA effects which fail to confirm an anti-auxin nature

Effect	*Author*
Opposite sequence of activity of halogen substituted benzoic acids in producing flower formation and abscission-inducing activity.	Gorter (1951)
Lack of auxin counteraction in flowering	Audus (1953)
Synergistic effect with IAA in growth test	Thimann and Bonner (1948) Linser (1954)
Inhibition (rather than promotion) of root growth	Aberg (1953) Audus and Shipton (1952)
Inhibition of root hair growth	Gorter (1949)

After numerous experiments Audus (1953) indicated that there is no justification for assuming that TIBA acted on flowering by counteracting the natural auxins and that it probably affected flowering directly as does auxin itself in the instance of pineapple and Wintex barley. The most convincing evidence against TIBA as an anti-auxin comes from Gorter (1951) who found that the sequence of activity in *flower formation* of the three halogen substituted benzoic acids (I, Cl, and Br) was Cl>Br>I, while the exact reverse of the *abscission-inducing* activity was found. One might expect that the abscission inducing effectiveness, where it appears the chemicals *are* acting as anti-auxins, should be manifest in the same sequence with regard to flower forming ability. This, apparently, is not the case.

Where combinations of TIBA and IAA have been used in elongation growth tests, there is no accordance of results. At a molar ratio of IAA to TIBA of 1:1, Galston (1947) found no effect while Thimann and Bonner (1948) obtained an enhancing effect with the *Avena* test. In the *Pisum* test Thimann and Bonner found a clear cut evidence of a stimulating effect of TIBA on IAA curvature when the substances were used in a molar ratio of 1:1. Linser (1954) tested several molar ratios of IAA and TIBA, NAA and TIBA, and 2,4,5-T and TIBA and found that there were synergistic effects shown with the *Avena* test from all combinations of growth promoting and inhibiting substances in low concentrations.

Similarly, Aberg (1953) obtained synergisms of TIBA and IAA in the inhibitions of root growth. TIBA alone gave an inhibition of growth in concentrations of 10^{-8} to 10^{-6} M, but at 10^{-6} to 10^{-5} M a plateau of inactivity was attained. From 10^{-5} to higher concentrations TIBA's inhibitory action increased. The plateau observed with TIBA alone seemed to be related to an antagonism between TIBA and IAA, and at higher concentrations, TIBA caused a distinct inhibition of growth which may not have been related to auxin *per se*.

If the inhibition at low concentrations is due to a synergism, then at this level the depressing effect of TIBA on the IAA content is not being exerted; otherwise a *stimulation* rather than an inhibition would be expected since auxin itself appears to inhibit root growth under natural conditions. (This explanation presupposes that auxins are effective in root growth and that this effect results from supra-optimal concentrations, conditions which have not yet been satisfactorily established experimentally.) Correspondingly, Audus and Shipton (1952) have reported that in root growth tests TIBA could not be demonstrated as an anti-auxin, and Gorter (1949) has observed inhibition of elongation of root hairs by TIBA.

Finally, Christie and Leopold (1965a), using the double inverse Lineweaver-Burk plot in experiments designed to detect TIBA effect on auxin

translocation, showed that even though TIBA was markedly effective its action was of a non-competitive rather than a competitive nature.

In essence, TIBA appears to reverse auxin action and this reversal can be generally overcome by the addition of excess auxin. In this sense, it may be properly called an anti-auxin. Yet, certain cases may be cited where auxin fails to reverse the TIBA action, where IAA and TIBA interact synergistically, and thus competitive antagonism is not exhibited in every case. One cannot say that TIBA acts as an anti-auxin in every test situation at every concentration, and thus the categorization of TIBA as an anti-auxin ultimately depends on how rigidly one desires to apply the rules. Even worse, as helpful as the concept of anti-auxins has been, it, too, may well be imperfect.

TIBA Effects on the Auxin Molecule

While the exact classification may be of only academic interest, the use of TIBA in a practical sense as a tool to study the nature of auxin action is of prime importance. Several theories, although partially related, have been advanced to account for the activity of TIBA and like compounds in regard to their effects on auxin and regulation of plant growth. Attempts have been made to correlate the observed activity with the known architectural structure of these molecules; however, there has not yet been developed any completely tenable explanation which proves how these materials react with the cell substrate and in what manner their structure is related to their activity.

In early work, Thimann and Bonner (1948) proposed that TIBA acted as an inhibitor of the respiratory processes of *Avena* coleoptile sections, while Skoog *et al.* (1942) claimed it acted as a "pseudo-auxin" which occupied active spots on enzymes, thereby inhibiting the auxins from combining with these enzymes.

Muir and Hansch (1953) tested TIBA and over 100 other compounds, while attempting to correlate chemical structure and plant growth regulating activity, and suggested that reaction with a -SH group of a protein cysteinyl unit with both ortho positions in the aromatic ring (two-point ortho reaction theory) was in good agreement with the reactivity of substituted phenols and benzoic acid. Leopold and Price (1957) found that TIBA could react non-enzymatically with sulfhydryl compounds such as cysteine, glutathione, and Coenzyme A. The TIBA reaction presumably involved a condensation of the aromatic nucleus of TIBA with the sulfur atom to form a thio ether.

These conclusions regarding the affinity of TIBA for the -SH group of a substrate are very closely related to the work which has been carried out on the nature and reactivity of IAA-oxidase. While studying the IAA-

IAAO relationships in regulating growth rate, Galston and Dahlberg (1954) found TIBA induced oxidase formation, but only at the relatively high concentration of about 5 x 10^{-6} M. Conversely, Pilet (1960, 1963) has shown that TIBA and glutatione are both active *inhibitors* of the enzymatic destruction of IAA, and when TIBA was added to an IAA-oxidase preparation, the inhibition produced increased with increasing IAA-oxidase activity.

It is almost universally accepted that externally applied TIBA blocks the transport of IAA past the point of application. This question has been investigated in excised plant stems and in intact, growing plants. Among those whose work has led to the conclusion that TIBA acts as an actual blocking agent of IAA movement are Hay (1956), Zwar and Rijven (1956), Niedergang-Kamien and Leopold (1957), Niedergang-Kamien and Skoog (1956) and Kuse (1954, 1959).

Using TIBA as a transport inhibitor, Hertel (1962) found that the movement of auxin *out* of tissues was preferentially inhibted and deduced that the transport of auxins was a secretive process and that TIBA was blocking this step. This preferential inhibition of IAA exit by TIBA was confirmed by Christie and Leopold (1965a) by kinetic studies which showed that TIBA selectively suppressed the exit phase since superior inhibition occurred when TIBA was incorporated in the receptor block rather than the donor block. According to Christie and Leopold (1965b), sulfhydryl antagonists such as TIBA and PCMB (p-chloromercuribenzoic acid) could be interfering with IAA exit by affecting secondary structural features of some protein material, presumably a constituent of the cell plasma membrane.

Although, one may not be prepared to accept this as the only explanation of how TIBA affects the auxin molecule, this is the most logical and best supported interpretation of how the drastic lowering of auxin contents is brought about. Moreover, the results produced by TIBA on the polarity of auxin transport are certainly consistent with the physiological and morphological alterations of plant growth and could account for the nature of the TIBA effect. It might also be possible that TIBA interference with IAA transport could cause enhanced auxin effects in cells where auxin is accumulated and *anti-auxin* effects in cells thus deprived of auxin. This would relate to the conflicting evidences of positive and negative effects of TIBA on the auxin systems discussed previously.

Conclusions

TIBA can bring about a host of morphological and physiological modifications which appear to generally result from an interaction with

auxin. However, the classification of TIBA as an anti-auxin appears to be largely a problem of semantics and may not even be a moot question.

The manner in which TIBA antagonizes the auxin molecule appears to be through interference with the exit of IAA from a particular tissue.

The ability of TIBA to induce profuse flowering and fruiting with concomitant loss of apical dominance in some species encourages the feeling that it may be of potential use in production of food and fiber.

Moreover, it has also filled an important role as a tool in elucidating the nature of auxin action in plant growth regulation and it is certain that this importance will continue to increase.

Literature Cited

Aberg, B. 1953. On the interaction of 2,3,5-triiodobenzoic acid and maleic hydrazide with auxins. Physiol. Plant. 6:277-291.

Audus, L. J. 1953. Plant growth substances. London, Leonard Hill Lion.

——— and R. Thresh. 1956. The effects of synthetic growth substances in the level of endogenous auxins in plants. Ann. Bot. 20:439-459.

——— and M. E. Shripton. 1952. 2,4-dichloranisole-auxin interactions in root growth. Physiol. Plant. 5:430-437.

Bedesam, P. P. 1958. Histogenic effects of 2,3,5-triiodobenzoic acid on the shoot apices and leaf primordia of tomato. Bull. Torrey Botan. Club 85:434-472.

Bonner. J. 1949. Further experiments on flowering in *Xanthium*. Bot. Gaz. 110:625-627.

——— and J. Thurlow. 1949. Inhibition of photoperiodic induction in *Xanthium* by applied auxin. Bot. Gaz. 110:613-624.

Christie, A. E. and A. C. Leopold. 1965a. On the manner of triiodobenzoic acid inhibition of auxin transport. Plant Cell Phys. 6:333-345.

——— and ———. 1965b. Entry and exit of indoleacetic acid in corn coleoptiles. Plant Cell Phys. 6:453-465.

Dedolph, R. R. 1959. Response of lima beans to growth regulators. Thesis Univ. of Maryland. 174 p.

Dostal, R. 1963. Use of 2,3,5-triiodobenzoic acid in studies on the growth correlation differences between epigeous and hypogeous seedlings *(Linum and Pisum)*. Biologia. Plant. (phaha) 5:68-76.

Esteves-de-Sousa, A. 1950. The action of synthetic auxin and synthetic anti-auxin in the flowering of *Kalanchoe rotundifolia Haw*. Portug. Acta. Biol. 3:91-108.

———. 1953. On the action of synthetic phytohormones upon the flowering stimulus of *Bryophillum daigremontianum*. Portug. Acta. Biol. 3:323-335.

Fisher, J. E. and W. E. Loomis. 1954. Auxin-florigen balance in flowering of soybean. Science 119:71-73.

Galston, A. W. 1947. Effect of 2,3,5-triiodobenzoic acid on the growth and flowering of soybeans. Amer. J. Bot. 34:356-360.

——— and L. Dalberg. 1954. Adaptive formation and physiological significance of indoleacetic acid oxidase. Amer. J. Bot. 41:373-380.

Gausman, H. W. and G. H. Dungan. 1954. Some effects of certain plant growth substances on maize. Bot. Gaz. 116:29-40.

Gorter, C. J. 1949. Action of 2,3,5-triiodobenzoic acid on growth of root hairs. Nature 164:800-801. 1949.

———. 1951. The influence of 2,3,5-triiodobenzoic acid on the growing points of tomatoes. II. The initiation of ring fasciations. Proc. Konimkl. Nederland. Akad. Wetenschap. 54:181-187.

Hay, J. R. 1956. The effect of 2,4-dichlorophenoxyacetic acid and 2,3,5-triiodobenzoic acid on the transport of indoleacetic acid. Plant Physiol. 31:118-120.

Hertel, R. 1962. Der Auxintransport in der Koleoptile von *Zea mays*. Doctoral Diss. Ludwig Maximilians Univ., Munich. 72 pp. (Cited by Christie, A. E. and A. C. Leopold).

Heslop-Harrison, J. and Y. Heslop-Harrison. 1957. Studies on flowering-plant growth and organogenesis. I. Morphogenetic effects of 2,3,5-triiodobenzoic acid on *Cannabis sativa*. Proc. Roy. Soc. Edinburgh 66:409-423.

Kuse, G. 1953. Effect of 2,3,5-triiodobenzoic acid on the growth of lateral bud and on tropism of petiole. Mem. Coll. Sci. Univ. Kyoto 20:207-215.

———. 1954. Bud inhibition and correlation growth of petiole in sweet potato stem. Mem. Coll. Sci. Univ. Kyoto 21:107-114.

Lang, A. 1952. Physiology of flowering. Ann. Rev. Pl. Physiol. 3:265-306.

Leopold, A. C. 1949. The control of tillering in grasses by auxin. Amer. J. Bot. 36:437-440.

——— and F. S. Guernsey. 1954. Flower initiation in the Aslaka pea. II. Chemical vernalization. Amer. J. Bot. 41:181-185.

——— and C. A. Price. 1957. Sulfhydryls in plants. I. Reactions with growth regulators. Plant Physiol. 32:520-525.

Linser, H. 1954. Mode of action of growth-stimulating and inhibiting substances. IV. The concentration-activity curves of some synthetic growth-stimulating compounds in the presence of synthetic inhibitors. Biochem. and Biophys. Acta. 15:25-30.

McRae, D. H. and J. Bonner. 1953. Chemical structure and anti-auxin activity. Physiol. Plant. 6:485-510.

Misra, G. and G. Sahu. 1957. Control of flowering in rice by plant-growth substances. Nature 180:816.

——— and ———. 1959. Physiology of growth and reproduction in rice. II. Effect of plant growth substances on three winter varieties. Plant. Physiol. 34:441-445.

Niedergang-Kamien, E. and F. Skoog. 1956. Studies on polarity and auxin transport in plants. I. Modification of polarity and auxin transport by triiodobenzoic acid. Physiol. Plant. 9:60-73.

——— and A. C. Leopold. 1957. Inhibitors of polar auxin transport. Physiol. Plant. 10:29-37.

Osborne, D. J. and R. L. Wain. 1950. Studies on plant growth regulating substances. II. Synthetic compounds inducing morphogenic responses in the tomato plant. J. Hort. Sci. 26:60-74.

Pilet, P. E. 1963. Action of 2,3,5-triiodobenzoic acid and of glutathione on the morphology and auxin catabolism of tissues cultured *in vitro*. Physiol. Plant. 16:299-310.

———. 1960. Le catabolisme auxinique. Bull. Soc. frane. Physiol. veg. 6:119.

Resende, F. and M. J. Viana. 1952. Some effects of synthetic auxins and anti-auxins on the floral state. Bol. Soc. Portug. Cience. Nat. 4:74-88.

Roberts, E. H. 1959. Geotropic and morphological alterations in rice seedlings caused by plant growth regulators. Nature 183:1197-1198.

———. 1960. Xylem regeneration in stem wound response in Coleus. Bot. Gaz. 121:201-205.

Seeley, R. C. and R. L. Wain. 1955. Studies on plant growth-regulating substances. IX. Investigations with pollen extracts and synthetic growth substances on the hop plant *(Humulus lupulus* L.) Ann. Appl. Biol. 43:355-365.

Skoog, F. 1942. Interactions of auxins in growth and inhibition. Amer, J. Bot. 29:568-576.

Snyder, W. E. 1949. Some responses of plants to 2,3,5-triiodobenzoic acid. Plant Physiol. 24:195-206.

Thimann, K. V. and W. D. Bonner. 1948. The action of triiodobenzoic acid on growth. Plant Physiol. 23:158-161.

——— and F. Skoog. 1933. Studies on the growth hormone in plants. Proc. Natl. Acad. Sci. 19:714-716.

Wardlaw, C. E. 1953. Action of triiodobenzoic and trichlorobenzoic acids in morphogenesis. New Phytol. 52:210-217.

Weintraub, R. L. 1952. Studies on the relation between molecular structure and physiological activity of plant growth-regulators. I. Abscission-inducing activity. Bot. Gaz. 113:348-362.

Whiting, A. G. and M. A. Murray. 1948. Abscission and other responses induced by 2,3,5-triiodobenzoic acid in bean plants. Bot. Gaz. 109:447-473.

Wittwer, S. H. and I. G. Hillyer. 1954. Chemical induction of male sterility in cucurbits. Science 120:893-894.

Zimmerman, P. W. and A. E. Hitchcock. 1942. Substituted phenoxy and benzoic acid growth substances and the relation of structure to physiological activity. Contrib. Boyce Thompson Inst. 15:353-361.

Zwar, J. A. and A. H. G. C. Rijven. 1956. Inhibition of transport of indole-3-acetic acid in the etiolated hypocotyl of *Phaseolus vulgaris* L. Austral. J. Biol. Sci. 9:528-538.

8-68/1¼M