SYNTHESIS AND STRUCTURAL CHARACTERIZATION OF ORGANOTIN AND GROUP IVB METALLOCENE DICHLORIDES WITH KINETIN

DONNA M. CHAMELY

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By

Donna M. Chamely

A Thesis Submitted to the Faculty of
The Charles E. Schmidt College of Science
in Partial Fulfillment of the Requirements for the Degree of
Master of Science

Florida Atlantic University
Boca Raton, Florida
August 2000

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This thesis was prepared under the direction of the candidate's dissertation advisor, Dr. Charles Carraher Jr., Department of Chemistry and Biochemistry, and has been approved by the members of her supervisory committee. It was submitted to the faculty of the Charles E. Schmidt College of Science and was accepted in partial fulfillment of the requirements for the degree of Master of Science.

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7/25/00

Date

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Vice Provost

ABSTRACT

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Title: Synthesis and Characterization of Organotin and Group IVB Metallocene Dichlorides with Kinetin.

Institution: Florida Atlantic University

Dissertation Advisor: Dr. Charles E. Carraher Jr.

Degree: Master of Science

Year: 2000

Metal containing polymers were synthesized by reaction of organotin and group IVB metallocene dichlorides with kinetin, a plant growth hormone (PGH), via the interfacial polycondensation process. Product yields were in the range of 40-63% and degrees of polymerization were from 20 to 150. The structures of the products were determined using infrared and mass spectroscopy. Further characterization involved determination of solubility, degree of polymerization and electrical conductivity. The biological potential of the products was tested against selected microorganisms and Balb/3T3 cell lines. One of the products showed activity in the 0.2μg/ml range, one hundreth of that of similar compounds. Germination experiments using the PGH incorporated polymers were performed on sawgrass and cattail seeds as well as selected vegetable seeds and compared to a control. Sawgrass germination, typically in the 0-2% range were increased to about 60% through the treatment with kinetin-containing polymers.

ACKNOWLEDGEMENTS

The author would like to extend her appreciation to the faculty of the Chemistry Department at Florida Atlantic University for their helpfulness and for the knowledge imparted to her. She would like to thank Dr Herbert Stewart for his help in the viability and germination experiments for her thesis. She specially thanks Dr. Jerome E. Haky for his guidance about the IR as well as his sound advice throughout her undergraduate and graduate career.

The author must extend her sincerest appreciation to her advisor Dr. Charles E. Carraher Jr. for his valuable time and guidance throughout the process of the research and the writing of this thesis as well as his wisdom in life. The author will always treasure his support, encouragement and kindness.

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DEDICATION

To my parents Edmund and Josephine, for their sacrifices, love, and guidance over the past years.

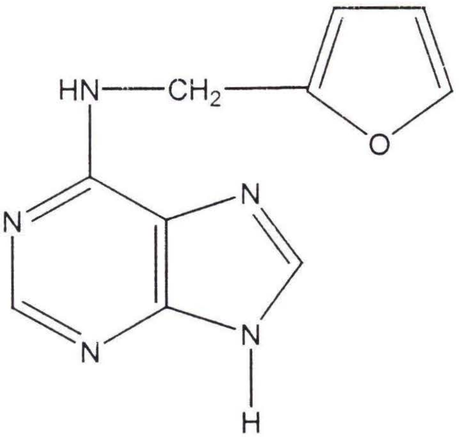
PART I:

INTRODUCTION

1.1: Plant Growth Hormones.

Plant Growth Hormones (PGH) are organic compounds that are created in one part of a plant, translocated to another part of the plant, where in small concentrations they stimulate a physiological response (6). There are currently five recognized groups of plant hormones: **auxins, gibberellins, cytokinins, abscisic acid (ABA)** and **ethylene.** In addition to the five principal hormones, two other groups appear to be active in regulating plant growth, the **brassinosteroids** and the **polyamines** (1). The PGH that was used in this study was kinetin. Kinetin comes from the PGH group of Cytokinins, all derived from the nitrogenous purine base adenine.

Cytokinins are N^6- substituted derivatives of the nitrogenous- purine base adenine and are characterized by their ability to stimulate cell division in tissue cultures (1). Their LD 50's are greater than 5000mg/kg, consistent with them not being very toxic compounds (13). Kinetin was the first cytokinin to be discovered. The structure of kinetin is shown in figure one:



Structure of kinetin

Figure 1

Folke Skoog and Carlos Miller, from the University of Wisconsin, were the discoverers of kinetin. They found that autoclaved herring sperm DNA had a powerful cell division-promoting effect on cultured tobacco pith tissue. Skoog and Miller identified a small molecule, which in the presence of an auxin, stimulated proliferation of tobacco pith cells. It was named kinetin and was identified as being an adenine derivative, 6-furfurylaminopurine (31).

Since their discovery, Cytokinins have been shown to have effects on many other physiological and developmental processes including leaf senescence, nutrient mobilization, apical dominance, the formation and activity of shoot apical meristems, floral development, the breaking of bud dormancy and seed germination (1).

Through the use of chemical technology, large increases have been reported in recent decades in the production of agronomic crops in the United States (16). The development and use of endogenous PGR's, which occur naturally in plants, and the development and use of new and existing synthetic PGR's, may help to make up deficiencies in crop production due to the decrease use of insecticides and fungicides in the future (8).

In order for a PGR to elicit optimum physiological response from a plant, it must satisfy at least two requirements:

1) It must be present in carefully controlled amounts in a given tissue.
2) It must be available continuously at the site of action.

These two requirements may possibly be met by the controlled release from a polymer of a biologically active agent, a PGR (8).

Carraher et al has been incorporating PGR's into polymers and measuring the responses against the non incorporated PGR itself (7). Since many of these plant responses are concentration dependent, a comparison of the response of the polymeric material containing the PGR and the PGR itself gives an indication of the amount of PGR released. Further, since the chemical bonds connecting the PGR in the polymer are analogous to those employed in studies involving drug delivery for human treatment, it is possible that extensions of these results may be appropriately extended to the drug delivery systems intended for human treatment (7).

1.2: Tin Containing Polymers

Carraher et al have synthesized a variety of metal-containing polymers using the interfacial condensation technique. Among these polymers are tin containing polymers. The emphasis on the use of tin containing polymers include:

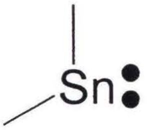
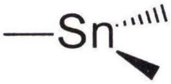
1) Tin-containing compounds can form both ionic and covalent bonds
2) Tin-containing compounds offer a ready variety of stable valences namely +2 and +4.
3) A wide variety of organostanne compounds exist which would allow structure-property studies
4) Tin-containing compounds generally exhibit biological activities that may prove advantageous.

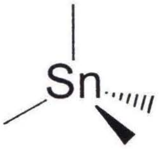
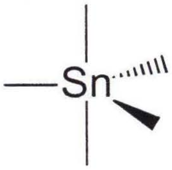
Characteristics of organotin compounds

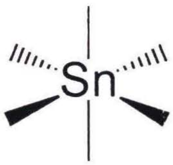
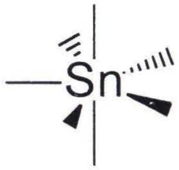
Organotin compounds are generally found in two oxidation states, Sn^{2+} (stannous) and Sn^{4+} (stannic). Organotin compounds offer a wide variety of structural types in which the tin atom is two to seven coordinated (11). Geometries of organotin compounds include bent, trigonal, tetrahedral, trigonal bipyramidal, octahedral and pentagonal bipyramidal for two to seven-coordination, as described in Figure 2:

Figure 2

Geometries of organotin compounds.

Coordination number	2	3
		
Geometry	bent	Trigonal

Coordination Number	4	5
		
Geometry	tetrahedral	trigonal bipyramidal

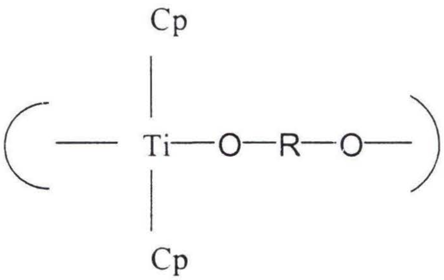
Coordination number	6	7
		
Geometry	octahedral	pentagonal bipyramidal

1.3: Organometallic compounds

Organometallic derivatives of titanium have been widely investigated, primarily as a result of ability to act as excellent catalysts in polymerization reactions. The interest in organotitanium derivatives was largely initiated by the discovery of the Ziegler-Natta catalysts (18,28). A majority of the research with organomettalic derivatives of titanium involves л-cyclopentadienyl derivatives, such as the bis-(cyclopentadienyl) derivatives. These derivatives are the most stable of the organotitanium derivatives, and thus allow for their isolation (10). Carraher et al have synthesized a wide variety of titanocene-containing polymers derived from the condensation of the bis(cyclopentadienyl)titanium dichloride with diols (22, 17), diamines (14) and salts of dicarboxyllic acids (21), the structures of which are as follows:

Figure3.

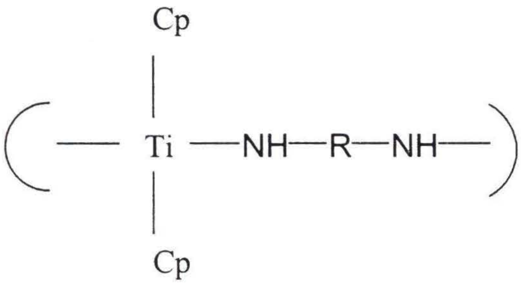
Representation of a diol – Cp_2Ti containing polymer.



$$\left(- \overset{\mathrm{Cp}}{\underset{\mathrm{Cp}}{\mathrm{Ti}}} - \mathrm{O} - \mathrm{R} - \mathrm{O} - \right)$$

Figure 4.

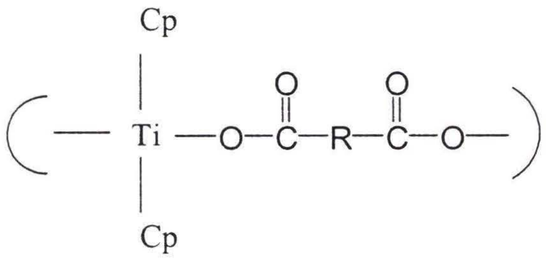
Representation of a diamine –Cp_2Ti contining polymer



$$\left(- \overset{\mathrm{Cp}}{\underset{\mathrm{Cp}}{\mathrm{Ti}}} - \mathrm{NH} - \mathrm{R} - \mathrm{NH} - \right)$$

Figure 5.

Representation of a dicarboxyllic acid- Cp_2Ti containing polymer



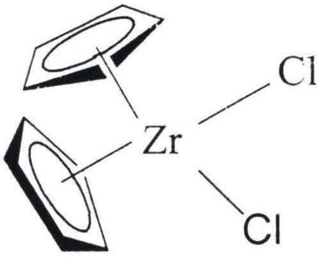
$$\left(- \overset{\mathrm{Cp}}{\underset{\mathrm{Cp}}{\mathrm{Ti}}} - \mathrm{O} - \overset{\mathrm{O}}{\overset{\|}{\mathrm{C}}} - \mathrm{R} - \overset{\mathrm{O}}{\overset{\|}{\mathrm{C}}} - \mathrm{O} - \right)$$

Compounds containing the Cp_2Ti moiety are used as catalysts, as anticancer agents (experimental stage), and in the manufacture of paints, fibres, textiles, wood products and paper to add temperature, pressure and light stability (24,15). For the present purpose, the Cp_2Ti moiety will be considered as a relatively inactive connective-carrier material.

The bis(cyclopentadienyl) compounds of zirconocene and hafnocene dichlorides are the most widely used zirconium and hafnium organometallic compounds (18). These compounds, and their alkyl-substituted counterparts, are somewhat air-stable, and exist as tetrahedral structures(18). As in the case of the analogs of Cp_2TiCl_2, the products have an essentially tetrahedral arrangement of the ligands about the metal for Cp_2ZrCl_2 and its hafnium analog (18). The structure of Cp_2ZrCl_2 is as follows:

Figure 6.

Structure of Bis(cyclopentadienyl) Zirconium Dichloride.



In some way these metallocene products can be considered as tetrahedral sandwich-like products, where the M-Cp is connected through the π- bond on the Cp so that the Cp is flat against the metal rather than being pointed towards the metal.

Bis(cyclopentadienyl) Compounds Containing the Metal- Nitrogen Bond.

Lappert and co-workers have developed the organometallic chemistry of the amido complexes of titanium, zirconium and hafnium to a large extent (5,23,26). The utility of the amido complexes in organometallic synthesis lies in their behavior with weakly acidic hydrocarbons (pK_a up to 20) such as cyclopentadiene and with compounds such as alcohols and thiols (18).

Kinetin was used in combination with group (IVB) metallocene dichlorides and organotin dichlorides to form polymers. Once the polymers were formed, physical and structural characterizations were performed, and applications using the polymers were performed on a variety of seeds, including the sawgrass and cattail from the Everglades.

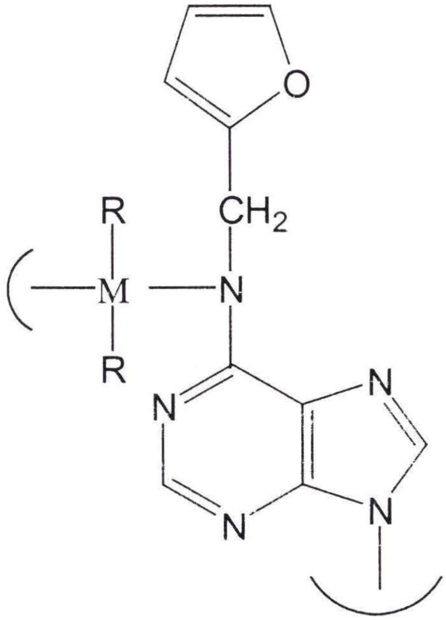
Controlled release polymers can offer a longer "shelf life", greater retention of the active agent (particularly in nature where the non-polymer form is soluble in water) due to the insolubility or lowered solubility of the polymer, sustained release of the active agent, and the co-reactant could impart some special additional property to the system (such as algal and fungal control for polymers containing the organotin moieties) (2).

1.4: Current Research

The current study involves incorporation of kinetin into members of Group IVB metallocene and organotin-containing polymers of the following form:

Figure 7

Structure of polymer formed from incorporation of kinetin with organotin and Group IVB metallocene compounds.



The major goals for this research are:

1) the synthesis and the structural characterization of the polymers
2) the preliminary evaluation of these polymer products with respect to the germination of sawgrass and cattail seeds.
3) The preliminary evaluation of these polymer products with respect to the germination of selected agronomic seeds.
4) The preliminary evaluation of the biological activity of the products with respect to their behavior in the presence of selected cells (as pertaining to cancer) and selected bacteria (as pertaining to antibacterial activity).

PART II:

EXPERIMENTAL

2.1: MATERIALS.

The following chemicals were used without additional treatments.

1) Acetone, Fisher Chemicals, New Jersey, 07410
2) Chloroform, Fisher Chemicals, New Jersey 07410
3) Dilauryltin dichloride, Aldrich Chemical Co. Milwaukee, WI 53233
4) Dibutyltin dichloride, Aldrich Chemical Co. Milwaukee, WI 53233
5) Diethyltin dichloride, Aldrich Chemical Co. Milwaukee, WI 53233
6) Dimethyl sulfoxide, Fisher Chemicals, New Jersey 07410
7) Dimethyltin dichloride, Aldrich Chemical Co. Milwaukee, WI 53233
8) Dioctyltin dichloride, Aldrich Chemical Co. Milwaukee, WI 53233
9) Hafnocene dichloride [bis(cyclopentadienyl)hafnium dichloride] , Thiokol/Ventron Division, Alfa Products, Danvers, MA 01923
10) Hexane, Fisher Chemicals , New Jersey 07410
11) Kinetin, 99%, Aldrich Chemical Co., Milwaukee, WI 53233
12) Methanol, Fisher chemicals, New Jersey, 07410
13) (Methyl sulfoxide)-d_6 99.9%, Aldrich Chemical Co., Milwaukee, WI 53233
14) Potassium bromide, Fisher Chemicals, New Jersey, 07410
15) Sodium hydroxide, Fisher Chemicals, New Jersey 07410
16) Talcum powder- Laboratory grade, Fisher Chemicals, New Jersey 07410
17) Titanocene dichloride [bis(cyclopentadienyl)titanium dichloride] , 97%, Aldrich Chemical Co., Milwaukee, WI 53233
18) Zirconocene dichloride [bis(cyclopentadienyl)zirconium dichloride], Ventron, Alta Products, Beverly MS.

2.2: SYNTHESES

All of the condensation polymerizations were carried out in a one quart Kimax emulsifying jar, placed on a Waring blender (Model 1120), with a no load speed of 18,000 r.p.m.

Products from Metallocenes (IV B) Dichlorides and Kinetin.

Compound #1

A red chloroform solution (50ml) containing Cp_2TiCl_2 (1.245g, 0.0050mol) was added to a stirring aqueous solution (50 ml) containing kinetin (1.075g, 0.0050mol) and standardized sodium hydroxide (0.40g, 0.010mol). The resulting solution was blended for thirty seconds. The color of the solution changed from red to pale yellow. The yellow precipitate was recovered using vacuum filtration and washed several times with de-ionized water and chloroform, to remove any excess unreacted materials. The product was then placed in a pre-weighed petri dish and left in a fume hood overnight to dry. The product weighed 1.27g and the yield calculated as 55% .

Compound #2

A chloroform solution (50ml) containing Cp_2ZrCl_2 (1.4615g, 0.0050mol) was added to a stirring aqueous solution (50ml) containing kinetin (1.075g, 0.0050mol) and standardized solution sodium hydroxide (0.40g, 0.010mol). The resulting solution was blended for thirty seconds. The precipitate was recovered using vacuum filtration and washed several times with de-ionized water and chloroform to remove any excess unreacted materials. The product was then placed in a pre-weighed petri dish and left overnight in a fume hood to dry. The product weighed 1.59g and the yield calculated as 63 % .

Compound #3

A chloroform solution (50ml) containing Cp_2HfCl_2 (1.8989. 0.0050mol) was added to a stirring aqueous solution (50ml) containing kinetin (1.075g, 0.0050mol) and standardized solution sodium hydroxide (0.40g, 0.010mol). The resulting solution was blended for thirty seconds. The precipitate was recovered using vacuum filtration and washed several times with de-ionized water and chloroform, to remove any excess unreacted materials. The product was then placed in a pre-weighed petri dish and left overnight in a fume hood to dry. The product weighed 1.85g and the yield calculated as 63 %.

Products of Organotin Dichlorides and Kinetin.

Compound #4

A chloroform solution (50ml) containing $(CH_3)_2SnCl_2$ (1.100g, 0.0050mol) was added to a stirring aqueous solution (50ml) containing kinetin (1.075g, 0.0050mol) and standardized solution sodium hydroxide (0.40g, 0.010mol). The resulting solution was blended for thirty seconds. The precipitate was recovered using vacuum filtration and washed several times with de-ionized water and chloroform, to remove any excess unreacted materials. The product was then placed in a pre-weighed petri dish and left overnight in a fume hood to dry. The product weighed 1.58g and the yield calculated as 43 %.

Compound #5

A chloroform solution (50ml) containing $(C_2H_5)_2SnCl_2$ (1.245g,0.0050mol) was added to a stirring aqueous solution (50ml) containing kinetin (1.075g, 0.0050mol) and standardized solution sodium hydroxide (0.40g, 0.010mol). The resulting solution was blended for thirty seconds. The precipitate was recovered using vacuum filtration and washed several times with de-ionized water and chloroform, to remove any excess unreacted materials. The product was then placed in a pre-weighed petri dish and left overnight in a fume hood to dry. The product weighed 2.22 g, and the yield calculated as 56 %.

Compound #6

A chloroform solution (50ml) containing $(C_4H_9)_2SnCl_2$ (1.525g, 0.005mol) was added to a stirring aqueous solution (50ml) containing kinetin (1.075g, 0.0050mol) and standardized solution sodium hydroxide (0.40g, 0.010mol). The resulting solution was blended for thirty seconds. The precipitate was recovered using vacuum filtration and washed several times with de-ionized water and chloroform, to remove any excess unreacted materials. The product was then placed in a pre-weighed petri dish and left overnight in a fume hood to dry. The product weighed 2.85g, and the yield calculated as 63 %.

Compound #7

A chloroform solution (50ml) containing $(CH_3(CH_2)_{11})_2$ $SnCl_2$ (2.6380g, 0.005mol) was added to a stirring aqueous solution (50ml) containing kinetin (1.075g, 0.0050mol) and standardized solution sodium hydroxide (0.40g, 0.010mol). The resulting solution was blended for thirty seconds. The precipitate was recovered using vacuum filtration and washed several times with de-ionized water and chloroform, to remove any excess unreacted materials. The product was then placed in a pre-weighed petri dish and left overnight in a fume hood to dry. The product weighed 4.23g, and the yield calculated as 65 %.

Compound #8

A chloroform solution (50ml) containing $(C_8H_{17})_2$ $SnCl_2$ (2.078g, 0.005mol) was added to a stirring aqueous solution (50ml) containing kinetin (1.075g, 0.0050mol) and standardized solution sodium hydroxide (0.40g, 0.010mol). The resulting solution was blended for thirty seconds. The precipitate was recovered using vacuum filtration and washed several times with de-ionized water and chloroform, to remove any excess unreacted materials. The product was then placed in a pre-weighed petri dish and left overnight in a fume hood to dry. The product weighed 2.19g, and the yield calculated as 66 %.

2.3: PHYSICAL CHARACTERIZATIONS:

Infrared Spectra (IR)

Infrared spectra were plotted using a Mattson Instruments, Galaxy Series 4020 FTIR spectrometer using films, neat and potassium bromide (KBr) pellets. All spectra were recorded at an instrumental resolution of 4 cm^{-1} using 32 scans.

Mass Spectra (MS)

High-resolution electron impact (HREI) positive ion mass spectral analyses were carried out at the Washington University Resource for Biomedical and Bioorganic Mass Spectroscopy, St Louis M.O., using a Du Point 21-496-B double focusing mass spectrometer. The samples were inserted as a solid in a glass ampule using a direct insertion probe (DIP-EI-MS). HREI scans were recorded up to 450 ^{0}C

Solubility Tests

A wide variety of organic solvents were used in an attempt to dissolve the products. These solvents included aprotic dipolar and non-polar solvents. Solubilities were determined by placing approximately 1 mg of solid product in 3 to 5 ml of liquid, shaking vigorously and observing the mixture over a period of time.

Electrical Conductivity

Bulk DC resistancs were obtained using a RCA Model UV-SIIA Pico-ammeter and Keithley 173 A Multimeter coupled through a custom built resistivity cell connected to a Hewlett-Packard Model 6516A DC power supply. Bulk specific resistance was calculated from the relationship

$\rho = R\delta lt$

where:

R is the resistance (ohms)

δ is the surface area of the pellet (cm^2)

t is the pellet thickness (cm)

ρ is the bulk specific resistance below that of the range of the assembly (ca 10^{12} ohm-cm)

Light Scattering Photometry.

Weight average molecular weights were obtained employing a Brice-Phoenix BP 3000 Universal Light Scattering Photometer. Refractive indices were obtained using a Bauch & Lomb Abbe Model 3-L Refractometer.

2.4: BIOLOGICAL CHARACTERIZATION.

Bacterial.

Bacterial studies were conducted as follows. Culture plates containing a suitable growth medium were seeded with a suspension of test organism to produce an acceptable lawn of test organism after 24 hours of incubation at $37\,^{0}$ C. Shortly after the plates were seeded, 0.1 mg of the solid test compounds was introduced. The plates were then incubated at $37\,^{0}$ C for 24 hours. Inhibition was determined by measuring the distance from the physical edge of the compound being tested to the area where organism growth began.

Cell Lines.

Normal mouse fibroblast Balb/3T3 cells were used (ATCC CCL 163). The Balb/3T3 cells were grown in Dulbecco's Modified Eagle medium containing 10% calf serum. They were maintained at 35^{0}C in a 5% carbon dioxide atmosphere. To test the compounds, cells were harvested, counted and planted into 35mm dishes at a concentration of 50,000 cells per dish on day one. On the second day, cells were counted (2 dishes) and the compounds were added to half of the remaining dishes. A stock-solution of the compounds was prepared in DMSO at a concentration of 20mg/ml. This was diluted as needed with cell culture medium and added to half of the dishes at various concentrations. To the remaining control dishes, a fresh medium containing the same amount of DMSO was added as the dishes with the compound. The control dishes and compound dishes were counted for several days and inhibition/ growth was measured. Counting was performed by removing the medium and adding 0.2ml of 1% trypsin in phosphate-buffered saline to each dish. Then 0.8 ml of medium was added per dish, the cells were mixed and aliquots were counted using a Coulter counter.

2.5: GERMINATION STUDIES

Germination Experiments

The germination experiments were carried out in the FAU greenhouse. Regular "tap" water was used. The water level was maintained to the level of the seeds and represented what is called a "saturated " situation. Watering was performed so that water would come through the bottom of the trays minimizing the movement of the treatments.

The seeds underwent a preparatory procedure that included washing with Clorox ™ (5). For cattail (*Typha domingensis Pers, 1996*), viable seeds were identified as those that sank to the bottom of the blender that was used to separated the seeds from fruit parts (25). For sawgrass (*Cladium jamicense Crantz, 1995*), mature seeds were identified by visual observation. Immature seeds were discarded along with bracts and other fruit parts using a mortar and pestle. Mature seeds were tested for viability using a dye staining technique (4).

Both cattail and sawgrass seeds were obtained from the area known as Water Conservation Area 2A, (WCA 2A). The seeds were treated with talc-mixtures containing varying amounts of test material corresponding to the levels where the responses have been previously reported for other seeds (13). The experiment was set up in January. Rectangular plastic pots with holes in the bottom were used to allow the exchange of water. Four inches of potting soil (Jungle Growth Potting Soil) was added to allow for seedling development to occur without need for immediate replanting. A combined seed arrangement was used where 50 pre-treated seeds of each cattail and sawgrass, were placed in the corresponding labeled pots based on the different treatments. The pots were placed in rows in miniature pools. The water -level of the pools were maintained at about four inches (10 centimeters), presenting what is referred to as "saturated conditions". Five identical replicates of the experiment was set up. The sawgrass and cattail seeds were allowed to germinate for a three –month period and the results were recorded.

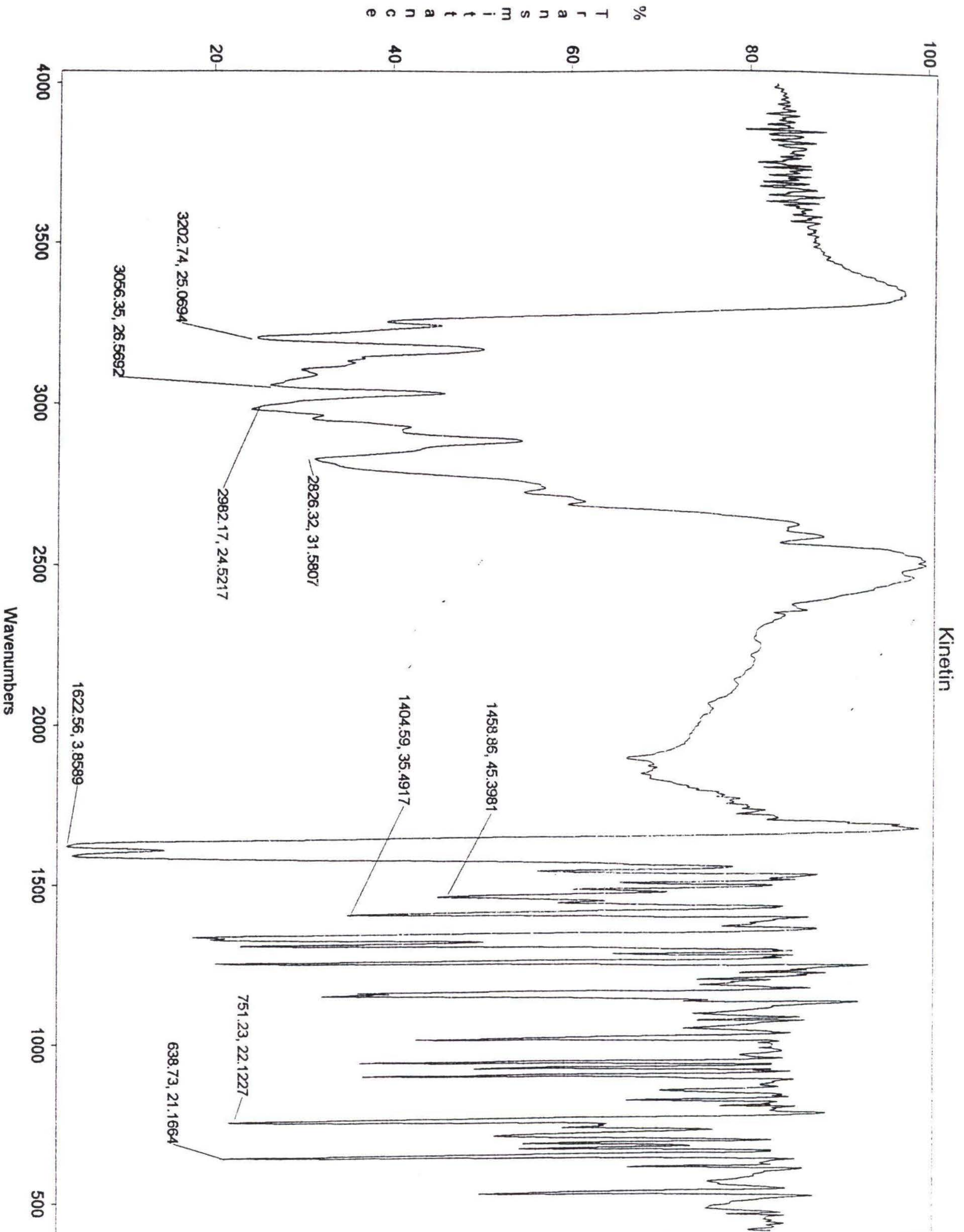
The above procedure was repeated, but this time, a variety of vegetable seeds were tested and there were only three replicates, 25 seeds in each pot for the experiment. The seeds were allowed to germinate for a two-month period and the results recorded. The seeds that were tested included: mustard, turnips, jaggar wheat, bad wheat, soybeans and broccoli.

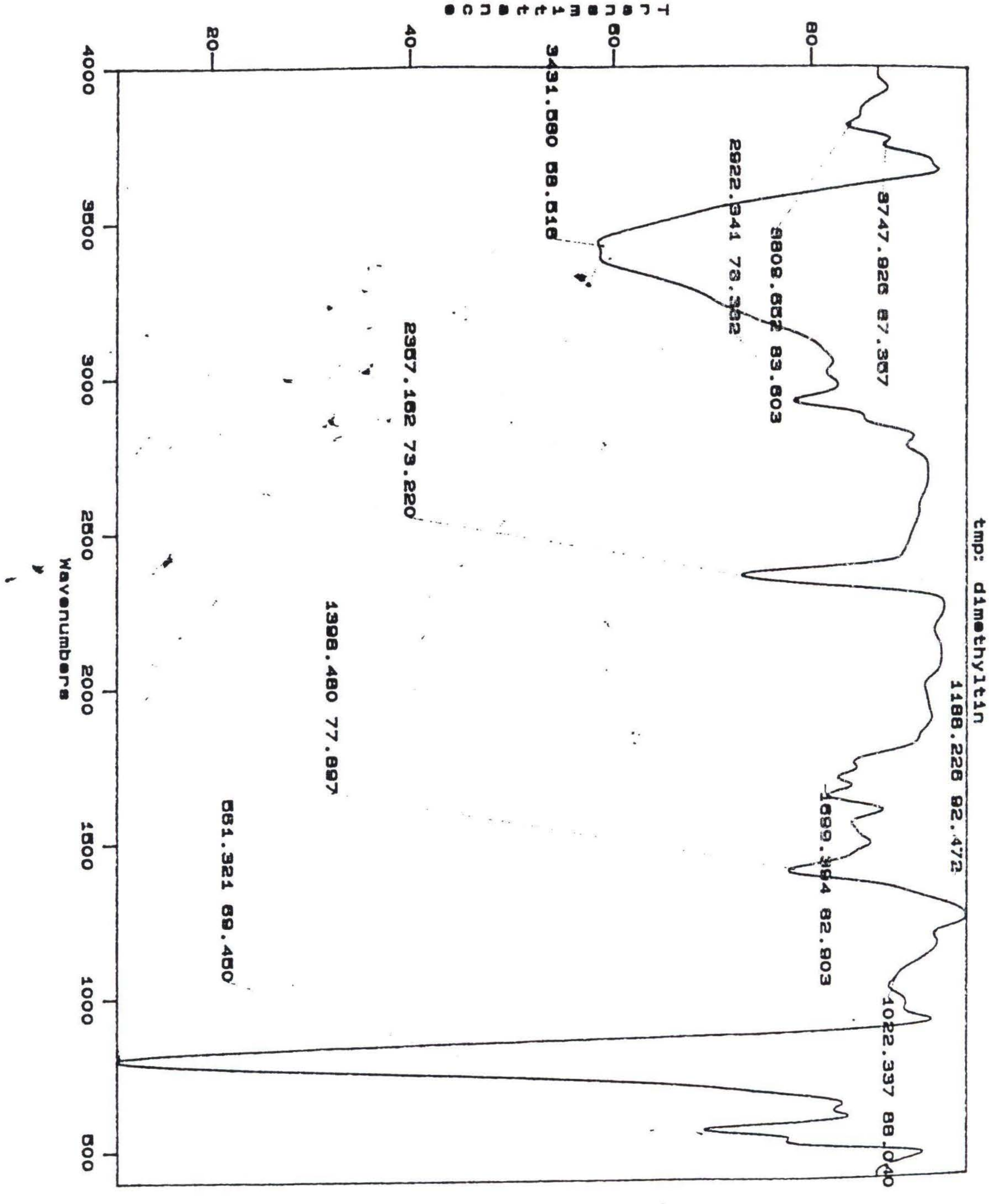
PART III:

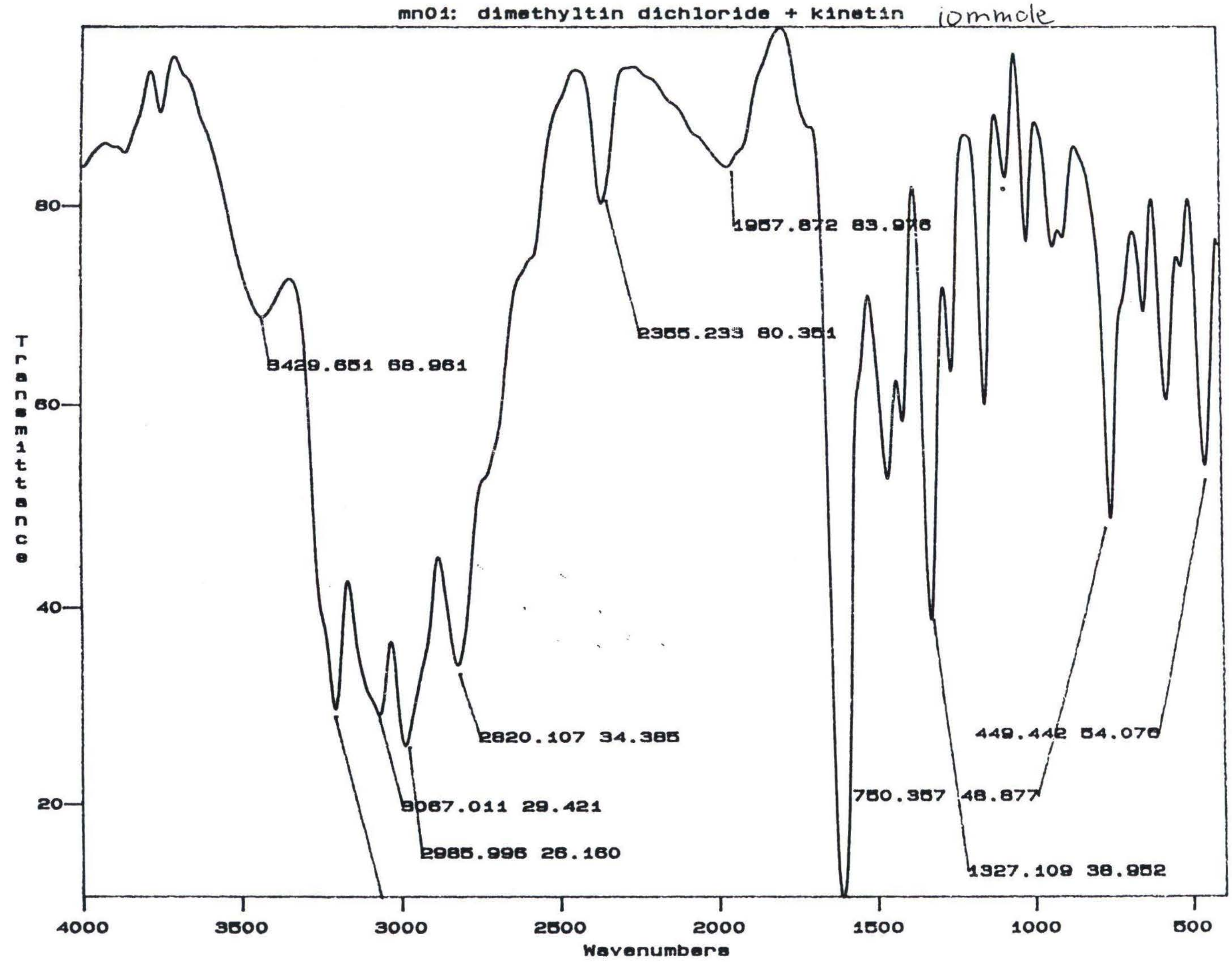
RESULTS AND DISCUSSION

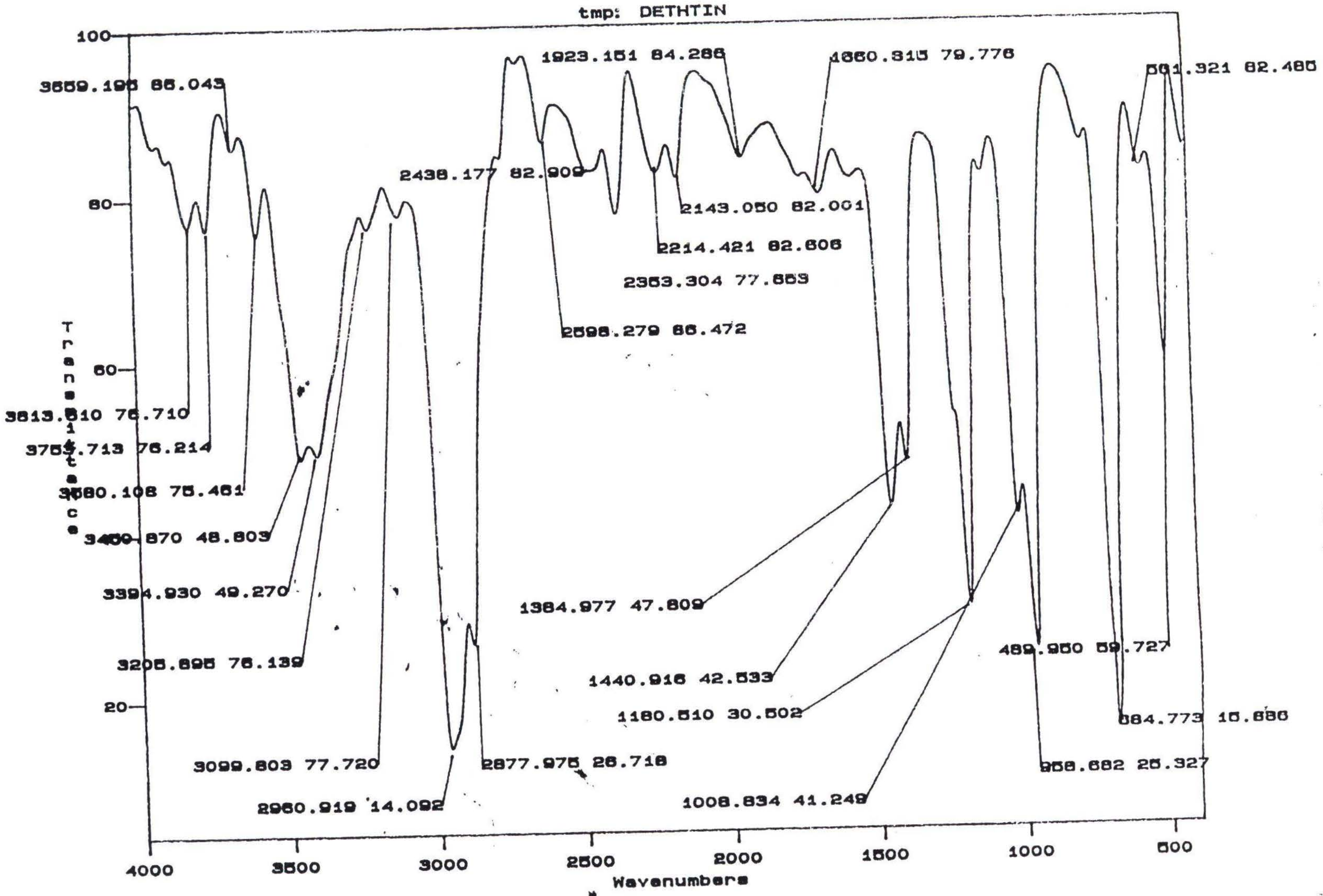
3.1: Infrared Spectroscopy

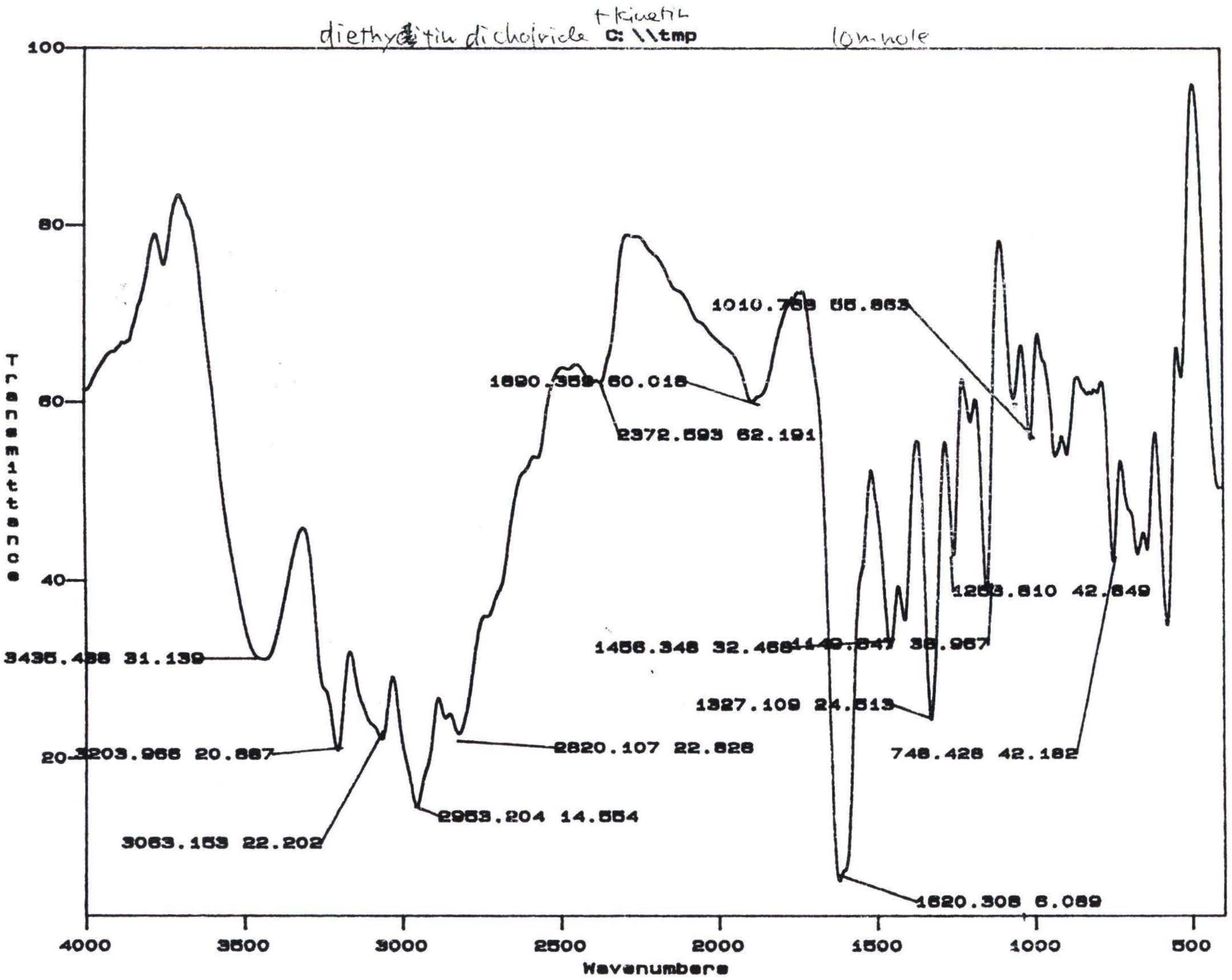
Polymers can be structurally characterized by infrared spectroscopy (IR). The vibrational motion of the polymer bonds is similar to those of small molecules including the monomers. Organotin compounds have been widely investigated (35-37). The IR spectra of these compounds exhibit characteristic stretching frequency or deformation modes associated with the aliphatic or aromatic groups attached to the metal. The IR spectra of products from the reactions of organometallic dichlorides with kinetin are shown in Figures 8-14. The spectra of the products are consistent with the presence of moieties from both kinetin and organometallic compounds.

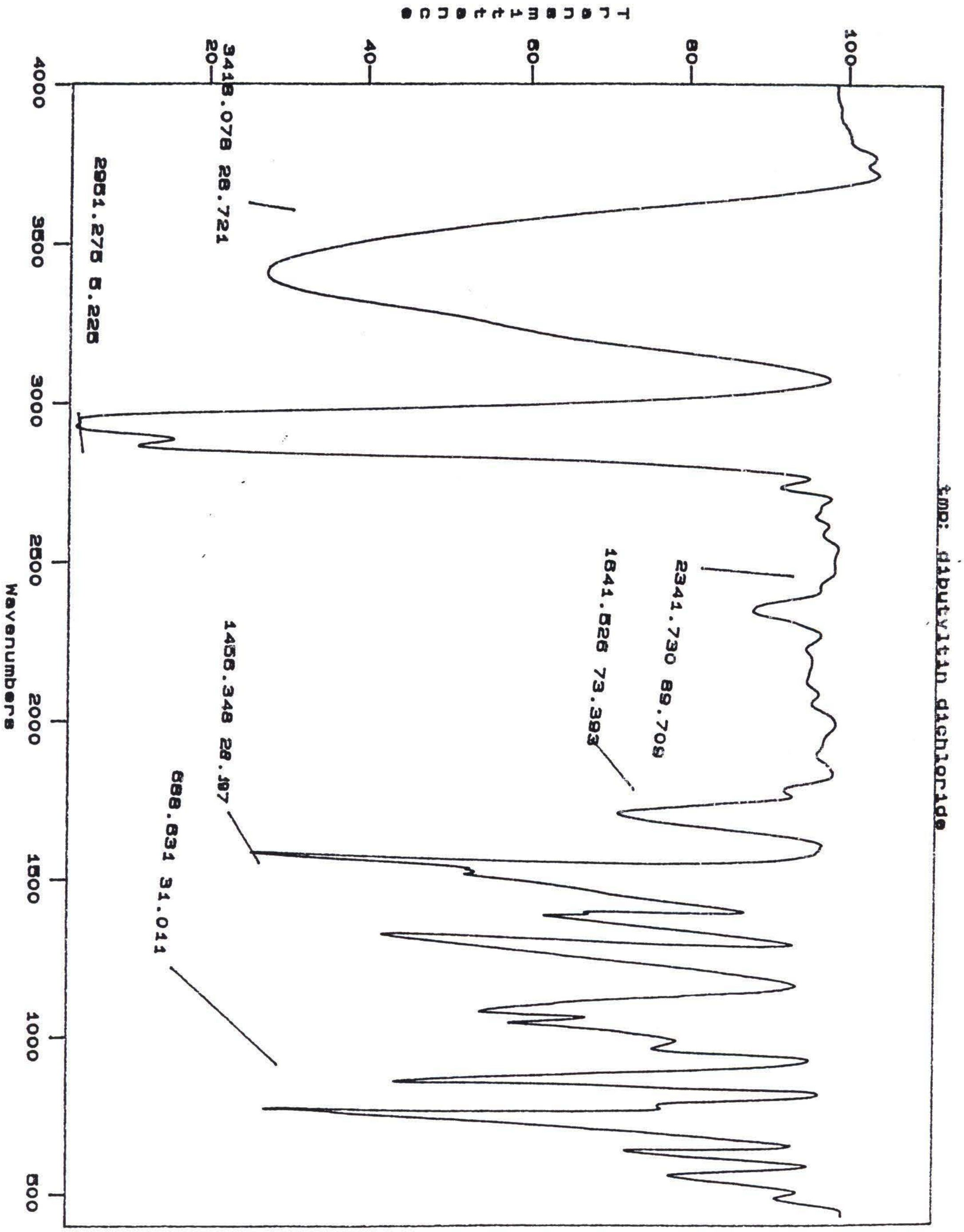

Kinetin
% Transmittance
100
80
60
40
20
4000
3500
3000
2500
2000
1500
1000
500
Wavenumbers
3202.74, 25.0694
3056.35, 26.5692
2982.17, 24.5217
2826.32, 31.5807
1458.86, 45.3981
1404.59, 35.4917
1622.56, 3.8589
751.23, 22.1227
638.73, 21.1664


tmp: dimethyltin
Transmittance
80
60
40
20
4000
3500
3000
2500
2000
1500
1000
500
Wavenumbers
3747.826 87.357
3808.652 83.803
2922.341 78.302
3431.580 58.518
2357.182 73.220
1398.480 77.897
1188.228 92.472
1639.384 82.803
581.321 69.450
1022.337 88.040


mn01: dimethyltin dichloride + kinetin
10mmole
Transmittance
80
60
40
20
Wavenumbers
4000
3500
3000
2500
2000
1500
1000
500
3429.651 68.961
3067.011 29.421
2965.996 26.160
2820.107 34.385
2355.233 80.351
1957.872 83.976
1327.109 38.952
750.357 48.877
449.442 54.076


tmp: DETHTIN
Transmittance
100
80
60
20
4000
3500
3000
2500
2000
1500
1000
500
Wavenumbers
3659.195 86.043
3813.510 76.710
3753.713 76.214
3580.108 75.461
3450.870 48.803
3394.930 49.270
3205.895 76.139
3099.803 77.720
2960.919 14.092
2877.975 26.718
2438.177 82.909
2598.279 86.472
2353.304 77.853
2214.421 82.606
2143.050 82.001
1923.151 84.286
1660.315 79.776
1384.977 47.809
1440.916 42.533
1180.510 30.502
1008.834 41.249
489.950 59.727
958.682 25.327
684.773 10.886
501.321 82.480


diethyl tin dichoiride
+ kinetin
C: \\tmp
10m.mole
Transmittance
100
80
60
40
20
3435.498 31.139
3203.966 20.887
3063.153 22.202
2953.204 14.554
2820.107 22.828
1890.359 60.018
2372.593 62.191
1010.759 55.863
1456.348 32.468
1140.547 38.967
1263.810 42.849
1327.109 24.513
748.428 42.182
1620.308 6.089
4000
3500
3000
2500
2000
1500
1000
500
Wavenumbers


tmp: dibutyltin dichloride
Transmittance
Wavenumbers
100
80
60
40
20
4000
3500
3000
2500
2000
1500
1000
500
3418.078 28.721
2951.275 5.226
2341.730 89.709
1641.526 73.393
1456.348 28.197
688.831 31.011

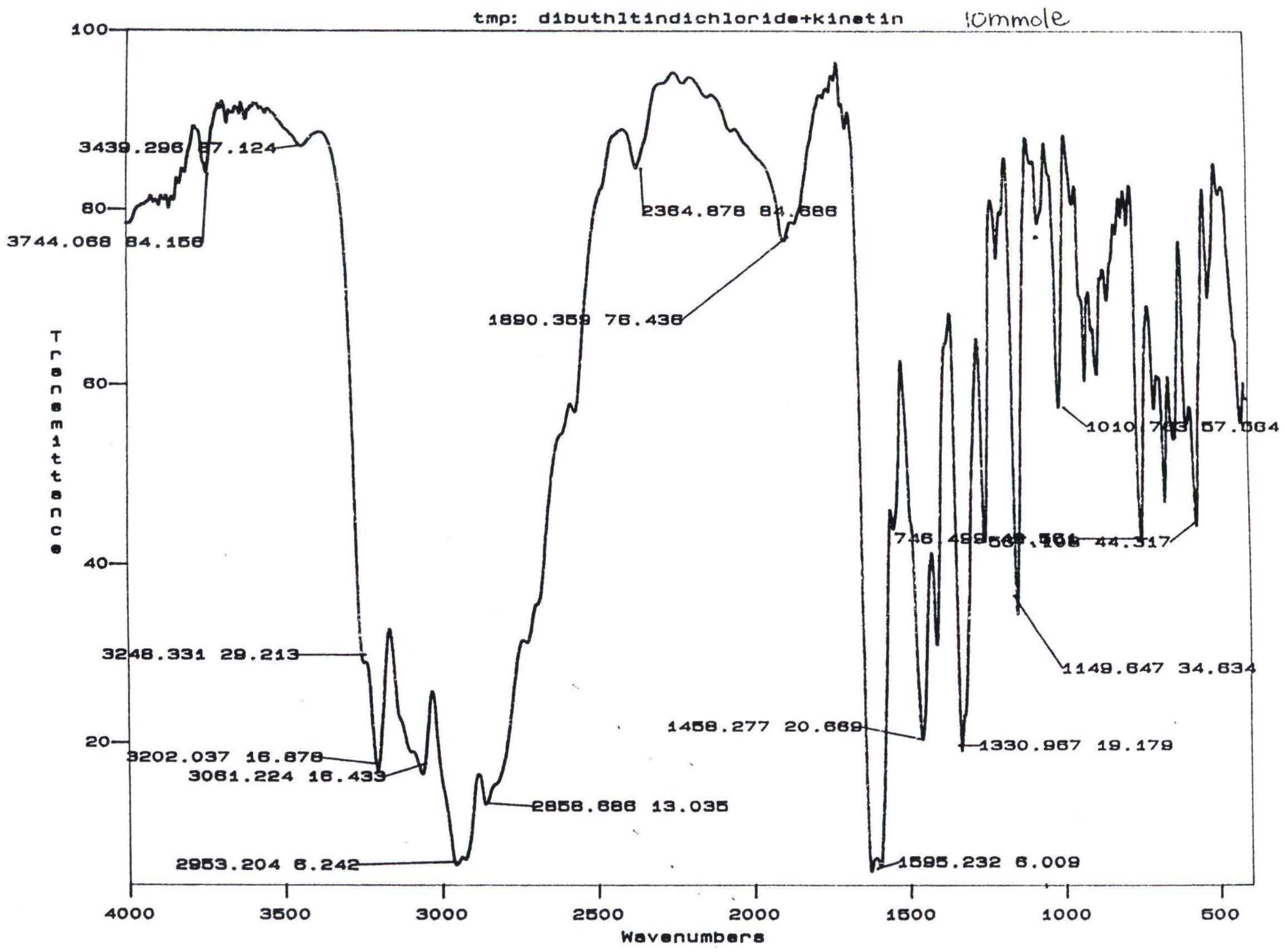

tmp: dibuthltindichloride+kinetin
10mmole
100
80
60
40
20
Transmittance
4000
3500
3000
2500
2000
1500
1000
500
Wavenumbers
3439.296 87.124
3744.068 84.156
3248.331 29.213
3202.037 16.878
3061.224 16.433
2953.204 6.242
2858.686 13.035
2364.878 84.886
1890.359 76.436
1595.232 6.009
1458.277 20.669
1330.967 19.179
1149.647 34.634
1010.763 57.564
746.489
44.317

Table 1

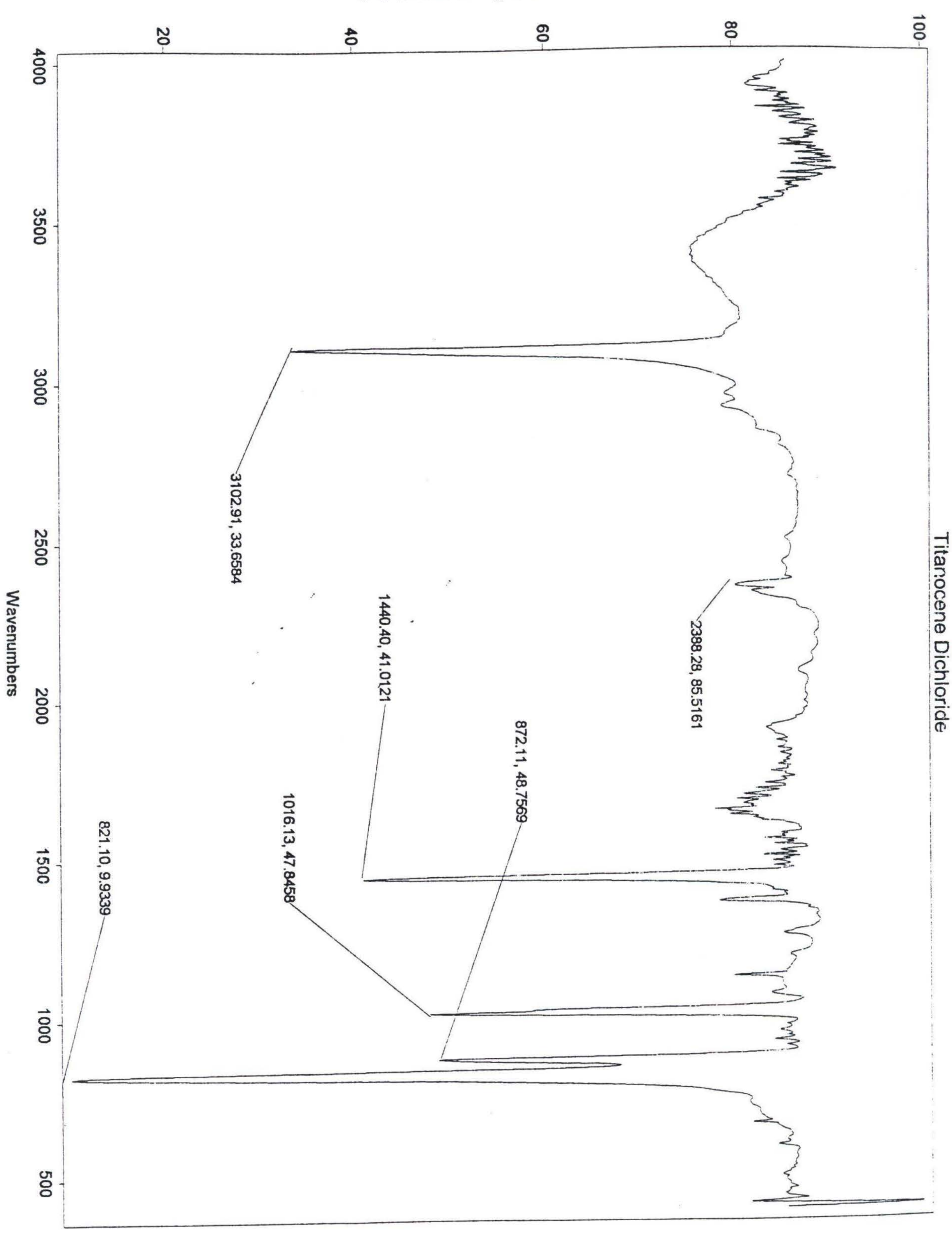
Infrared spectral band assignments for the products derived from organotin dichlorides and kinetin

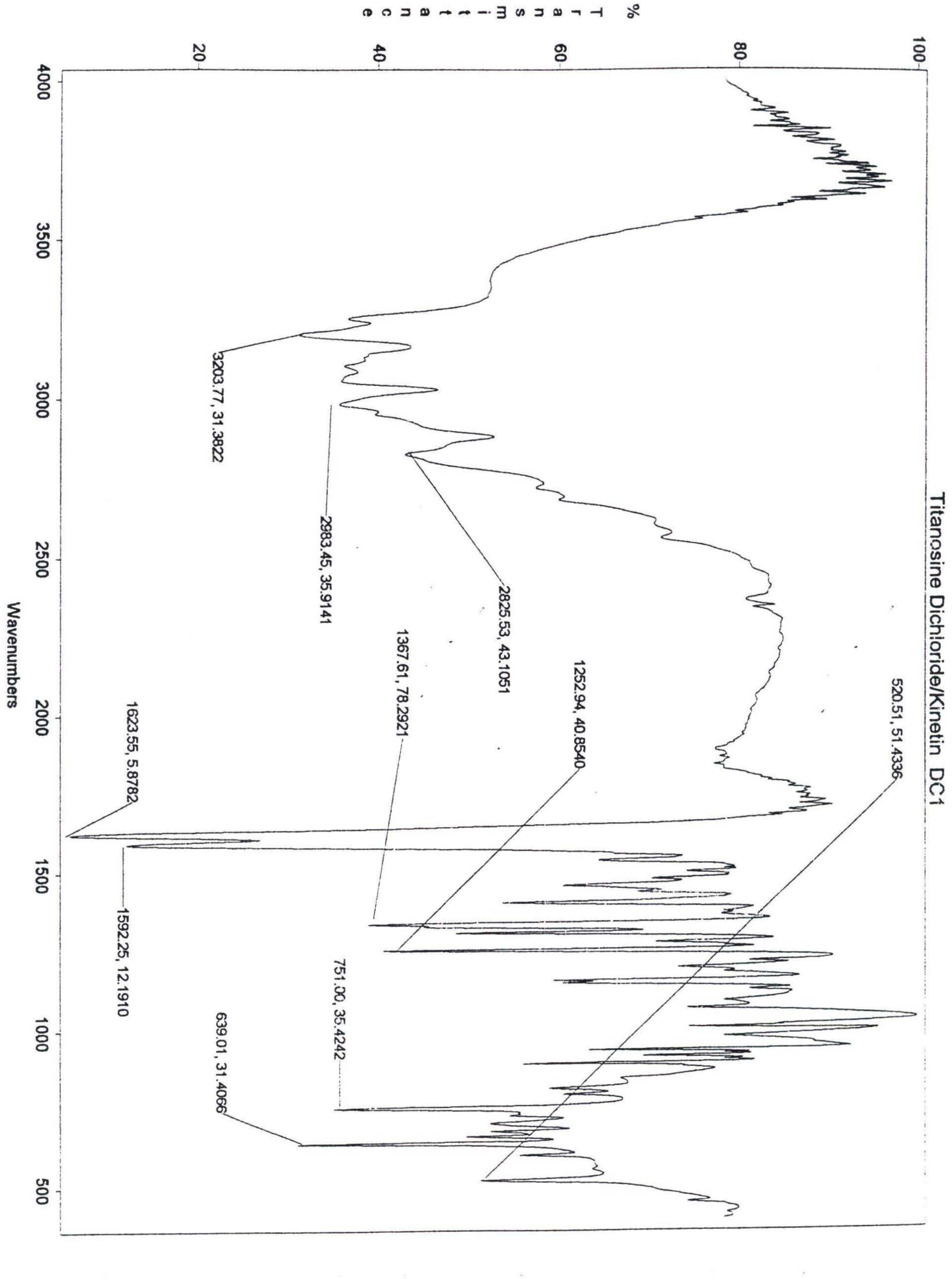
Assignment	Kinetin	Bu_2Sn/Kinetin	Et_2Sn/Kinetin	Me_2Sn/Kinetin
NH stretch	3450	-	-	-
CH Stretch-Aromatic	3150-3080	3200-3060	3080	3180,3070
CH Stretch, aliphatic	2970	2950-2860	2760,2840	2990,2820
CC, CN aromatic breathing, Purines	1610,1480	1610,15801460,1430	1610,1480	1590,14901420
Epoxy-ring Breathing	1250	1260	1260	1260
Sn-N	-	1150	1120	1120
Skeletal vibration, Furanes	1010	1010	1020	1000
Assymetric ring stretching, Furanes	920	920	950	920
CH out of plane, Purines	740,710	760,710	760, 710	750, 710
Sn-C Stretching	-	520	540	560

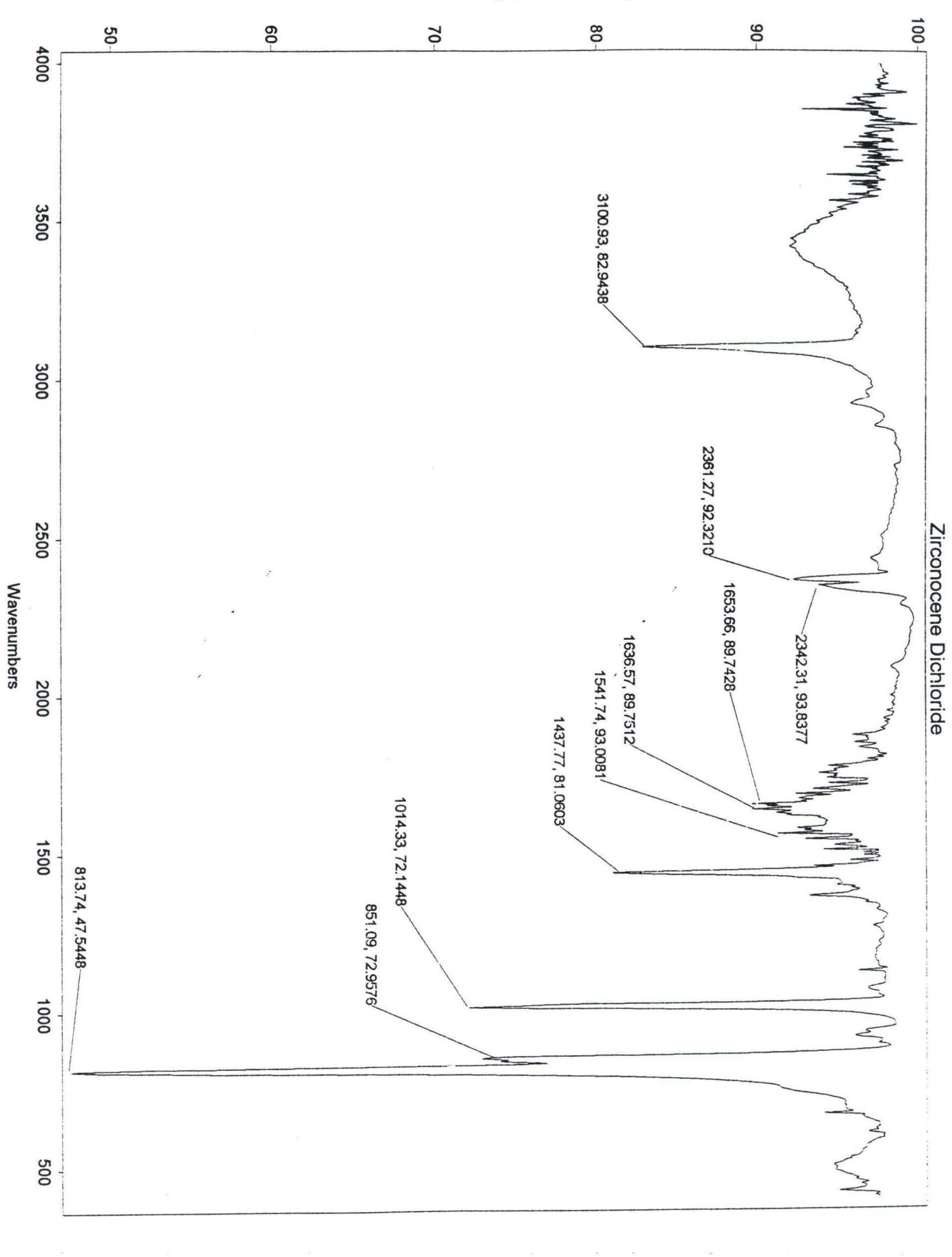
Table 1 gives the band assignments for the diorganotin dichloride products in comparison to those of kinetin itself. The spectra are similar. A new band assigned to the formation of the Sn-N linkage is seen in the area of 1120 to 1150 (all IR bands are given in units of cm^{-1}) consistent with the proposed structure. For dibutyltin the Sn-N linkage is found at 1150 cm^{-1} and for dimethyl and diethyltin the Sn-N linkage is found at 1120 cm^{-1} . As expected, the spectra differ in the 2800 to 3000 region assigned to the aliphatic C-H stretching. This is also the region for the N-H stretching (generally about 3400-3450). The N-H stretching bands are absent in this region for the products.

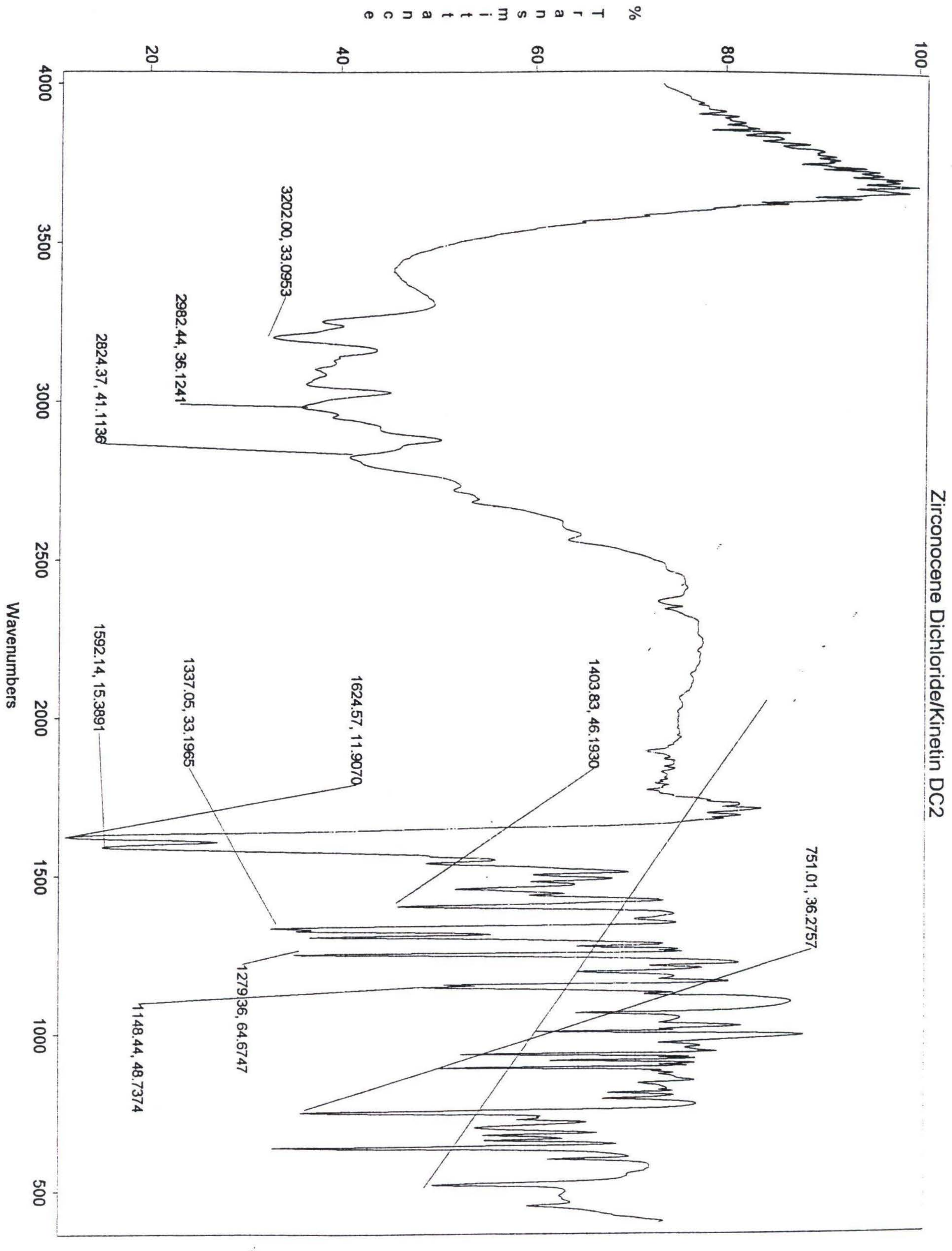
Purines show several characteristic aromatic CC, CN ring vibration bands. Kinetin shows bands at 1610, 1480 and 1430. There is a slight band shift for the dibutyltin product from 1480 to 1460 for the 1480 kinetin associated band. The C-H out of plane deformation vibrations associated with purine rings occur at about 760-710. The characteristic ring skeletal vibration for furanes occurring at about 1010 remains essentially unchanged. The asymmetric ring stretching in furanes occurs at about 920 to 950. A band characteristic of epoxy breathing for furanes occurs at 1250 to 1260.

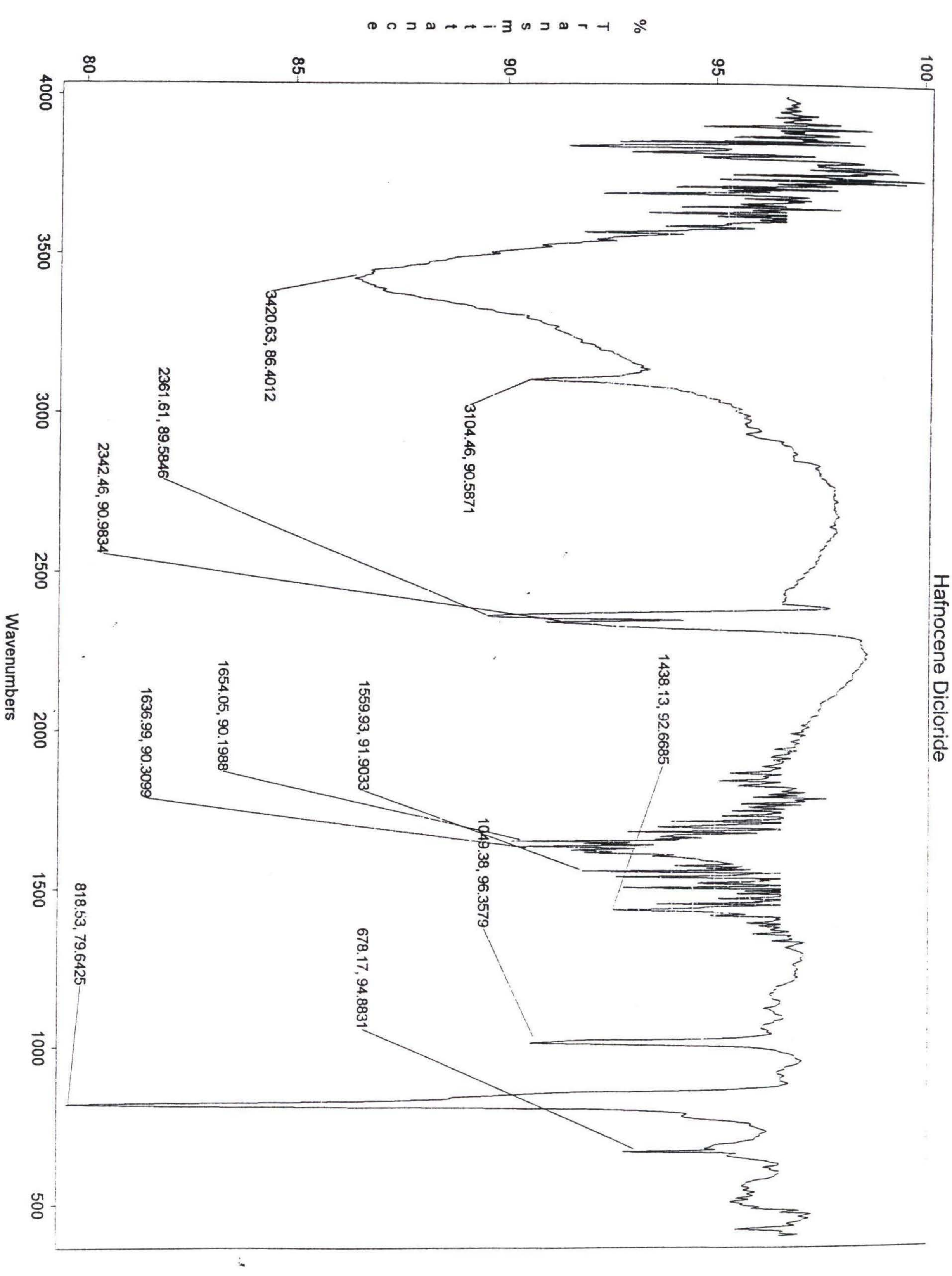
The IR spectra's of products from the Group IVB metallocene dichlorides and kinetin are shown in Figures 15-20. The spectra of all of the products are consistent with the presence of both moieties from the kinetin and the Group IVB metallocene compounds.


Titanocene Dichloride
%
T
r
a
n
s
m
i
t
t
a
n
c
e
100
80
60
40
20
4000
3500
3000
2500
2000
1500
1000
500
Wavenumbers
3102.91, 33.6584
2388.28, 85.5161
1440.40, 41.0121
872.11, 48.7569
1016.13, 47.8458
821.10, 9.9339


Titanosine Dichloride/Kinetin DC1
% Transmittance
100
80
60
40
20
4000
3500
3000
2500
2000
1500
1000
500
Wavenumbers
3203.77, 31.3822
2983.45, 35.9141
2825.53, 43.1051
1367.61, 78.2921
1252.94, 40.8540
520.51, 51.4336
1623.55, 5.8782
1592.25, 12.1910
751.06, 35.4242
639.01, 31.4066


Zirconocene Dichloride
%
Transmittance
100
90
80
70
60
50
4000
3500
3000
2500
2000
1500
1000
500
Wavenumbers
3100.93, 82.9438
2361.27, 92.3210
2342.31, 93.8377
1653.66, 89.7428
1636.57, 89.7512
1541.74, 93.0081
1437.77, 81.0603
1014.33, 72.1448
851.09, 72.9576
813.74, 47.5448


Zirconocene Dichloride/Kinetin DC2
% Transmittance
100
80
60
40
20
4000
3500
3000
2500
2000
1500
1000
500
Wavenumbers
3202.00, 33.0953
2982.44, 36.1241
2824.37, 41.1136
1624.57, 11.9070
1592.14, 15.3891
1403.83, 46.1930
1337.05, 33.1965
1279.36, 64.6747
1148.44, 48.7374
751.01, 36.2757


Hafnocene Dicloride
% Transmittance
100
95
90
85
80
4000
3500
3000
2500
2000
1500
1000
500
Wavenumbers
3420.63, 86.4012
3104.46, 90.5871
2361.61, 89.5846
2342.46, 90.9834
1438.13, 92.6685
1559.93, 91.9033
1654.05, 90.1988
1636.99, 90.3099
1049.38, 96.3579
678.17, 94.8831
818.53, 79.6425

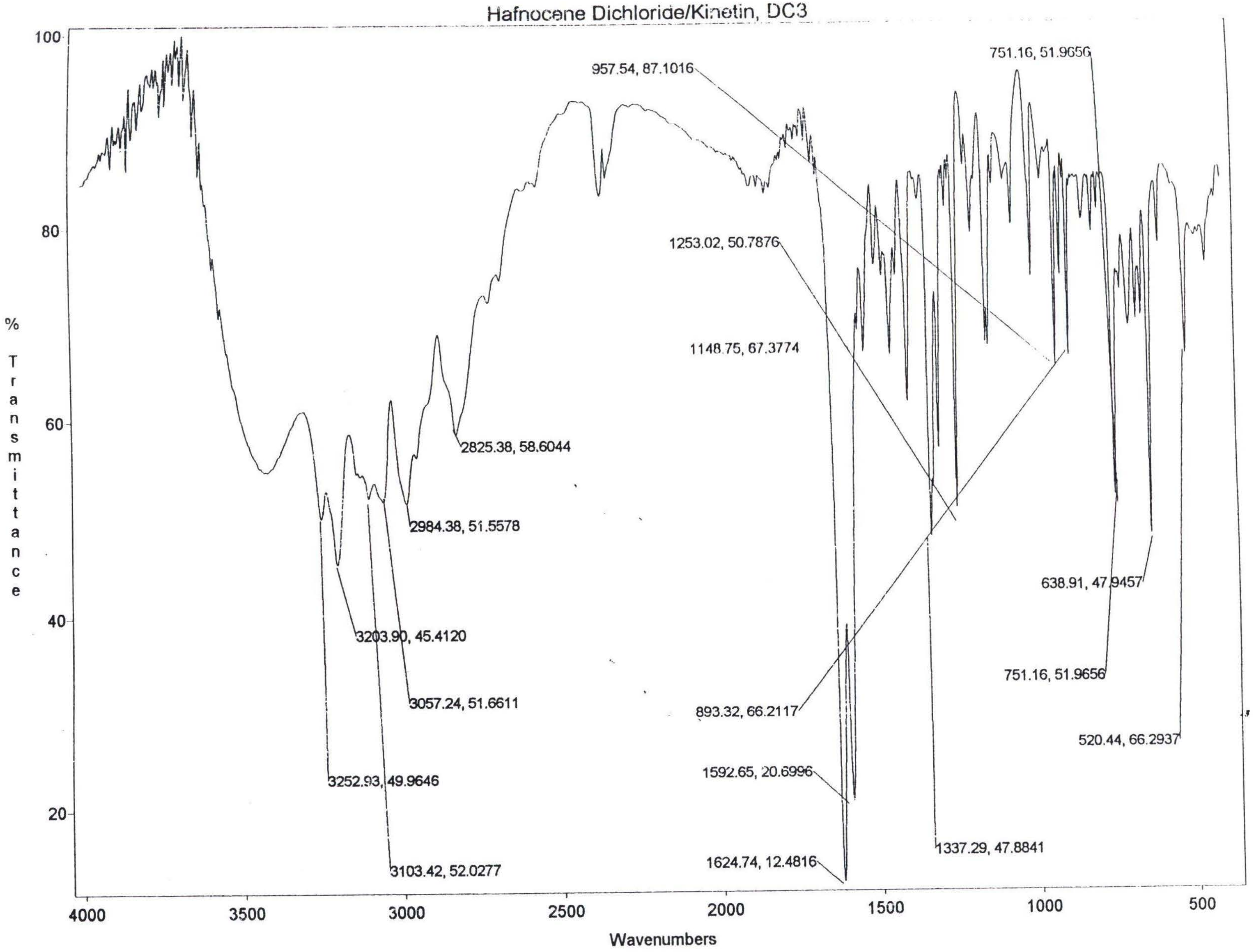

Hafnocene Dichloride/Kinetin, DC3
% Transmittance
100
80
60
40
20
4000
3500
3000
2500
2000
1500
1000
500
Wavenumbers
957.54, 87.1016
751.16, 51.9656
1253.02, 50.7876
1148.75, 67.3774
2825.38, 58.6044
2984.38, 51.5578
638.91, 47.9457
3203.90, 45.4120
751.16, 51.9656
3057.24, 51.6611
893.32, 66.2117
520.44, 66.2937
1592.65, 20.6996
3252.93, 49.9646
1337.29, 47.8841
1624.74, 12.4816
3103.42, 52.0277

Table 2

Infrared spectral band assignments for products derived from Group IVB Metallocene dichlorides and kinetin.

Assignment	Kinetin	Cp_2TiCl_2	Cp_2ZrCl_2	Cp_2HfCl_2
N H stretch	3450	-	-	-
C H stretch Aromatic	3056	2983	2982	3057
C H stretch aliphatic	2970, 2982, 2826	2825	2824	2984,2825
CC, CN aromatic breathing,Purines	1622, 1610, 1458, 1404	1623,1592	1624,1592,1403	1624,1592
C O stretch	1325	1367	1337	1337
Skeletal Vibrations, Furanes	1010	1367	1403	1337
Assymetric ring stretching, Furanes	925			957
CH out of plane, Purines	751, 744	751	751	751

Table 2 gives the band assignments for the Group IVB metallocene products as well as those of kinetin itself. Again the spectra are similar. The spectra differ in the 2800 to 3000 region assigned to the aliphatic C-H stretching. This is also the region for the N-H stretching (generally about 3400-3450). The N-H stretching bands seen in kinetin at 3450 cm^{-1} are absent in the products for this region. The assignment of the metallocene-N band was not possible because fell below the 500 cm^{-1} range of the IR spectrophotometer. There is a shift in the aromatic CH stretch region for the products, from 3056 in kinetin to include the 2982-2983 region due to the inclusion of the cyclopentadiene moiety from the metallocene dichlorides.

3.2: Mass Spectroscopy

Mass spectra for the products of organotin halides and kinetin were obtained by electron impact ionization (EI-MS), with probe temperatures typically in the range of 400^0 to $450\,^0C$.

M. Gielen (23) studied the degradation of a number of different series of organotin compounds. From these studies he formulated a fragmentation rule which is useful in the interpretation of tin-containing mass spectra. He found that in most cases organotin compounds, RSnX or RSnR', produced fragments containing tin with a co-ordination number of 3 and a valence state of +4 by losing a radical ($R^{\cdot}$ or $X^{\cdot}$) from the next higher unfavorable valence state. A tin ion with a +2 valence state is also favored to be produced. The mass spectra results are consistent with the proposed structure, including both tin containing and polymer backbone associated fragments.

When run at lower temperatures (ca 200^0C) most of the ion fragments are assigned to the kinetin moiety, (all ion fragments given in m/z =1 or in Daltons). The only differences are associated with the "R" groups on the tin. Therefore, masses associated with the butyl groups are more prominent for the product formed from the dibutyltin dichloride. All of the mass spectra show ion fragments about 134 assigned to the Sn-N moiety. All of the products also show bands that are associated with the R-Sn-N moiety, R being methyl at 134, R being ethyl at 163 and R being butyl at 166. All of the products show an ion fragment at 148, which was assigned to the N-Sn-N group. Both of these are consistent with the formation of the Sn-N linkage between the organotin and kinetin. Table 3 below shows the most abundant ion fragments for the product of diethyltin dichloride and kinetin.

Table 3

Most abundant ion fragments for the product of diethyltin dichloride and kinetin

m/z	Relative Intensity, %	(Possible) Structure
53	31	C_4H_5
81	64	Furan-HCH
93	13	Furan-HCH-NH, minus 3H
96	18	Furan-HCH-NH
108	11	Purine, minus C
119	16	Kinetin minus Furan-HCH-NH
120	12	Above
135	13	Kinetin, minus Furan-HCH
160	23	Kinetin , minus C_3H_3O
186	84	Kinetin, minus O-CH
187	17	Above
215	100	Kinetin

Mass spectra for the metallocene dichlorides were also performed and the results were consistent with the proposed structure. Table 4 gives the most abundant ion fragments derived from the product of titanocene dichloride and kinetin. These ion fragments are mainly derived from kinetin, but unlike kinetin itself, which exhibits no ion fragments above 117 (kinetin plus 2 protons), the results from the product shows ion fragments up to and beyond 1000. Because kinetin is not a symmetrical molecule, two geometries are possible for the repeat unit linkage.

Figure 21.

Possible geometries of titanocene dichloride /kinetin products.

F-N-Ti-N-F F-N-Ti-N-P and P-N-Ti-N-P

where –N-F designates attachment through the furan-containing moiety and –N-P designates attachment through the purine-containing moiety of the kinetin. Ion fragments consistent with all two geometries are present. While tin contains isotopes, the most abundant is 33 %. Because of this and the fact that the ion fragments are in low abundance, isotopic abundance ion fragmentation patterns are not evident.

Table 4

Most abundant ion fragments derived from the product of titanocene dichloride and kinetin .

M/e	% Relative Abundance	(Possible) Assignment
53	100	C_4H_5
65,66	50,97	Cp
81	100	Furan plus Me
92,93,96	52,100,100	Furan plus Me, N
108	81	Purine, minus C
119,120	100,93	Purine
135	97	Kinetin minus Furan, Me
159,160	58,100	Kineitn minus C_3H_3O
186,187	100,99	Kinetin minus OCH
214,215,216	99,100,89	Kinetin

The presence of the Ti-N linkage is found in the lower molecular weight ion fragments. Table 5 and 6 shows some of these ion fragments.

Table 5

Ion fragments found in the product of titanocene dichloride and kinetin indicating the Ti-N linkage

	N	N,N	N,P	P,P	P
Cp_2Ti	192	206	310	412	393
CpTi	127	141	243	349	231
Ti	62	76	181	284	166

Table 6 shows the higher molecular weight ion fragments for the titanocene dichloride and kinetin product and their possible assignments and include ion fragments containing two and three units.

Table 6

Higher molecular weight ion fragments for the titanocene dichloride and kinetin product.

M/e	PossibleAssignment	M/e	PossibleAssignment
509	U +P	817-822	2U+N Me
558,560	U +P, Ti	823	2U +Ti
572	U +P,Ti, N	834-837	2U +P minus Cp
587,589	Cp$_2$Ti,K,Cp$_2$Ti,N	854-856	2U+F
622	U+P,CpTi	867-872	3U minus Cp$_2$Ti,PNMe
636	U+CpTi,P,N	881-884	3U minusCp$_2$Ti,PN
655,657	2U minus 2Cp	893	3U minusCp$_2$Ti,P
679	P,Ti,U,P	899	2U+CpTi
688,690	U +P,Cp$_2$Ti	905	2U+P
702,704	U+PN,Cp$_2$Ti	916,917	2U+PN
715,717	2U minus Cp	930-934	3Uminus2Cp,P
741-743	P +CpTi,U,P	938,943	3Uminus CpTi,K
753	P+CpTi,U,PN	978	3U minus P,N,Cp
767	P+Cp$_2$Ti,U,PNMe	996	3U minus P,Cp
783	2U	1017	3U minus Cp$_2$Ti,N
806,808	P+Cp$_2$Ti,U,P	1044	3U minus 2Cp

where "U" represents one repeating unit, "F" represents a furan ring, "P" represents a purine ring, and "Me" represents a methylene.

Ion fragments were also found at 35, 36, 37 and 38, which could correspond to Cl (35), HCl (36), Cl (37) and HCl (38). The expected ratio of Cl(36)/Cl(37) and HCl (36) and HCl (38) should be 3 corresponding to the natural abundance ratio of Cl (35) to Cl (37). Instead, it was found that the ratio of the 35/37 was 0.04 and for 36/38 it was 0.06. This is consistent with the ion fragments in this range not being derived from chlorine- containing species.

The ion fragmentation pattern for cyclopentadiene, shown in Table 7, is in reasonable agreement with the known ion fragmentation pattern for cyclopentadiene.

Table 7

Fragmentation patterns for cyclopentadiene found in comparison to the standard

66	65	39	40	38	63	67	62	31	M/e
100	47	32	27	8	8	6	6	5	Standard (Relative %)
100	51	61	26	-	7	3	5	5	Found (Relative %)

Titanium has five naturally occurring isotopes, but only one is relatively abundant in comparison to the others. The natural abundance of Ti (48) is 74% in comparison to the next most abundant isotope Ti (46) is 8%. Since there is such a small relative abundance of higher mass ion fragments, and because one titanium isotope accounts for almost 75% of the total isotopic abundance, the isotopic abundance ratios were not determined for the product.

Similar results were found for the mass spectra for the analogous products of the reaction of zirconocene dichloride and hafnocene dichloride with kinetin.

For the product of hafnocene dichloride and kinetin, Table 8 shows the most abundant ion fragments. Again, as seen with titanocene dichloride and kinetin product, the majority are derived from kinetin.

Table 8

Most abundant ion fragments for hafnocene dichloride and kinetin product

M/e	Relative Abundance, %	(Possible) Assignment
51,52,53	11,10,55	C_4H_x
65,66	11,16	Cycopentadiene
81	83	Furan + Me
92,93	11,22	Furan +Me, N
159,160	10,31	Kinetin minus C_3H_3O
186,187	100,17	Kinetin minus OCH
214,215,216	15,99,12	Kinetin

Ion fragments consistent with the Hf-N linkage are also found and are given in Table 9. These include ion fragments containing all of the possible linkages between the non-symmetrical kinetin and hafnocene moiety.

Table 9

Ion fragments from the hafnocene dichloride and kinetin product indicating the presence of the Hf-N linkage

	N	N,N	N,P	P,P	P
Cp_2Hf	324	338	442	544	428
CpHf	259	273	378	481	363
Hf	194	206	311	417	297

Table 10 shows the higher molecular weight ion fragments derived from the product of hafnocene dichloride and kinetin and their possible assignments.

Table 10.

Possible assignments for the mass spectra derived from the hafnocene dichloride and kinetin product

M/e	(Possible) Assignment	M/e	(possible) Assignment
605,606	U –Cp, +P,N,Me	779,780	U+ CpHf, N
617,618	U +F, N, Me	805	2U –P,Cp
643,644,648	U +P	817	2U – P,Cp,N
655,656	U +P,N	828,833	U + Hf,P, N
660	2U – Cp_2Hf, F, Me	865	2U- Cp, P
667	U +P,N, Me	886	U + CpHf, P
678,679,680	U + CpHf –F,Me,N	893,895	2U – P, Cp, +NMe
689	U +CpHf –F,Me	905	2U –2Cp, N
705,706	U +Hf	918	2U –2Cp
717	U –2Cp,P,F,Me	929	2U –2Cp +N
729,733,736	U +P,N,F	997	2U – Cp,N
767,769	U +CpHf	1031	2U –N

While there are ion fragments over the range 35-38, as seen in the case of the titanocene product, the ratios obtained of 35/37 =0.26 and 36/38 = 0.096 are consistent with the ion fragments not being due to the presence of chlorine.

The fragmentation pattern for cyclopentadiene is in mild agreement with the expected pattern seen in Table 11. Note that the standard pattern for cyclopentadiene is run at about 250^{0}C and not for the 400 to 450^{0}C range for our present compounds.

Table 11.

Ion fragments found for cyclopentadiene in comparison to the standard.

66	65	39	46	38	63	67	62	31	M/e
100	47	32	27	8	8	6	6	5	Standard (Relative %)
100	65	80	32	32	6	-	3	5	Found (relative %)

As in the case for the titanium product, the ion fragments that contain hafnium are widely spread and divided into isotopic-abundance bundles according to the natural isotopic abundance and they are of low intensity. Thus, few good isotopic abundance matches are expected and none found.

3.3: Solubility.

Solubility tests were performed on the 6 polymers, using a range of organic solvents. All of the products derived from the reaction of the kinetin with the metallocene dichlorides are insoluble in all tested liquids. The results are given in Table 12. Poor solubility for such metal-containing polymers appears to be typical and is probably due to a tendency for such metals to be highly crystalline. Products derived from the dihalo organostanne are soluble in DMSO.

Table 12.

The Solubility of the Group IVB Metallocene Dichloride and Kinetin in Some Polar Solvents

	$CpTiCl_2$/kinetin	$CpZrCl_2$/kinetin	$CpHfCl_2$/kinetin
DMF	-	-	-
DMA	-	-	-
HMPA	-	-	-
DMSO	-	-	-
Acetone	-	-	-
Acetonitrile	-	-	-

\- : insoluble, all of the testing under room temperature

DMF : Dimethyl formamide

DMA : dimethyl acetamide

HMPA : Hexamethyl phosphoramide

DMSO : Methyl sulfoxide

3.4: Electrical Conductivity

A number of analogous metal-containing polymers have been found to be semiconductors. As such, materials are routinely measured for bulk resistivity to see if they are conductive materials for further study.The zirconocene-kinetin product exhibited bulk resistivity of about 1.2 x 10^3 ohm-cm within the mid-range for semiconductors. For comparison, non-doped cis-poly-acetylene has a bulk resistivity of about 10^9 ohm-cm whereas the trans-polyacetylene has a bulk resistivity of about 10^5 ohm-cm. When doped, the trans-isomer becomes a conductor with bulk resistivity of 10^{-2} to 10^{-4} ohm-cm. Thus further studies of this product using doping might be considered. Table 13 shows the results for the electrical conductivity experiments.

Table 13

Results from electrical Conductivity Experiments

Sample	**Thickness (mm)**	**Electrical Conductivity**
Kinetin	1.20 mm	N.C
Me_2Sn/K	1.25 mm	N.C
Et_2Sn/K	1.26 mm	N.C
Bu_2Sn/K	1.15 mm	N.C
$TiCl_2/K$	1.30 mm	N.C
$ZrCl_2/K$	1.57 mm	616.7 KΩ
$HfCl_2/K$	1.26 mm	N.C

N.C – Non conductive.

3.5: Product Yield and Molecular Weight.

The synthesis of the polymers derived from the reaction of diamines and organotin dichlorides and Group IVB metallocene dichlorides has been described previously in the experimental section. Product yields ranged from about 43% to 63% (Tables 14 and 15).

Table 14

Product yields for the kinetin and organotin dichloride products

Product	Percent Yield
Me_2SnCl_2/kinetin	43%
Et_2SnCl_2/kinetin	56%
Bu_2SnCl_2/kinetin	63%

Table 15.

Product yields for the kinetin and metallocene dichloride products.

Product	Percent Yield
Cp_2TiCl_2/kinetin	55%
Cp_2ZrCl_2/kinetin	63%
Cp_2HfCl_2/kinetin	63%

Light scattering photometry was performed to determine the molecular weight of the soluble compounds. Based on the molecular weights obtained, the average degree of polymerization was determined. Table 16 shows the results from the light scattering photometry and the average degree of polymerization.

Table 16

Product yield as a function of organotin and concentration

Organotin Dichloride	Percentage Yield 1 mmole	Percentage Yield 10 mmole	Molecular weight for 10 mmole pdt.	Average degree of Polymerization
Dimethyltin	43	95	9100	23
Diethyltin	56	100	55,00	150
Dibutyltin	63	100	15,000	33

The organotin products are moderate polymers with a degree of polymerization ranging from about 20 to 150. This is consistent with degrees of polymerization found for other similar organotin polyamides. (26-27)

The Group IVB metallocene dichlorides were insoluble in all liquids so light scattering photometry could not be performed.

3.6: Biological Characterization.

The biological activities of organostannes are well known and have been widely investigated (28). Generally inorganic tin compounds are non-toxic or only slightly toxic towards mammals, insects and fungi whereas organotin compounds usually show various biological activities. For alkylorganostannes, toxicity is varied for different alkyl groups. Usually an increase in the alkyl chain length produces a progressive toxicity to all living species with few exceptions. One exception is that for fungi, butyltin compounds are generally the most active fungicides. Different numbers of alkyl groups on organostannes also give different fungicidal properties. High fungicidal activity is found for trialkyltins, R_3SnX and much less for compounds of type $RSnX_3$. Therefore the order of fungicidal activity in organostannes is $R_3SnX. > R_2SnX_2 > RSnX_3$ (29).

Polymeric organostannes may also exhibit biological activity. Carraher et al reported that the products from poly (vinyl alcohol), modified through the reaction with organostanne halides showed good toxicity towards the yeast causing C. Abicans (30).

In the present study, the biological activity of the products from organotin dichlorides with kinetin and Group IV B metallocene dichlorides was investigated. Their effectiveness was measured by the size of the zone of inhibition on test plates. Those products that exhibit the largest zone of inhibition are considered the most effective towards that particular microorganism. Bacterial studies were conducted using plates containing a suitable growth medium such as tryptic soy agar or Mueller

Hinton agar. The plates were seeded with a suspension the test organism that produces an acceptable lawn of test organism after 24 hours of incubation at 37 ^{0}C. Shortly after the plates were seeded, the test compounds were introduced as solids directly, the plates were incubated and the inhibition was noted.

The microorganisms used in the testing are Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureaus, and Vanco res. They are described in Table 17.

Table 17.

The nature of the microorganisms used in the biological characterization of organotin and metallocene dichlorides with kinetin products.

Name	Species	Type
Staphylococcus aureaus	Bacterium	Gram-negative rods
Escherichia coli	Bacterium	Gram-positive cocci
Pseudomonas aeruginosa	Bacterium	Gram-negative rods
Vanco res.	Bacterium	Enterococcus

The testing results for the biological activity of the products are shown in Table 18.

Table 18.

Biological results for selected bacteria.

Compounds Tested	S.Aureus	P. Aueriginosa	E. coli
Me_2SnCl_2	2.0	1.5	
Et_2SnCl_2	1.8	1.5	1.5
Bu_2SnCl_2	0.6	Stac	1.0
Kinetin	N	N	N
Me_2SnCl_2/kinetin	1.0	N	
Et_2SnCl_2/kinetin	N	0.3	0.2
Bu_2SnCl_2/kinetin	N	N	N

As expected kinetin itself is not active. While the organotin chlorides are active, this activity is believed to be due to the hydrolysis of the organotin chloride forming hydrochloric acid, which is known to damage living matter. The product from diethyltin dichloride is active while the product from dibutyltin dichloride is not. This is consistent with the bacterial studies where diethyl is more active than dibutyl. Table 19 shows the results from the Group IVB metallocene dichloride and kinetin products and their response to selected bacteria.

Table 19.

Biological results for Selected Bacteria.

Compounds Tested	S. Aureus	P. Auerigionosa	Vanco Res.
Cp_2TiCl_2/kinetin	N	N	N
Cp_2ZrCl_2/kineitn	N	N	N
Cp_2HfCl_2/kinetin	N	N	N

No activity is seen with the metallocene dichloride products. Again this is to be expected.

Cell line experiments.

In the past, a number of Group IVB and organotin-containing polymers have been found to inhibit selected cell lines. As such, the present polymers were screened for cell line inhibition. The organotin and group IVB metllocene dichloride polymers with kinetin were tested using Balb/3T3 cells and Table 20 shows the results for the organotin polymers.

Table 20

Results for the cell line experiments for organotin/kinetin polymers.

Me_2SnCl_2/ kinetin	Day 1 Cells per dish x 10^4	Day 2 Cells per dish	Day 3 Cells per dish	Day 4 Cells per dish	Day 5 Cells per dish
control	5	13.7	22.7	52.0	116.5
25μg/ml	5	13.7	11.6	27.8	98.4
10μg/ml	5	13.7	22.2	47.9	114.1

Et_2SnCl_2/ kinetin	Day 1 Cells per dish x 10^4	Day 2 Cells per dish	Day 3 Cells per dish	Day 4 Cells per dish	Day 5 Cells per dish
Control	5.0	10.0	25.6	76.1	193.5
25μg/ml	5	15.6	16.8	18.1	20.1
15μg/ml	5.0	10.0	12.9	24.3	41.6
10μg/ml	5.0	10.0	16.4	32.7	79.6
5μg/ml	5.0	10.1	19.7	47.4	105.1

Bu_2SnCl_2/ kinetin	Day 1 Cells per dish x 10^4	Day 2 Cells per dish	Day 3 Cells per dish	Day 4 Cells per dish	Day 5 Cells per dish
Control	5	9.6	18.6	51.3	119.0
25μg/ml	5	9.6	2.6	3.2	3.3
2.5μg/ml	5	9.6	8.6	6.1	5.4
Control	5	8.3	29.9	56.1	130
0.20μg/ml	5	8.3	18.5	35.8	67.2
0.25μg/ml	5	8.3	18.7	30.8	48.5
0.50μg/ml	5	6.9	9.2	13.0	20.0
Control	5	12.5	22.2	50.0	117.4
0.05μg/ml	5	12.5	24.0	44.1	101.8
0.15μg/ml	5	12.5	22.1	46.1	100.6

For the Me_2SnCl_2/kinetin, there was very little effect seem at 10μg/ml concentration but slight effect at 25μg/ml concentration for the Balb/3T3 cell lines. Moderate effect was seen for the Et_2SnCl_2/kinetin polymer at a concentration of 10μg/ml. For the Bu_2SnCl_2/kinetin polymer, at 25 and 2.5μg/ml there was almost 100% effectiveness. When the concentration was dropped to 0.20 and 0.25μg/ml they still proved to be effective against the Balb/3t3 cell lines. This is significant in that activity is seen at a low concentration of the product, lower than or about the same as that for traditional drugs such as cis-platin and taxol, both of which are more toxic than our products. In conclusion, it was found that the larger the alkyl groups attached to the kinetin, the more toxic the polymer, possibly as they become more hydrophobic. Because such high activity was see in the Bu_2SnCl_2 product at such low concentrations, di-octyl and di-lauryltin polymers were synthesized and are presently in testing for effectiveness against the Balb/3T3 cell line.

Table 21 shows the results for the Group IVB metallocene polymers.

Table 21

Results for the cell line experiments for Group IVB metallocene dichloride/kinetin polymers.

Cp_2HfCl_2/ kinetin	Day 1 Cells per dish x 10^4	Day 2 Cells per dish x 10^4	Day 3 Cells per dish x 10^4	Day 4 Cells per dish x 10^4	Day 5 Cells per dish x 10^4
Control	5	14.1	29.9	103.6	214.3
5µg/ml	5	14.1	31.6	107.8	202.6
15µg/ml	5	11.4	29.8	90.4	209.1
25µg/ml	5	11.4	26.4	85.4	181.9

Cp_2TiCl_2/ kinetin	Day 1 Cells per dish x 10^4	Day 2 Cells per dish x 10^4	Day 3 Cells per dish x 10^4	Day 4 Cells per dish x 10^4	Day 5 Cells per dish x 10^4
Control	5	9.1	28.0	70.4	179.4
25µg/ml	5	9.2	26.0	67.3	132.9
10µg/ml	5	9.1	22.0	73.4	166.3

Not much activity was seen for the Group IVB metallocene dichloride polymers but slight activity was seen at a concentration of 25μg/ml for the Balb/3T3 cell line.

3.7: Germination Experiments for sawgrass and cattail.

Table 22 shows the results for the maximum germination data for the organotin dichloride and kinetin products with sawgrass and cattail. It indicates the maximum percentage germination in comparison to the treatment provided.

Table 22

Maximum germination percentages for organotin dichloride/kinetin products with sawgrass and cattail

Treatment Organotin/ppm	% Germination Sawgrass	% Germination Cattail
Me_2Sn/ 0.1	14	16
Me_2Sn/ 10	9	23
Me_2Sn/ 1000	17	38
Et_2Sn/0.1	12	34
Et_2Sn/ 10	21	29
Et_2Sn/1000	16	37
Bu_2Sn/0.1	16	28
Bu_2Sn/10	10	20
Bu_2Sn/1000	12	17
Kinetin/0.1	15	37
Kinetin/10	19	36
Kinetin/1000	20	36
Control (talc only)	12	23

It was found that, in general there was an overall increase in percentage germination for the seeds that were treated with either kinetin or kinetin bound to the polymer in comparison to that of the control. Results were varied where concentration was concerned. No specific trends were found. There seemed to be no change in the amount of fungi growth for those that contained either kinetin or kinetin bound polymer.

Table 23 shows the T and P values for the data presented in Table 22 comparing the results with the control.

Table 23

T and P values for data given in Table 22

Treatment organotin/ppm	Sawgrass		Cattail	
	T	P	T	P
Me_2Sn/ 0.1	0.38	0.37	0.38	0.37
Me_2Sn/ 10	1.0	0.21	1.0	0.21
Me_2Sn/ 1000	6.9	0.01	4.8	0.02
Et_2Sn/0.1	0.14	0.45	1.5	0.13
Et_2Sn/ 10	1.7	0.12	4.3	0.02
Et_2Sn/1000	1.5	0.13	2.6	0.06
Bu_2Sn/0.1	1.2	0.17	3.0	0.05
Bu_2Sn/10	0.46	0.34	1.7	0.11
Bu_2Sn/1000	14	0.45	1.6	0.13
Kinetin/0.1	3.5	0.03	1.9	0.10
Kinetin/10	3.2	0.04	15	0.004
Kinetin/1000	3.9	0.03	2.6	0.06

Table 24, gives the maximum percentage germination for the results of the metallocene dichloride/kinetin products with sawgrass and cattail.

Table 24

Maximum germination percentages for metallocene dichloride/kinetin products with sawgrass and cattail

Treatment metallocene/ppm	% germination Sawgrass	% germination Cattail
Cp_2Ti/20	47	30
Cp_2Ti/200	52	21
Cp_2Ti/2000	8.7	11
Cp_2Zr/20	59	45
Cp_2Zr/200	0.67	38
Cp_2Zr/2000	13	16
Cp_2Hf/20	31	15
Cp_2Hf/200	21	20
Cp_2Hf/2000	28	44
Kinetin/20	0.66	17
Kinetin/200	21	37
Kinetin/2000	32	19
Control (talc only)	0	12

Table 25 shows the T and P values for the data presented in Table 24 comparing the results with the control. Differences are present for both the polymers containing kinetin and for kinetin itself.

Table 25

T and P values for data given in Table 24.

Treatment Metallocene/ppm	Sawgrass		Cattail	
	T	P	T	P
$Cp_2Ti/20$	2.84	0.023	0.60	0.289
$Cp_2Ti/200$	2.72	0.021	0.80	0.214
$Cp_2Ti/2000$	1.00	0.187	0.99	0.189
$Cp_2Zr/20$	2.42	0.036	1.89	0.066
$Cp_2Zr/200$	2.45	0.035	1.65	0.087
$Cp_2Zr/2000$	1.59	0.093	1.22	0.145
$Cp_2Hf/20$	1.41	0.115	1.80	0.073
$Cp_2Hf/200$	1.08	0.170	0.23	0.414
$Cp_2Hf/2000$	1.78	0.075	1.74	0.078
Kinetin/20	1.63	0.089	1.63	0.089
Kinetin/200	1.09	0.069	0.86	0.220
Kinetin/2000	1.00	0.187	0.29	0.392

Again it was found that in general, there was an increase in germination for the seeds that were treated with kinetin or kinetin bound to the polymer, in comparison to the control. The lower concentrations of polymer bound to kinetin proved better for germination than kinetin itself. The control for the experiment for sawgrass was 0% germination. When treated with polymer bound to kinetin, there was an increase in germination up to 60%. This is a significant increase in germination, since the typical germination of sawgrass is 0-2%. A significant positive is that for the Everglades restoration, this research allows for the seeding of large areas by boat or air easily through simply treating the seeds with low concentrations of tin/kinetin polymer materials. For the cattail seeds, the control was 12% germination and the kinetin bound to polymer showed an increase in germination as well, but only up to 38%.

Differences are present for both the polymers containing kinetin and for kinetin itself. The increase in germination percentages for some of the polymers is consistent with the polymers acting as control release agents, since only "free" kinetin is believed to act as a plant growth regulator. Further this is consistent with the kinetin and selected polymers, influencing the percentage germination of both sawgrass and cattail. In both cases, this increase is positive with respect to increasing germination fraction.

Germination Results for beans and seeds.

Seven types of vegetable and wheat seeds were tested. They were mustard, turnips, jaggar wheat, bad wheat, soybeans, peas and broccoli. Of the seven tested seeds, there was only germination found for four, the jaggar wheat, mustard peas and turnip seeds. Table 26 gives the results for the average maximum germination percentages for the seeds.

Table 26

Results for seed germination experiment.

Treatment/ ppm	% germination MUSTARD	% germination TURNIPS	% germination JAGGAR WHEAT
Control (talc only)	22.7	4	9.3
Kinetin 10	34.7	16	25.3
Kinetin 1000	26.7	24	10.7
Me_2Sn/K 10	28	30.7	0
$Me_2SnK/1000$	9.3	10.6	12
Et_2Sn/K 10	25.3	18.6	6.7
$Et_2SnK/1000$	16	22.6	0
$Bu_2SnK/10$	36	22.6	14.6
Bu_2Sn/K 1000	26.7	10.6	17.3
$TiCl_2K/10$	25.3	17.3	9.3
$TiCl_2K/$ 1000	32	17.3	20
$ZrCl_2K/$ 10	18.6	9.3	9.3
$ZrCl_2K/1000$	36	26.7	16
$HfCl_2K/10$	17.3	21.3	6.7
$HfCl_2K/1000$	40	24	13.3

Table 27 shows the T and P values for the information presented in Table 26.

Table 27

T and P values for Mustard , turnip and jaggar wheat seeds when

Treated with kinetin bound polymer in comparison to a control.

	Mustard		Turnips		Jaggar wheat	
Treatment	T	P	T	P	T	P
Kinetin 10	1.1	0.18	3.0	0.4	3.5	0.037
Kinetin 1000	0.58	0.31	1.6	0.12	1.0	0.21
Me_2Sn/K 10	0.47	0.34	2.7	0.054	1.7	0.11
$Me_2SnK/1000$	1.1	0.18	1.0	0.21	0.76	0.26
Et_2Sn/K 10	0.55	0.31	3.0	0.046	1.0	0.21
$Et_2SnK/1000$	0.35	0.37	1.7	0.11	1.7	0.11
$Bu_2SnK/10$	0.89	0.23	1.4	0.0025	0.72	0.27
Bu_2Sn/K 1000	0.50	0.33	5.0	0.019	1.3	0.16
$TiCl_2K/10$	0.36	0.37	3.7	0.031	0.00	1.0
$TiCl_2K/$ 1000	1.3	0.15	1.2	0.16	0.73	0.27
$ZrCl_2K/$ 10	0.23	0.42	1.5	0.13	0.00	1.0
$ZrCl_2K/1000$	1.0	0.20	17	0.0015	0.76	0.26
$HfCl_2K/10$	0.49	0.33	1.8	0.10	0.76	0.26
$HfCl_2K/1000$	2.4	0.066	1.8	0.10	1.7	0.11

Responses to a PGH vary from plant to plant and with concentration. With respect to concentration, activity generally is somewhat "bell-shaped" so that at low concentration there is little or no activity and as concentration increases, so does activity up to a point where greater concentration decreases the activity. The concentrations employed in the present study are within the range where activity is often found for kinetin in commercial applications (25).

While many aspects might be considered as a measure of activity, the current study focuses primarily on germination and secondarily on observation (only) with respect to seedling development. This was performed because of rapidity, ease of measurement, and importance of germination. Table 28 is a listing of seeds and sources for the seeds employed in the current study. Table 29 is a listing of the viability results for the seeds. Germination results were given in table 26 and statistical treatment of the data was given in Table 27.

Table 28

Seeds obtained from Wesley Learned used in the experiments.

Name	Year	From	Type
Soybeans	1997	Johnstons seed company	
Turnip seeds	1995-1996	Johnston seed company	Purple top
Little marvel peas	1996	Johnstons seed co.	
Jaggar wheat	1998	Flying Circle L ranch	
Radish	1994	Johnstons seed Co.	Cherry Belle
Mustard	1994	Johnston seed Co.	Florida Broad Leaf
Black beans	1994	Johnston seed Co.	Jackson Wonder

Table 29

Viability results for seeds.

Name	Total #	Total alive	% viability
Marvel peas	22	20	91
Bad wheat	57	35	61
Radish	50	1	2
Mustard	50	7	14
Jaggar wheat	22	7	32
Black beans	42	32	76
Pinto beans	17	16	94
Rice	13	1	8
Pigeon peas	71	70	99
Lentils	39	38	97

In all cases, the seedling development appeared to be independent of the particular seed treatment. While 7 different seeds were tested, results are reported here for only three (or four) since the other seeds did not germinate, consistent with the low viability percentages found for them, Table 28. Statistically important results (significance values > 0.1) were found for some of the seed treatments.

For the mustard seeds treated with kinetin and kinetin containing polymers, in general the treated seeds germinated to the same or greater percentage compared to the control. While some of the polymers gave the highest percentage germination, they were not appreciably greater than those treated with kinetin itself.

For the turnips, all of the kinetin and kinetin containing polymer treated seeds did better than the control. Again, while some of the greatest germination percentages were for polymer containing kinetin seeds, they were not appreciably greater than those for seeds treated with kinetin alone.

In summary, in almost all cases, PGH treated seed groups gave greater percentages of germination when compared with the control. Thus, treatment with either kinetin or polymers containing kinetin is advantageous, and is one method of increasing the germination of older and damaged seeds. By comparison, seeds treated with the polymer –bound kinetin did not germinate to an appreciably greater extent in comparison to kinetin.

The use of a polymer- bound PGH is a viable way of delivering the PGH since the germination results are similar to those of free PGH's. On the negative side, there is the increase in cost of using a polymer-bound PGH, due to the additional preparation of bound PGH.

PART IV:

CONCLUSIONS

The products synthesized by reaction of kinetin with organotin and Group IVB metallocene dichlorides for the tin-containing products gave yields of 40-60% with chain lengths of 20-150. The mass spectral results are consistent with the assumed structures and show presence of moieties derived from both kinetin and the organostanne reactants. Mass spectral analysis and infrared spectral results show the presence of the M-N linkages between the organostanne and Group IVB metallocene (mass spectral results only) and kinetin.

In biological studies, the diethyltin proved to be more active than the dibutlytin with respect to bacterial activity, consistent with other bacterial studies, while the group IVB metallocene dichloride products were inactive against the tested bacteria. For the cell line experiments, it was found that the larger the alkyl group attached to the kinetin, the more toxic the polymer for the organotin polymers. The dibutyltin – kinetin polymer showed activity to the 0.2 μg/ml level, fully one hundredth that found for other similar products. Group IVB metallocene dichloride polymers were not very active against the Balb/3T3 cell lines, but slight activity was seen for the Cp_2TiCl_2/kinetin at 25μg/ml.

For the germination experiments, the Group IVB metallocene dichloride products seemed effective in promoting germination in sawgrass, increasing germination up to 60 % in comparison to 0% germination for the control. In most cases, polymer bound PGH treated seeds gave greater germination percentages in comparison to the control.

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