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Comparison of Zeatin Indoleacetate with Zeatin and Indoleacetic Acid in the Tobacco Bioassay[1]

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RUTH Y. SCHMITZ AND FOLKE SKOOG
Institute of Plant Development, Birge Hall, University of Wisconsin, Madison, Wiscosin 53706
SIDNEY M. HECHT[2] AND NELSON J. LEONARD
School of Chemical Sciences, University of Illinois, Urbana-Champaign, Illinois 61801

ABSTRACT

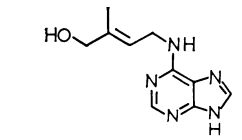
Zeatin indole-3-acetate, 6-[4-(indole-3-acetoxy)-3-methyl-*trans*-2-butenylamino]purine, is at least as effective as zeatin on a molar basis in satisfying the cytokinin requirement for growth and bud formation in tobacco bioassays. It is less effective than indole-3-acetic acid and is needed as a variable function of the cytokinin concentration for satisfying the optimal requirement of an auxin. Comparisons of the types of growth and yield of tissue obtained with serial concentration of the ester and with equimolar mixtures of its free base and acid indicate that the relative requirement for auxin changes with the concentration of cytokinin and is related to the types of callus growth and differentiation which occur. The results also suggest that the ester serves as a source of auxin only after modification, presumably by hydrolysis to indoleacetic acid.

In a study of the activity of esters of zeatin (Scheme I) (5), the indoleacetic acid ester, Z-*O*-IAA[3] (Scheme II), was synthesized and tested for cytokinin activity in the tobacco bioassay. Like the formate, acetate, and propionate esters of zeatin and the 2-chloro-substituted derivatives of the latter two compounds, it was found to be fully as active or even slightly more active than zeatin itself.

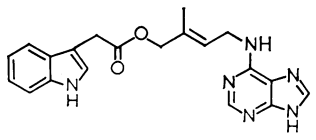
The substance is of special interest in representing a possible easily reversible and unique position for auxin attachment to transfer ribonucleic acids in plants. For example, zeatin, which is present in certain species of tRNA in plants, might be esterified with indoleacetic acid. It also is of interest in having the structural features of both an auxin and a cytokinin incorporated into one molecule, thus being able to serve as a single source of IAA and zeatin in physiological experiments.

MATERIALS AND METHODS

Bioassay Procedure. Cytokinin activity was determined by the tobacco bioassay (3), based on fresh weight yields of cytokinin-dependent callus tissue derived from *Nicotiana tabacum* var. Wisconsin No. 38.



Scheme I



Scheme II

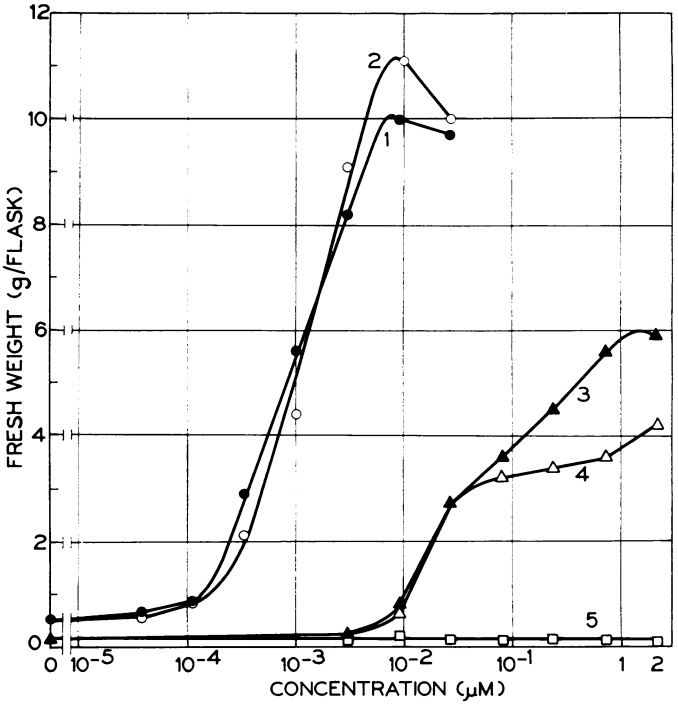


FIG. 1. Effect of zeatin (Scheme I) and zeatin indoleacetate (Scheme II) on tobacco callus growth in the presence and absence of indoleacetic acid. Curve 1: zeatin with 11 μM IAA; curve 2: Z-*O*-IAA with 11 μM IAA; curve 3: zeatin and IAA in equimolar combinations; curve 4: Z-*O*-IAA without IAA; curve 5: zeatin without IAA.

The medium contained the mineral salts and organic compounds specified by Linsmaier and Skoog (ref. 3, Table 6, A and B). The indole-3-acetic acid was dissolved in water and added to the media before autoclaving. The zeatin and zeatin ester were dissolved in Me_2SO,[3] 3-fold serial dilutions were

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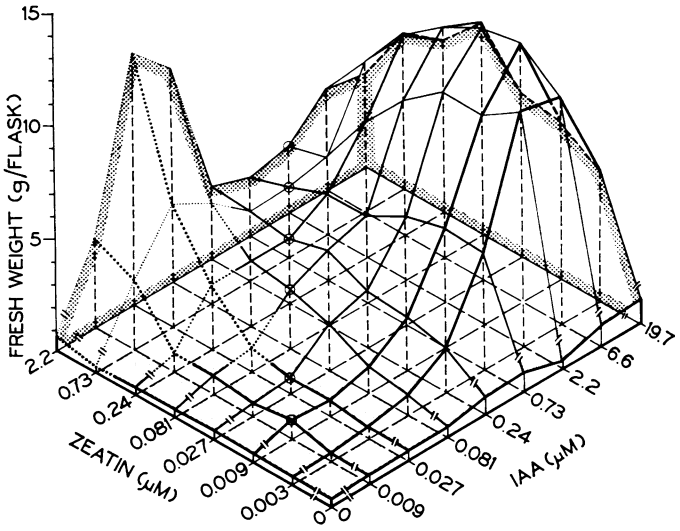
[2] National Institutes of Health predoctoral Research Fellow. Present address: Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Mass., 02139.

[3] Abbreviations: Me_2SO: dimethylsulfoxide; Z-*O*-IAA: zeatin indole-3-acetate, 6-[4-(indole-3-acetoxy)-3-methyl-*trans*-2-butenylamino]purine.



made, and the materials were added to the cooling, autoclaved media at the uniform rate of 0.05% (v/v), a concentration of Me_2SO which does not affect growth of the tobacco tissue (4). This procedure protected the compounds from possible degradation by heat.

Each treatment consisted of 12 pieces of callus, about 40 mg each, planted three apiece in 125-ml culture flasks containing 50 ml of medium, and cultured for a 4- or 5-week period in the dark at 28 C, with only brief occasional exposure to light to permit observation during the culture period. The stock tissue consisted of callus which had been derived from pith tissue and maintained on the above media supplemented with 300 μg/l of kinetin, followed by two 3-week passages on media supplemented with 30 μg/l of kinetin.

Compounds. Indole-3-acetic acid was purchased from Nutri-

FIG. 2. Fresh weight yields of tobacco callus cultured on various concentrations of zeatin and IAA. Yields for equimolar concentrations are indicated by small circles; points joined by dotted lines indicate the concentration combinations which resulted in budding.

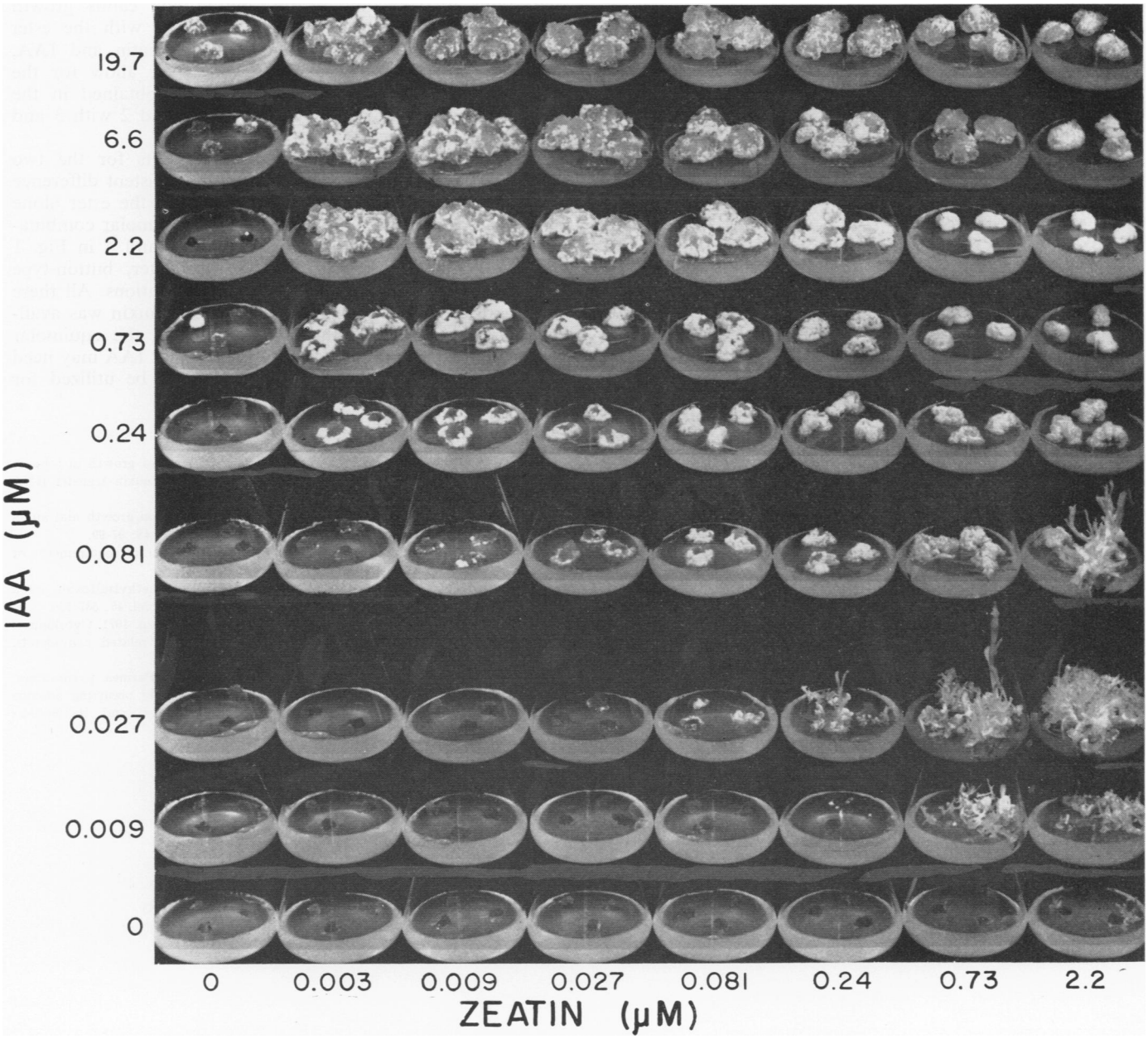


FIG. 3. Representative callus cultures illustrating the types of growth obtained with various combinations of zeatin and IAA. Ordinate: zeatin (μM); abscissa: IAA (μM).

tional Biochemicals Corporation. Zeatin indole-3-acetate and zeatin were obtained by previously published methods (5, 6).

RESULTS AND DISCUSSION

As shown in Figure 1, in the presence of the standard 11 μM concentration of IAA, the growth response curves of zeatin, curve 1, and of Z-*O*-IAA, curve 2, are practically identical. In contrast to zeatin, however, the ester is also active when added to media without IAA (compare curves 4 and 5 with curves 1 and 2). In the low portion of the concentration range (0.003–0.03 μM) the yield curves for the ester alone (curve 4) and for equimolar combinations of zeatin and IAA (curve 3) are nearly identical, but in the range from 0.05 to 1 μM the yields obtained for the ester alone suggest that these tissues have reached a growth plateau, while the yields for the zeatin and IAA combinations continue to rise at a fairly uniform rate. Following this plateau, the yield curve for the ester again accelerates so that in the 2 μM region the yields obtained with the ester alone and with the equimolar combinations of zeatin and IAA are nearly the same.

It is clear from the rapid growth of callus supplied with low concentrations of the ester in the presence of an ample supply of IAA (Fig. 1, curve 2) that the ester can function as, or perhaps releases, a nearly equimolar quantity of zeatin and that it is the concentration of auxin which limits growth on media supplied with the ester but without additional IAA.

A possible explanation of the growth plateau obtained with moderate concentrations of the ester alone was derived from a study of callus tissues grown on media supplying varying proportions of zeatin and IAA (Fig. 2). It was apparent that the molar concentration of IAA required for growth was greater than that of zeatin, and that the relative requirement for IAA changed with the concentration of zeatin.

The growth pattern of the callus reflected this changing requirement (Fig. 3). The "growth form" of so-called undifferentiated callus may vary from a watery or jelly-like mound of large, translucent cells to a compact mass of small, white cells, referred to as a "button." Intermediate types between these extremes range from a watery mass with interspersed small-celled meristematic centers to tissues with a watery center and peripheral layers of firm white callus. As would be expected, differentiation of buds occurred only on media with high zeatin: IAA ratios (Figs. 2 and 3). It should be noted that in the presence of very high cytokinin concentrations (6.6 and 19.7 μM), synthesis of auxin by the tissue is stimulated (1, 2). Furthermore, the buds that develop on the callus also produce auxin, so that once budding has been initiated in tissue cultured without exogenous IAA, callus growth is enhanced.

Except in budding cultures, very little growth occurred on low IAA media. At intermediate IAA levels such as 0.2 to 0.7 μM, which were suboptimal for growth, increasing zeatin levels promoted "button" formation. At optimal IAA levels, 2.2 to 20 μM, vigorous callus growth was promoted in the presence of adequate zeatin supplies (0.003 to 0.1 μM) and only at higher zeatin concentrations (0.7–2.2 μM) did button-type growth predominate. In general, a 30- to 100-fold excess of IAA over zeatin was required to obtain large mounds of fast growing tissue. Therefore, in treatments with equimolar concentrations of IAA and zeatin, button-type callus growth predominated. This explains why treatments with the ester alone or with equimolar combinations of zeatin and IAA, regardless of the concentration, would never allow for the large maximum yields of tissue that were obtained in the presence of added IAA (compare curves 1 and 2 with 3 and 4 in Fig. 1).

The difference in the relative requirements for the two hormones can also explain the slight but consistent difference in the growth pattern of callus supplied with the ester alone as compared with that of callus receiving equimolar combinations of zeatin and IAA (compare curves 3 and 4 in Fig. 1 and see Fig. 4). In the presence of the ester, button-type growth appeared at somewhat lower concentrations. All these effects are consistent with the view that less auxin was available to the tissue from the ester than from the equimolar combinations of zeatin and IAA, and thus that IAA may need to be released from the ester before it can be utilized for growth.

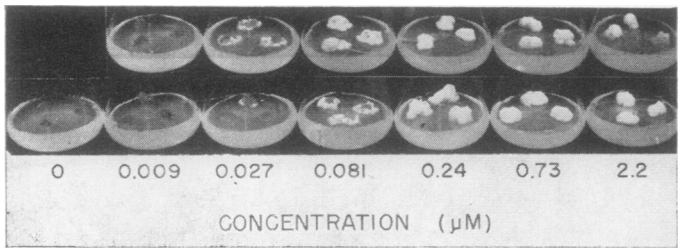


FIG. 4. Representative callus cultures for comparison of the types of growth obtained with the ester alone (upper row) and with equimolar combinations of zeatin and IAA (lower row).

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