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Synthesis of some Indole derivatives and examining their impact on the growth and metabolism of carbohydrates and proteins in the cells of maize seedlings

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Abstract : This paper contains synthesis of two indole derivatives; which are the following:
3-Acetylindole, which is synthesized by the acylation of indole and converted to that previously unknown compound3-(4-HydroxyPhenyl)-1-(1H-indol-3-yl) prop-2-en-1-oneby Aldole condensation reaction.
The infrared spectra (IR), nuclear magnetic resonance (^{1}H-NMR) and mass spectrometry (MS) of these compounds were studied.
The synthesized compounds show vital efficiency as plant hormones, their presence in growth medium causes increase of maize growth and their use in incubation medium of maize clips causes the increase of the quantity of cumulative protein in these incubated cells. In the presence of compound (II) in incubation medium, the quantity of hexose and pentose and pentozanes decreased, and the quantity of these hexose, pentose and pentozanes increased in the presence of compound (III) in incubation medium. At the same time, the presence of any of these compounds (II) and (III) did not have any impact on the amount of starch or cellulose carbohydrates in maize clips.
Key words: Indole, 3-Acetyl Indole, Hormones, Protein, Carbohydrates, Maize.

1. Introduction

Indoles are one of the most important nitrogen containing heterocyclic molecules. Indole is the commonly used name for the benzopyrrole ring system, consisting of a benzene ring fused to the 2, 3-positions of a pyrrole ring[1]. The interest and development in indole chemistry began in mid-nineteenth century, with intensive studies on indigo, a violet-blue dye, originally derived from Indigofera species in India[1]. Useful investigations of indole chemistry started when indigo was successfully oxidized to isatin which was then reduced to oxindole[1].

Indole structures are present in a great number of compounds of biological importance, e.g., the plant growth hormone indoleacetic acid (IAA),2 the pineal gland hormone melatonin, serotonin and tryptophan[1-4].

The interesting chemical properties of indole have inspired chemists to design and synthesize a variety of indole derivatives. Indole derivatives represent many important classes in medicinal chemistry such as anticancer, antioxidant, anti-rheumatoidal, and anti- HIV antagonist, anti-allergic, antibacterial, antifungal, anti-inflammatory[1,2,4-9]. Furthermore, some indole derivatives, such as melatonin and serotonin, influence many important biochemical processes. They act as antioxidant and play an important role in the immune system[1,2,4].

2. Experimental Section:

2.1. *Materials*

Indole, Acetic Acid, Anhydrous Acetic Acid, Sodium Hydroxyl, 4-hydroxybenzaldehyde, Maize, Sulfuric Acid, Hydro Chloric Acid.

Solvents: Ethanol, Methanol, Ethyl Acetate, Chloroform, Iso Propanol, Di methyl sulfoxide, n-Hexane. All compounds were purchased from Merk and Aldrich Chemicals.

Coomassie reagent (Bradford reagent)

Coomassie reagent reacts with the proteins forming a blue complex. Dissolve 100 mg of coomassie crystals in 50 ml of Ethanol (96%), and then add 100 of H_3PO_4 (85%) and complete the volume with dual distilled water[10].

Anthron reagent (Anthron: $C_{14}H_{10}O$):

Anthron reagent reacts with the hexoses forming a complex blue – greenish. Dissolve 200 mg of anthron crystals in 100 ml of H_2SO_4 (83%), and then cooled[11].

Orcinol reagent or(Bial's Reagent):

Dissolve 3 g orcinol in 500 mL concentrated HCl, add 2.5 mL of a 10% solution of ferric chloride hexahydrate, and dilute to one liter with water; this is approximately 6 M HCl[11].

2.2. General

Melting points are uncorrected and were recorded by open capillary tube method using Electro-thermal Melting Point apparatus. Infra-red spectra (KBr) were recorded on Jasco FT/IR 300 E Fourier Transformer spectrophotometer. The ^{1}H-NMR spectra were recorded on a 400 MHz and 300 MHz Bruker spectrometer using Methanolsolvent and TMS as internal standard. The chemical shifts were expressed in ppm units. Mass spectra were recorded on LC-MS Shimadzo mass spectrometer using Methanol/Water/Acetic Acid 60/40/0.1 as mobile phase, column C_{18}, temperatures of column 40^{o}C. All the reactions were monitored by TLC usingseveral solvent systems, TLC plates were prepared by the spreading method.

2.3. Synthesis of the Compounds

2.3.1. Synthesis of 1,3-Di acetyl indole (I)

Indole 10 g (0.0862 mol), acetic acid (25 ml) and acetic anhydride (100 ml) were refluxed for 24 h, the solvent was removed under vacuum and the residue was crystalline. It was recrystallized from toluene. 1,3-Diacetyl indole (8.6 g, 86 %) was obtained as colorless needles, m.p. 148^{o}C. The reaction was monitored by TLC using solvent system (Ethyl Ether/ n-Hexane; 7/5) (See chart-1)[12].

2.3.2. Synthesis of 3-acetyl indole (II)

1,3- Di acetyl indole (I) 1 g (0.005) was suspended in ethanol (35 ml) and sodium hydroxide (10 ml) of 2 N was added. The mixture was stirred and warmed until the di acetyl indole had dissolved; the product after being precipitated by dilution with water, collected and crystallized from ethanol 0.7 g was obtained as white needles, m.p. 190^{o}C (60.2 %). (See chart-1).The reaction was monitored by TLC using the same solvent system (Ethyl Ether/ n-Hexane; 7/5) [12]. (Notice: the reactions are repeated using more grams of indole for the following reactions).

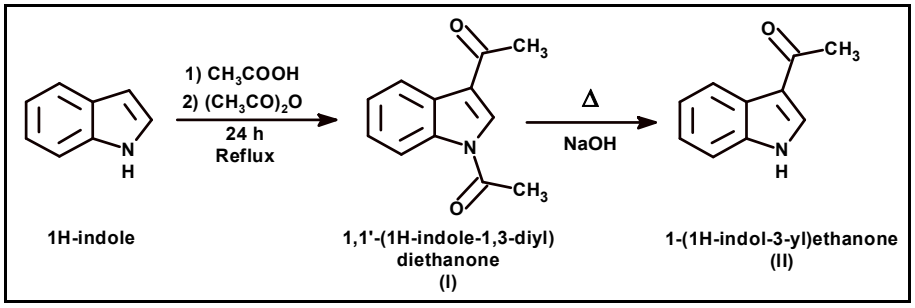

Chart-1 synthesis steps of Compound (II)

2.3.3. Synthesis of 3-(4-hydroxyphenyl)-1-(1H-indole-3-yl) prop-2-en-1-one (III)

3-acetyl indole (II)0.5 g (0.00315 mol) was solved in methanol (40 ml) drops of sodium hydroxide (10%) and 0.4 g of 4-hydroxybenzaldehydewere added. The mixture was refluxed for 24 h, the reaction mixture was concentrated under vacuum to quarter its volume and diluted with cold water. The dried crude was recrystallized from ethanol. The product was obtained as yellow crystals with a yield of 0.2 g (40%), m.p 182^{o}C. The reaction was monitored by TLC using chloroform. (See chart-2)

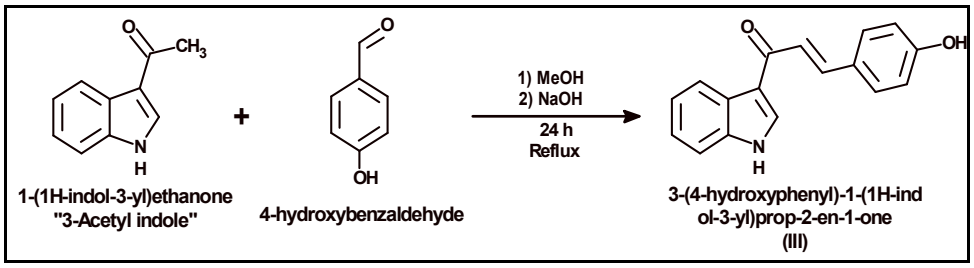

Chart-2synthesis steps of Compound (III)

2.4. Vital Study:

2.4.1. Methods

For seedling used in the research, maize seeds are soaked for 3 hours at 50ºC, and then grown in darkness with continuous ventilation at 30ºC for 6 days.

2.4.2. Preparation plant sections

Seedling clips of 1cm long are taken from the seedling stalk in the area under the node, one gram of plant these sections is weighed to extract and carbohydrates calibration.

These clips are incubated in water medium and incubated in medium of synthesized compounds at gradient concentrations (1,3,5 mg/l) witch souled first in 1 ml of DMSO and complete the volume with distilled water.

2.4.3. Extraction of proteins from clips seedling and calibrate it

After 24 hour (incubated time) the clips fixed in boiling ethanol (98%) for an hour, then crashed and washed with many organic solvents. After this period, potassium hydroxide solution is added (10%) to extract proteins with the solutions of HCl and complete the volume with distilled water[10].

As for calibration, proteins form with Coomassie detector a blue complex where its color intensity of absorbance is measured at 595 nm wavelength, after taking 1.5 ml of coomassie in a test tube with 1.5 ml of sample, and after a good combination is left in darkness for exactly 10 minutes[10].

2.4.4. Extraction of carbohydrates from clips seedling

2.4.4.1. Soluble carbohydrates

Dissolved Hexoses and pentoses extracted by 82% ethanol solution, after the evaporation of ethanol**,** solve the precipitate with distilled water to a certain size complements[11].

2.4.4.2. Starch and pentozanes

These are extracted by sulfuric acid solution (1.5N) and the volume is completed with distilled water[11].

2.4.4.3. Cellulose

The structural polysaccharides such as cellulose extracted by acid hydrolysis with boiling,sulfuric acid solution 80.7%is used for this purpose[11].

2.4.5. Calibration of carbohydrates

Hexoses calibrated by anthron detector, where the complex is formed in green, measured intensity of the absorbance at 620 nm wavelength, after taking 5ml of anthron in a test tube with 1ml of sample, and after a good combination is put in boiling water bath for exactly 10 minutes, then immediately cooled in an ice bath[11].

Pentoses calibrated by orcinol detector, where the complex is formed in blue, measured intensity of the absorbance at 660 nm wavelength, after taking 3ml of orcinol in a test tube with 1ml of sample, and after a good combination is put in boiling water bath for exactly 30 minutes[11].

3. Results and Discussions

3.1. Organic Synthesis

3.1.1. Synthesis of 3-Acetyl indole (II)

In this work we first prepared the basic 3-acetyl indole (II) compound with good yield starting with indole and acetic acid reaction in the presence of anhydrous acetic acid, 1,3-di acetyl indole (I) as intermediate compound, which have acetyl group (Chart-1) [12].

The infrared spectra (IR), nuclear magnetic resonance (^{1}H-NMR) and mass spectrometry (MS) of these compounds were studied.

Noting the absorption band clique carbonyl belonging to the acetyl group and mass spectrum showed value of 159 for the approval of the molecular weight of this compound. The ^{1}H-NMR spectrum (Figure -1) for this compound include chemical shifts for methyl and aryl groups, and this is consistent with the reference data for this compound[12].

(MS): m/z=159

IR (KBr pellets, cm^{-1}): 3386.39 (N-H), 1704.76 (C=O), 1215.9 (C-N), 757.88 (C-H_{Ar}).

H^{1}NMR: (Methanol, ppm): 2.518 (s, 3H, CH_3); 7.191-7.458 (m, 4H, C-H_{Ar}); 8.127 (s, -αH); 8.234 (s, N-H).

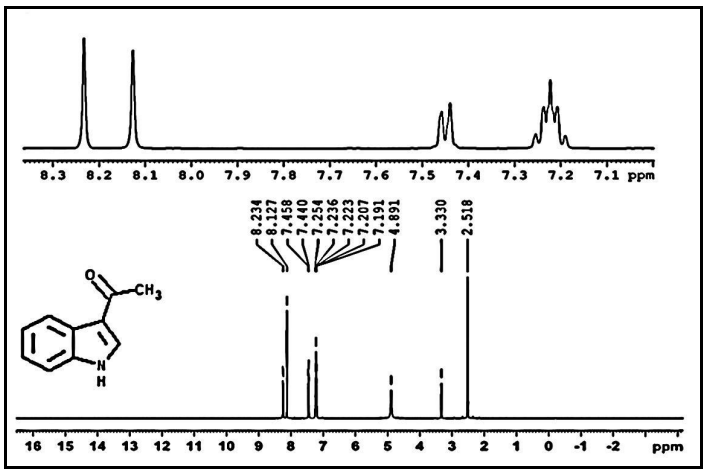

Fig-1 ^{1}H-NMR spectrum for Compound (II)

3.1.2. Synthesis of 3-(4-hydroxyphenyl)-1-(1H-indole-3-yl) prop-2-en-1-one (III)

The compound (III) was synthesized starting with 3-acetyl indole (II) (which previously synthesized) and 4-hydroxybenzealdehyde by aldole condensation (Chart-2).

The infrared spectra (IR), nuclear magnetic resonance (^{1}H-NMR) and mass spectrometry (MS) of these compounds were studied.

Noting the absorption band clique carbonyl belonging to the acetyl group and appearance absorption band for alyl and hydroxyl group and mass spectrum showed value of 263 for the approval of the molecular weight of this compound. It is noticed in ^{1}H-NMR spectrum (Figure -2) that all the signals of protons and functional groups are in accordace with the structure of this compound.

(MS): m/z=263

IR (KBr pellets, cm^{-1}): 2832.92 (N-H), 1694.16 (C=O), 3155.94 (-OH), 1440.56 (C=C_{Ar}), 1241.93 (C-N), 754.031 (C-H_{Ar}).

H^{1}NMR: (Methanol, ppm): 7.191-7.254 (m, 4H, C-H_{Ar}); 7.313-7.374 (m, 4H,C-H_{Ar}); 7.528-542 (d, =CH); 8.127(s, N-H); 8.164-8.178(d, =CH); 8.255 (s, -αH); 9.614 (s, -OH).

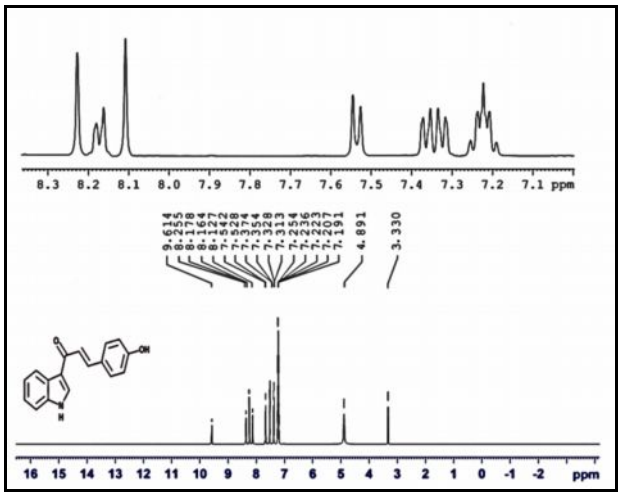

Fig-2 ^{1}H-NMR spectrum for Compound (III)

3.2. CalibrationResults

3.2.1. Impact of compounds (II) and (III) on maize seedlings growth

To make sure of the vital effectiveness of the two synthesized compounds [II] and [III] on the physiological properties of the plant, a series of experiments was made on seedlings plant maize (Zea mays L.) during 6 days of the growth of the seedling in the aqueous medium and in DMSO solution (0.1%) containing gradient concentrations of these two compounds (1,3,5) mg/l.

To determine the possibility of using DMSO as hormones's solvent in the experiments to be conducted, it has been shown that its use in concentration of (0.1%) does not show any effect on the speed and growth rate of corn seeds, compared with the speed and growth rate of these seeds in aqueous media.So it was used in this concentration as a solvent for the studied hormones when determining their hormonal effectiveness "Auxinal".

At the end of the development, the length of the grown plants was measured, (Table-1) and (Figure-3) show the growth rate of the studied seedling during 6 days of development in different mediums.

Table-1: lengths of maize seedlings in aqueous and DMSO solution with the studied compounds (II) and (III) and without them

Compound (III) in DMSO (0.1%) (mg/l)			Compound (II) in DMSO (0.1%) (mg/l)			DMSO medium	H_2O medium	
5	3	1	5	3	1			
15.2	16.5	13.1	18.1	15.1	13.3	15	14.8	Length (cm)

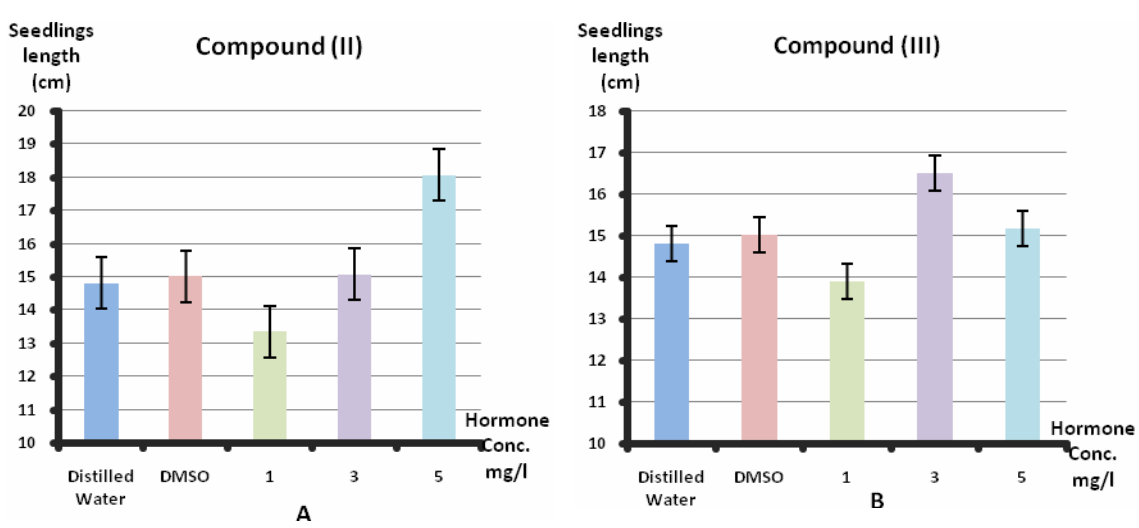

Fig-3: Growth of maize seedlings in aqueous and DMSO solution with the studied compounds (II) and (III) and without them

3.2.2. Impact of compounds (II) and (III) on maize stalk clips growth

A study is made on the effectiveness of the synthesized hormones on the growth of corn stalk clips in age of 6 days and which have been cultivated in the aqueous medium and in DMSO (0.1%) solution and in solutions containing gradient concentrations 1,3,5 mg/l of dissolved compounds in DMSO (0.1%).

Seedling clips of 1cm long taken from the area under the node and above seed with one gram of plant sections per sample were incubated at the rate of two samples per concentration for 24 h in an incubator in darkness with continuous ventilation in the media mentioned above.

To determine the effect of compounds on the maize stalk clips growth at the end of incubation period, the length of the incubated clips was measured after dried on filter paper.

Table-2 and Figure-4 shows growth percentage in various sections of experimental conditions:

Table-2: lengths of maize stalk clipsincubated in aqueous and DMSO solution with the studied compounds (II) and (III) and without them

Compound (III) in DMSO (0.1%) (mg/l)			Compound (II) in DMSO (0.1%) (mg/l)			DMSO medium	H_2O medium	Control	
5	3	1	5	3	1				
11.08	10.64	10.72	11.00	10.76	10.04	10.64	10.60	10	Length (mm)

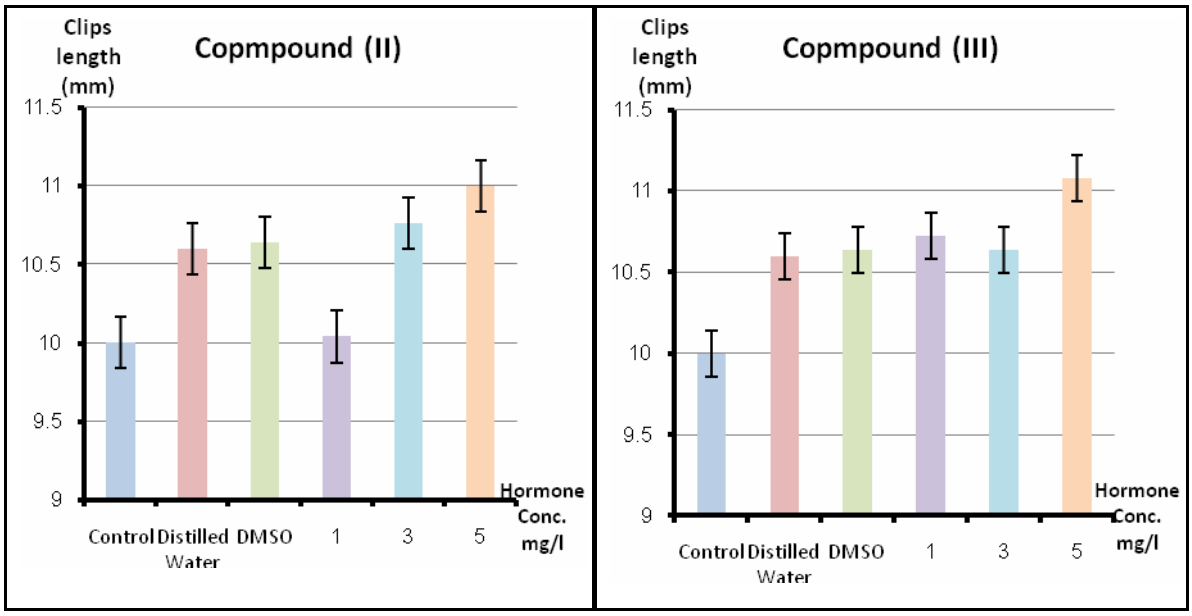

Fig-4: Growth percentage of maize stalk clipsincubated in aqueous and DMSO solution with the studied compounds (II) and (III) and without them

The results show that the growth percentage in both aqueous solution and DMSO (0.1%) was the same, i.e. an increase of up to 4% of the original length.

The compound (II) did not have a reliable effect on incubated clips growth; visual impact has reached its peak in concentration of 5 mg/l. Moreover, compound (III) did not have a significant effect on the growth of these clips.

3.2.3.Impact of compounds (II) and (III) on maize stalk clips total proteins content

Bradford method was used to calibrate the amount of total proteins in the incubated cells [12]. Test results that have been reached are shown in Table-3 and Figure-5:

Table-3: Total proteins content in maize stalk clipsincubated in aqueous and DMSO solution with the studied compounds (II) and (III) and without them

Compound (III) in DMSO (0.1%) (mg/l)			Compound (II) in DMSO (0.1%) (mg/l)			DMSO medium	H_2O medium	Control	
5	3	1	5	3	1				
2.19 (±0.01)	2.05 (±0.01)	1.41 (±0.01)	1.81 (±0.01)	2.01 (±0.01)	2.19 (±0.02)	0.90 (±0.01)	0.42 (±0.01)	2.28 (±0.00)	Protein Conc. (mg/g) (±SD)
3.95	10.09	38.16	20.61	11.84	3.95	60.53	81.58		Disintegration Percentage %

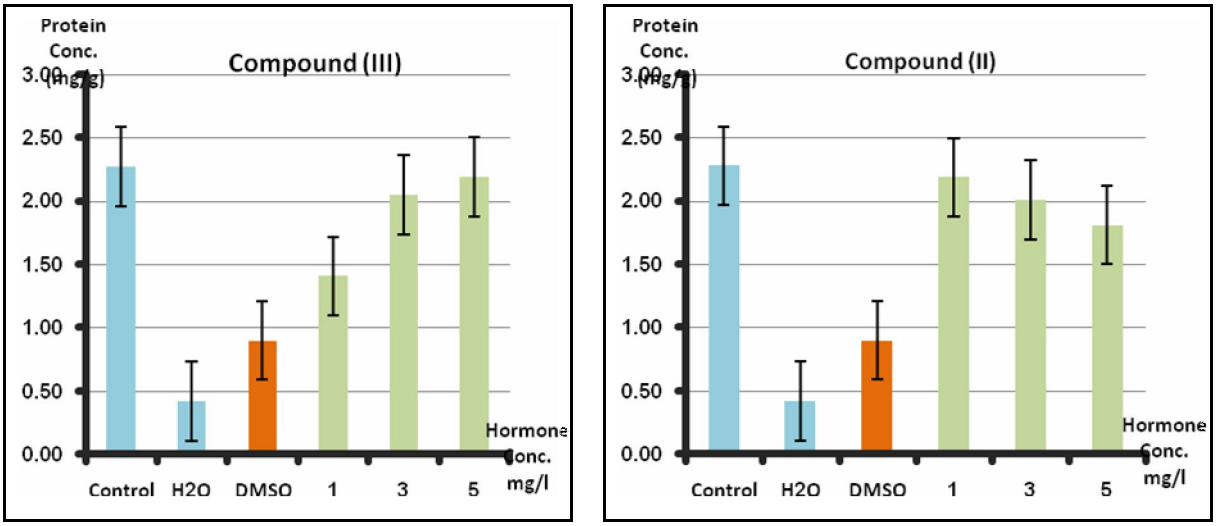

Fig-5: Total proteins content in maize stalk clipsincubated in aqueous and DMSO solution with the studied compounds (II) and (III) and without them

Regarding the amount of proteins in maize stalk clips incubated in the aqueous medium and in DMSO (0.1%) and solutions of the studied hormones, it's clear from the figure that the differences in the amount of degraded proteins during incubation period (24 h) in the samples incubated in the aqueous medium and in DMSO (0.1%) wasn't reliable, and has reached to approximately 71.99%.

The effect of the compound (II) dissolved in DMSO (0.1%) was convergent in solutions of all concentrations that have been used, where the presence of this compound (II) in the incubation medium was inhibited the disintegration of the proteins in the incubated cells, where the disintegration percentage of proteins in these cells to 12.13% from its original amount. As well as for compound (III) dissolved in DMSO (0.1%), where the disintegration percentage of proteins in these cells to 17.40% of its original amount.

3.2.4. Impact of compounds (II) and (III) on carbohydrate metabolism

In this group of experiments, was studied the impact of synthesized hormones on carbohydrate content (Hexoses, Pentoses, Starch, Pentozanes, Cellulose) in maize stalk incubated clips, according to the above-mentioned incubation conditions (temperature, ventilation, darkness, period, incubation media).

3.2.4.1. Impact of compounds (II) and (III) on soluble carbohydrate metabolism

The results of tests that have been accessed (Table-4 and Figure -6) that the hexoses amount in maize stalk cells in 6 days of age up to 17.50 mg / g, and which goes down when these sections incubated for 24 hours in aqueous solution or DMSO (0.1%) to about 9.64 mg/g.

Presence of compound (II) in incubation medium rushed to the disintegration of these hexoses reaching this amount to 2.48 mg/g. Conversely the presence of compound (III) in incubation medium was discouraged the disintegration of this hexoses, where its amount in incubated maize clips reached to 15.33 mg/g.

Table-4: Hexoses content in maize stalk clipsincubated in aqueous and DMSO solution with the studied compounds (II) and (III) and without them

Compound (III) in DMSO (0.1%) (mg/l)			Compound (II) in DMSO (0.1%) (mg/l)			DMSO medium	H_2O medium	Control	
5	3	1	5	3	1				
16.91 (±0.04)	15.27 (±0.05)	13.80 (±0.04)	2.96 (±0.05)	1.67 (±0.04)	2.81 (±0.05)	9.71 (±0.05)	9.57 (±0.06)	17.50 (±0.02)	Hexoses Conc. (mg/g) (±SD)
3.37	12.74	21.14	83.09	90.46	83.94	44.51	45.31		Disintegration Percentage %

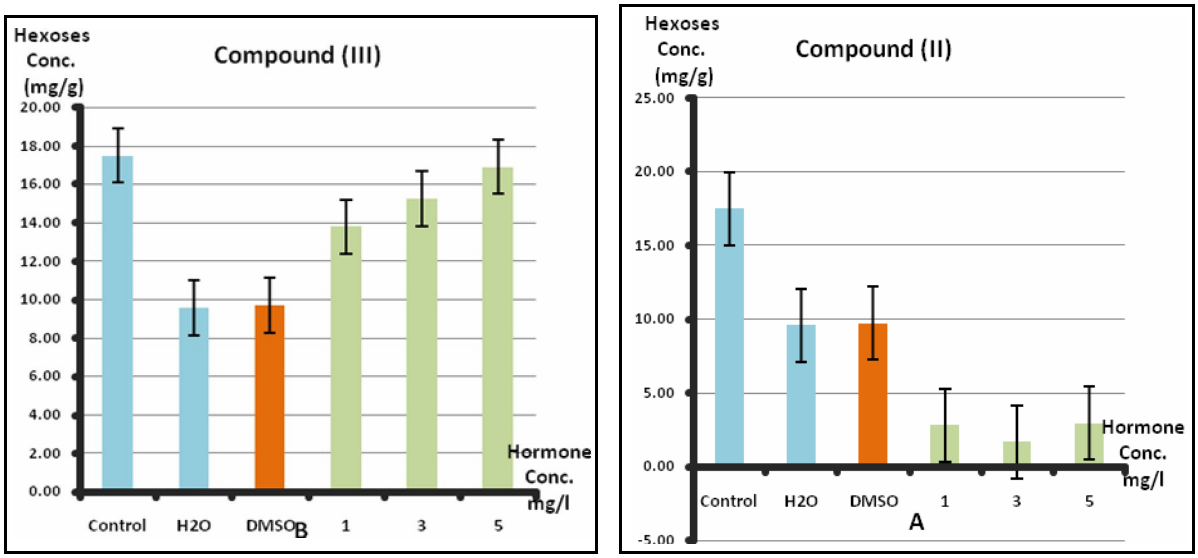

Fig-6: Hexoses content in maize stalk clipsincubated in aqueous and DMSO solution with the studied compounds (II) and (III) and without them

Regarding to the quantity of pentoses, the analyzes have been done on cells of maize stalk clips showed that their quantity up to 7.94 mg/g, as illustrated in Table-5 and Figure-7, and which goes down when these sections incubated for 24 hours in aqueous or DMSO (0.1%) solution to about 3.14 mg/g.

Presence of compound (II) in incubation medium rushed to the disintegration of these pentoses reaching this amount to 1.30 mg/g. Conversely presence of the compound (III) in incubation medium was discouraged the disintegration of this pentoses, where its amount in incubated maize clips reached to 5.04 mg/g.

Table-5: Pentoses content in maize stalk clipsincubated in aqueous and DMSO solution with the studied compounds (II) and (III) and without them

Compound (III) in DMSO (0.1%) (mg/l)			Compound (II) in DMSO (0.1%) (mg/l)			DMSO medium	H_2O medium	Control	
5	3	1	5	3	1				
5.24 (±0.03)	5.45 (±0.09)	4.44 (±0.05)	1.44 (±0.05)	1.14 (±0.04)	1.33 (±0.02)	3.13 (±0.04)	3.15 (±0.06)	7.94 (±0.04)	Pentoses Conc. (mg/g) (±SD)
34.01	31.36	44.08	81.86	85.64	83.25	60.58	60.33		Disintegration Percentage %

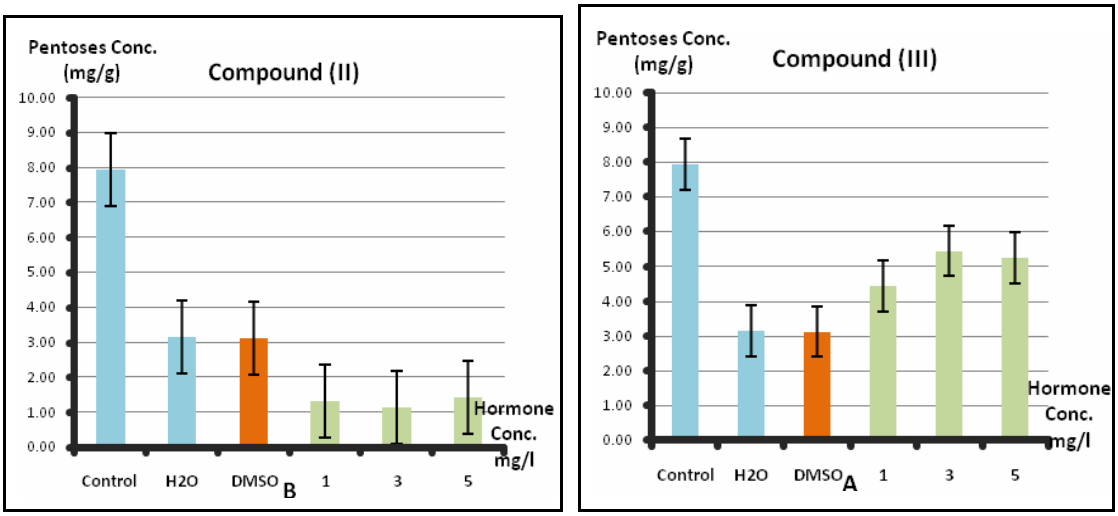

Fig-7: Pentoses content in maize stalk clipsincubated in aqueous and DMSO solution with the studied compounds (II) and (III) and without them

3.2.4.2. Impact of compounds (II) and (III) on starch and pentozanes metabolism

Reached the amount of starch in the cells of corn stalk to 24.33 mg / g, according to the results of experiments that have been done are described in Table -6 and Figure-8, which decreased quantity in incubated sections (24 hours) in aqueous or DMSO (0.1%) solution to about 10.10 mg/g.

Presence of compound (II) in incubation medium rushed to the disintegration of starch reaching this amount to 5.71 mg/g. As the presence of the compound (III) in incubation medium has had an accelerator impact on the disintegration of starch, where its amount in incubated maize clips reached to 9.66 mg/g, as illustrated in table-6 and figure-8.

Table-6: Starch content in maize stalk clipsincubated in aqueous and DMSO solution with the studied compounds (II) and (III) and without them

Compound (III) in DMSO (0.1%) (mg/l)			Compound (II) in DMSO (0.1%) (mg/l)			DMSO medium	H_2O medium	Control	
5	3	1	5	3	1				
7.33 (±0.16)	9.60 (±0.08)	12.05 (±0.16)	5.60 (±0.15)	6.20 (±0.15)	5.34 (±0.08)	10.24 (±0.09)	9.95 (±0.15)	24.33 (±0.07)	Starch Conc. (mg/g) (±SD)
69.87	60.54	50.47	76.98	74.52	78.05	57.91	59.10		Disintegration Percentage %

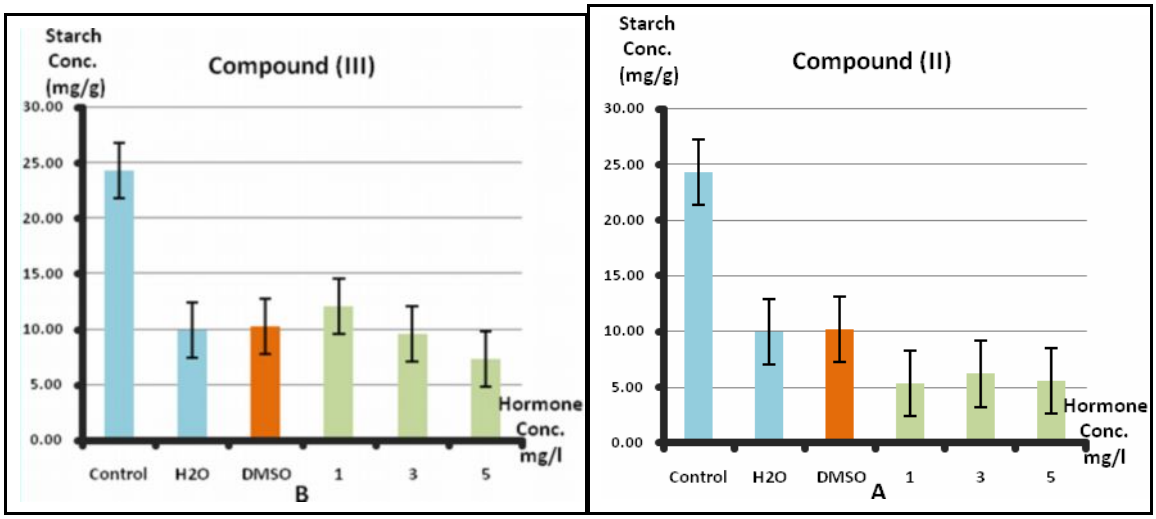

Fig-8: Starch content in maize stalk clipsincubated in aqueous and DMSO solution with the studied compounds (II) and (III) and without them

Regarding to the amount of pentozanes, results have shown (Table-6 and Figure-8), that two studied hormones have different effects from those on other carbohydrate groups. Analyses have shown that the amount of this pentozanes in the cells of corn stalk age 6 days is up to 6.77 mg/g, and goes down when these sections are incubated for 24 hours in aqueous solution or DMSO (0.1%) to about 4.07 mg/g.

The quantity of those pentozanes reached to 5.44 mg/g, in the presence of the compound (II) in incubation medium, which makes of this compound an inhibiting effect on the disintegration of these compounds. Here it should be noted that different concentrations of this compound has comparable efficacy in their inhibitory impact on the disintegration of the pentozanes, as well as for the effect of the compound (III), where the amount of these compounds reached to 5.78 mg/g.

Table-7: Pentozanes content in maize stalk clipsincubated in aqueous and DMSO solution with the studied compounds (II) and (III) and without them

Compound (III) *in* DMSO (0.1%) (mg/l)			Compound (II) in DMSO (0.1%) (mg/l)			DMSO mediu m	H_2O medium	Control	
5	3	1	5	3	1				
6.34 (±0.01)	5.62 (±0.02)	5.37 (±0.02)	5.07 (±0.02)	5.63 (±0.02)	5.62 (±0.01)	4.16 (±0.02)	3.97 (±0.02)	6.77 (±0.02)	Pentozanes Conc. (mg/g) (±SD)
6.35	16.99	20.68	25.11	16.84	16.99	38.55	41.36		Disintegration Percentage %

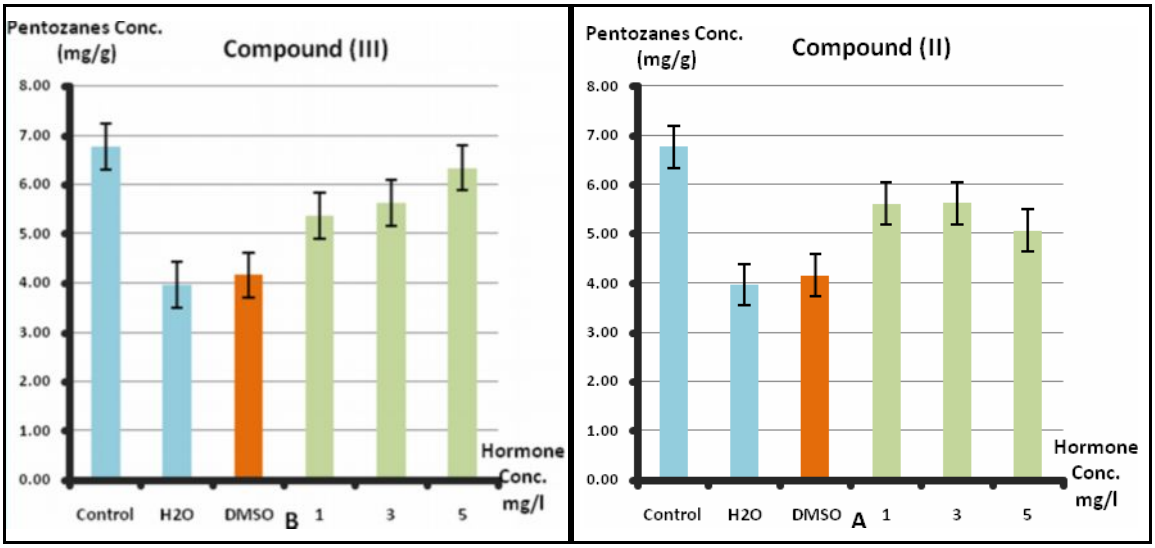

Fig-9: Pentozanes content in maize stalk clipsincubated in aqueous and DMSO solution with the studied compounds (II) and (III) and without them

3.2.4.3. Impact of compounds (II) and (III) on cellulose metabolism

The results of tests, as illustrated in Table-8 and Figure-10, showed that the amount of cellulose in corn stalk cells age of 6 days is up to 24.13 mg/g, which goes down when these sections are incubated for 24 hours in aqueous or DMSO (0.1%) solution was significantly increased to about 2005 mg/g.

compound (II); when in the incubation medium, had no significant effect on the changes in terms of the amount of cellulose in incubation conditions mentioned above, as well as for compound (III), reaching the amount of cellulose to about 19.66 mg/g.

Table-8: Cellulose content in maize stalk clipsincubated in aqueous and DMSO solution with the studied compounds (II) and (III) and without them

Compound (III) in DMSO (0.1%) (mg/l)			Compound (II) in DMSO (0.1%) (mg/l)			DMSO medium	H_2O medium	Control	
5	3	1	5	3	1				
23.29 (±0.12)	13.95 (±0.18)	21.73 (±0.06)	16.86 (±0.15)	13.93 (±0.07)	18.05 (±0.12)	20.64 (±0.09)	19.46 (±0.12)	24.13 (±0.05)	Cellulose Conc. (mg/g) (±SD)
3.48	42.19	9.95	30.13	42.27	25.20	14.46	19.35		Disintegration Percentage %

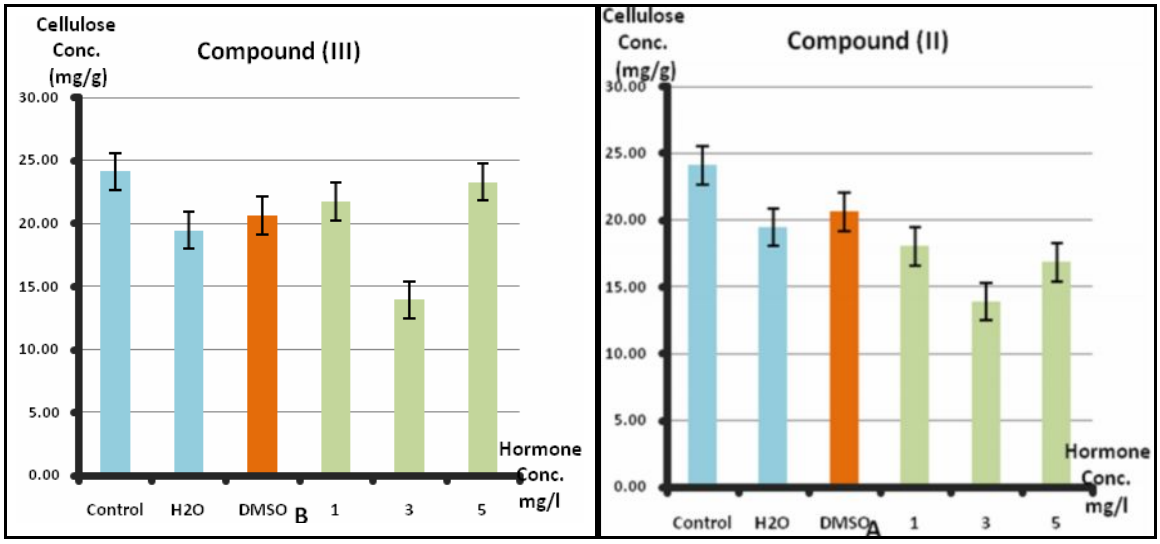

Fig-10 Cellulosecontent in maize stalk clipsincubated in aqueous and DMSO solution with the studied compounds (II) and (III) and without them

4. Conclusions and Recommendations

4.1. Two indolic derivatives were synthesized, one previously known3-acetyl indoleandthe other is 3-(4-hydroxyphenyl)-1-(1H-indole-3-yl) prop-2-en-1-one which was not knownpreviously.

4.2. The infrared spectra (IR), nuclear magnetic resonance (^{1}H-NMR) and mass spectrometry (MS) of these compounds were studied, and the spectral data confirmed the formation of the compounds.

4.3. The biological activity of the synthesized compounds was studied and has proven vital activity on maize plant growth hormones. Therefore, these synthesized two compounds can be used as plant growth hormones.

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