

3-Alkyl Derivatives of 6-Acetamido- and 6-Nitro-2-benzothiazolinones as Plant Growth Stimulators

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6-Acetamido- and 6-nitro-2-benzothiazolinones and their 3-substituted derivatives were synthesized. The compounds proved to be active as stimulators of fresh green mass and chlorophyll production in *Zea mays* L.; 6-nitro derivatives being more effective than the corresponding acetamides. Stimulation of the growth of green algae *Chlorella vulgaris* was not significant.

Some 3-substituted [1, 2] and 3,6-disubstituted [3] derivatives of 2-benzothiazolinone were found to be active as plant growth regulators and herbicides. Only a few precise data about biological activity are given in patents and they are seldom comparable among themselves for the great variety of the testing methods. These facts initiated us to synthesize some new 3-substituted 6-acetamido- and 6-nitro-2-benzothiazolinones as well as several easily available known ones (Table 1).

6-Nitro-2-benzothiazolinethione, prepared by nitration of 2-benzothiazolinethione was oxidized in alkaline media and the intermediate obtained by acidification, 6-nitro-2-benzothiazolesulfonic acid, after degradation by heating gave 6-nitro-2-benzothiazolinone. This new method is more advantageous than the method of preparing 6-nitro-2-benzothiazolinone by nitration of 2-benzothiazolinone. While the degradation of 2-benzothiazolesulfonic acid takes 5 h of heating, the degradation of its 6-nitro derivative occurs immedi-

Table 1. Characterization of the Prepared 3-Alkyl 6-Substituted 2-Benzothiazolinones

Compound	Alkyl	6-Substituent	Formula M_r	$w_i(\text{calc.})/\%$				Yield %	M.p. °C
				$w_i(\text{found})/\%$					
				C	H	N	S		
II	C_2H_5	CH_3CONH	$\text{C}_{11}\text{H}_{12}\text{N}_2\text{O}_2\text{S}$	55.91	5.12	11.86	13.57	89.7	192.5—195.5
			236.30	55.65	5.11	12.03	13.51		
III	$\text{CH}_2\text{—CH=CH}_2$	CH_3CONH	$\text{C}_{12}\text{H}_{12}\text{N}_2\text{O}_2\text{S}$	58.05	4.87	11.28	12.91	99.5	185.5—188.5
			248.31	57.77	4.82	11.28	12.93		
IV	$(\text{CH}_2)_3\text{CH}_3$	CH_3CONH	$\text{C}_{13}\text{H}_{16}\text{N}_2\text{O}_2\text{S}$	59.07	6.10	10.60	12.13	78.3	160.0—162.5
			264.35	58.78	6.00	10.80	11.85		
V	$\text{CH}_2\text{C}_6\text{H}_5$	CH_3CONH	$\text{C}_{16}\text{H}_{14}\text{N}_2\text{O}_2\text{S}$	64.41	4.73	9.39	10.75	96.2	180—182
			298.37	64.20	4.79	9.41	10.79		
IX	$\text{CH}_2\text{—CH=CH}_2$	NO_2	$\text{C}_{10}\text{H}_{10}\text{N}_2\text{O}_3\text{S}$	50.84	3.41	11.86	13.57	81.9	147.5—148.5
			236.25	50.99	3.32	12.00	13.83		
X	$(\text{CH}_2)_5\text{CH}_3$	NO_2	$\text{C}_{13}\text{H}_{16}\text{N}_2\text{O}_3\text{S}$	55.70	5.75	9.99	11.44	90.5	68—70
			280.35	55.77	5.76	10.01	11.46		
XI	$\text{CH}_2\text{C}_6\text{H}_5$	NO_2	$\text{C}_{14}\text{H}_{10}\text{N}_2\text{O}_3\text{S}$	58.51	3.51	9.75	11.16	95.7	158.5—160
			287.42	58.47	3.51	9.90	11.39		
XIII	CH_2COOH	NO_2	$\text{C}_9\text{H}_8\text{N}_2\text{O}_5\text{S}$	42.52	3.38	11.02	12.61	62.5	267—270
			254.23	42.33	3.28	11.02	12.65		
XIV	COOC_2H_5	NO_2	$\text{C}_{10}\text{H}_8\text{N}_2\text{O}_5\text{S}$	44.78	3.01	10.44	11.95	75.2	130.5—132 ^a
			268.25	44.81	2.95	10.51	11.98		

a) No data available [4].

Table 2. IR Spectral Data ($\bar{\nu}/\text{cm}^{-1}$) of Some Characteristic Vibrations of Compounds I—XIV

Compound	$\nu(\text{C}=\text{O cycl.})$	6-Substituent		3-Alkyl
I	1690 sh	$\nu(\text{C}=\text{O})$ 1665 s,	$\nu(\text{NH})$ 3280 m	—
II	1685 m	$\nu(\text{C}=\text{O})$ 1620 s,	$\nu(\text{NH})$ 3305 m	—
III	1685 m	$\nu(\text{C}=\text{O})$ 1620 s,	$\nu(\text{NH})$ 3300 m	—
IV	1675 sh	$\nu(\text{C}=\text{O})$ 1635 s,	$\nu(\text{NH})$ 3290 m	—
V	1690 s	$\nu(\text{C}=\text{O})$ 1640 s,	$\nu(\text{NH})$ 3300 m	—
VI	1703 s	$\nu_s(\text{NO}_2)$ 1340 s,	$\nu_{as}(\text{NO}_2)$ 1503 m	—
VII	1680 s	$\nu_s(\text{NO}_2)$ 1330 s,	$\nu_{as}(\text{NO}_2)$ 1502 m	—
VIII	1675 s	$\nu_s(\text{NO}_2)$ 1325 s,	$\nu_{as}(\text{NO}_2)$ 1502 m	—
IX	1665 s	$\nu_s(\text{NO}_2)$ 1310 s,	$\nu_{as}(\text{NO}_2)$ 1500 m	—
X	1680 s	$\nu_s(\text{NO}_2)$ 1320 s,	$\nu_{as}(\text{NO}_2)$ 1500 m	—
XI	1695 s	$\nu_s(\text{NO}_2)$ 1320 s,	$\nu_{as}(\text{NO}_2)$ 1500 m	—
XII	1680 s	$\nu_s(\text{NO}_2)$ 1322 s,	$\nu_{as}(\text{NO}_2)$ 1502 m	$\nu(\text{OH})$ 3550 m
XIII	1625 s ^a	$\nu_s(\text{NO}_2)$ 1325 s,	$\nu_{as}(\text{NO}_2)$ 1500 m	$\nu(\text{C}=\text{O})$ 1715 s
XIV	1708 sh	$\nu_s(\text{NO}_2)$ 1325 s,	$\nu_{as}(\text{NO}_2)$ 1507 m	$\nu(\text{C}=\text{O})$ 1750 s

a) The lower wavenumber of the carbonyl group in the benzothiazolinone cycle of compound XIII can be explained by intramolecular hydrogen bond with the $-\text{COOH}$ group.

ately. As for the nitration of 2-benzothiazolinone and 2-benzothiazolinethione, the procedures are rather similar as regards reaction time and necessity of cooling.

Alkylation of 6-nitro- (VI) and 6-acetamido-2-benzothiazolinones (I) with alkyl halides was carried out in aqueous ethanol in the presence of equivalent amount of potassium hydroxide at reflux temperature. IR spectral data are in accordance with the expected structures (Table 2).

The effect of the synthesized compounds upon growth and synthesized chlorophyll content of green algae *Chlorella vulgaris* and of maize plants was tested using a wide concentration range. It was found that all the compounds studied at relatively low concentrations (10^{-6} — 10^{-9} mol dm $^{-3}$) showed stimulating influence mainly upon fresh green mass production and to a certain extent upon the synthesized chlorophyll content in maize plants as well (Table 3). It was confirmed that in

general the effect of NO $_2$ substituent in position 6 of 2-benzothiazolinone on fresh green mass production is more pronounced than the effect of CH $_3$ CONH substituent. The most efficient were unsubstituted compound VI and its 3-ethyl derivative VIII.

Both series of compounds stimulate also the chlorophyll synthesis in maize plants. This effect with 6-nitro derivatives is still lower than the corresponding stimulation of plant growth (Table 2). The most efficient compound was 3-allyl-6-nitro-2-benzothiazolinone (IX). Further biological experiments showed that this compound stimulated also the growth and the chlorophyll synthesis of tissue cultures at simultaneous production of plant organs in these cultures [5].

Table 4. Influence of 3-Substituted 6-Acetamido- and 6-Nitro-2-benzothiazolinones upon Green Algae *Chlorella vulgaris*

Compound	Concentration range	Relative chlorophyll content	Absorbance
	mol dm $^{-3}$	% ^a	% ^a
I	10^{-5} — 10^{-13}	98.4 \pm 4.7	111.0 \pm 3.7
II	10^{-5} — 10^{-13}	109.6 \pm 7.9	106.1 \pm 5.8
III	10^{-5} — 10^{-11}	96.1 \pm 4.5	100.1 \pm 4.5
IV	10^{-5} — 10^{-13}	100.9 \pm 4.7	100.5 \pm 4.7
V	10^{-5} — 10^{-11}	109.3 \pm 9.5	110.3 \pm 8.4
VI	10^{-5} — 10^{-13}	106.6 \pm 7.9	106.4 \pm 6.4
VII	10^{-5} — 10^{-13}	119.3 \pm 7.9	119.8 \pm 9.8
VIII	10^{-5} — 10^{-13}	102.3 \pm 8.7	98.1 \pm 6.7
IX	10^{-5} — 10^{-13}	107.3 \pm 10.8	101.5 \pm 11.3
X	10^{-5} — 10^{-13}	102.2 \pm 6.5	96.2 \pm 5.9
XI	10^{-5} — 10^{-13}	105.0 \pm 4.6	101.6 \pm 3.4
XII	10^{-5} — 10^{-13}	102.0 \pm 4.7	97.4 \pm 7.0
XIII	10^{-5} — 10^{-9}	104.2 \pm 7.1	96.6 \pm 9.0
XIV	10^{-5} — 10^{-9}	108.6 \pm 4.5	97.6 \pm 5.2

Table 3. The Effect of 3-Substituted 6-Acetamido- and 6-Nitro-2-benzothiazolinones upon the Maize Growth and upon the Synthesized Chlorophyll Content

Compound	Concentration range	Relative chlorophyll content	Relative seedlings mass
	mol dm $^{-3}$	% ^a	% ^a
I	10^{-6} — 10^{-9}	104.9 \pm 2.3	119.2 \pm 9.4
II	10^{-6} — 10^{-9}	113.7 \pm 5.0	106.2 \pm 11.6
IV	10^{-6} — 10^{-9}	114.5 \pm 3.7	115.4 \pm 7.9
V	10^{-6} — 10^{-9}	113.2 \pm 4.8	111.6 \pm 6.9
VI	10^{-6} — 10^{-8}	109.0 \pm 6.5	132.9 \pm 6.5
VII	10^{-6} — 10^{-8}	107.7 \pm 2.9	122.6 \pm 3.6
VIII	10^{-6} — 10^{-8}	107.7 \pm 6.9	130.4 \pm 5.2
IX	10^{-6} — 10^{-8}	105.4 \pm 5.2	122.6 \pm 5.5
XI	10^{-6} — 10^{-8}	119.3 \pm 3.7	123.4 \pm 10.0
XII	10^{-6} — 10^{-8}	112.3 \pm 4.2	125.8 \pm 2.8

a) Related to the control with 95 % confidence interval.

a) Related to the control with 95 % confidence interval.

The effect of investigated compounds on the growth and the synthesized chlorophyll content of green algae *Chlorella vulgaris* was in wide concentration range (10^{-5} — 10^{-13} mol dm $^{-3}$) relatively small (Table 4); more pronounced stimulation was found only with compounds V and VII. This fact anticipates the application of most of the investigated compounds as plant growth regulators in agriculture without the risk of increased contamination of water sources with algae, which could happen in the case of a compound stimulating the growth of algae in low concentrations when it is washed from the soil by rain.

The fact that both series of synthesized compounds stimulate the growth of plants but do not significantly stimulate the growth of algae points to the probability of different mechanisms of action of the studied compounds on these photosynthesizing organisms.

EXPERIMENTAL

The starting compounds I and VI were prepared according to Refs. [6, 7]. Analytical data, melting points determined on a Kofler block, and the yields of 3-substituted 6-acetamido- and 6-nitro-2-benzothiazolinones are presented in Table 1. The infrared spectra of compounds I—XIV were measured on a spectrophotometer IR 75 (Zeiss, Jena) in nujol suspension (Table 2).

For the investigation of the stimulating effect of the prepared compounds on fresh green mass production of maize plants and on synthesized chlorophyll content the maize grains were germinated in dark for 72 h in coiled cylinders of filtration paper moistened with distilled water and placed in perforated polyethylene bags. Next, the roots of the germinated seedlings were immersed in hydroponia containing aqueous solutions of the studied compounds with 1×10^{-6} , 1×10^{-7} , 1×10^{-8} , and 1×10^{-9} mol dm $^{-3}$ concentrations and cultivated in the cultivation box with the following illumination regime: 14 h in light and 10 h in dark. After 6 d of cultivation the green portions of the seedlings were weighed and the synthesized chlorophyll contents determined after extraction with *N,N*-dimethylformamide. The results were related to the controls (Table 3). The concentrations studied were prepared from the stock solution of the compound in *N,N*-dimethylformamide ($c = 1 \times 10^{-2}$ mol dm $^{-3}$) by dilution with distilled water. The content of the organic solvent in the sample and in the control was equal. The results presented in Table 3 are given as the average values over the studied concentration range.

Green algae *Chlorella vulgaris* were stationary-cultivated at (25 ± 1) °C at the following light conditions: 14 h illuminated, 10 h in the dark in the Šetlik's [8] cultivating medium which contained 20 mmol KNO $_3$, 2.5 mmol KH $_2$ PO $_4$, 4.0 mmol MgSO $_4 \cdot 7$ H $_2$ O, 7.0 μ mol CaCl $_2 \cdot 6$ H $_2$ O, 34.6 μ mol FeSO $_4$, 34.6 μ mol Na $_2$ EDTA, 50 μ mol H $_3$ BO $_3$, 5.0 μ mol ZnSO $_4 \cdot 5$ H $_2$ O, 5.0 μ mol MnCl $_2 \cdot 4$ H $_2$ O, 1.5 μ mol (NH $_4$) $_6$ Mo $_7$ O $_{24} \cdot 4$ H $_2$ O, 5.0 μ mol CoCl $_2 \cdot 6$ H $_2$ O, 5.0 μ mol Na $_2$ MoO $_4 \cdot 2$ H $_2$ O in 1 dm 3 of solution. After 7 d of cultivation the samples were refilled with distilled water to the original volume (to compensate for evaporation losses), and the absorbance at $\lambda = 660$ nm was measured in the suspension of cells (corresponding to the algal cell numbers), along with determination of the chlorophyll content following its extraction with *N,N*-dimethylformamide from the centrifuged algal cells. The total chlorophyll content of the algae in the cultivating medium was evaluated as in [9]. The values of the above parameters found were compared to those of the simultaneously cultivated control samples.

6-Acetamido-3-alkyl-2-benzothiazolinones II—V

6-Acetamido-2-benzothiazolinone (6.3 g; 0.03 mol) was dissolved in the solution of potassium hydroxide (2.0 g; 0.03 mol) in the mixture of water (10 cm 3) and ethanol (40 cm 3). Alkyl halide (0.035 mol) was added to the mixture and after 3 h of reflux and 16 h of keeping at 5 °C raw 6-acetamido-3-alkyl-2-benzothiazolinone precipitated from the solution. The compounds were crystallized using charcoal from the mixture of alcohol with water or from the mixture of ethyl acetate with petroleum ether (benzyl derivative V).

3-Alkyl-6-nitro-2-benzothiazolinones VII—XIV

6-Nitro-2-benzothiazolinone (5.9 g; 0.03 mol) was dissolved in the solution of potassium hydroxide (2.0 g; 0.03 mol) in the mixture of water (10 cm 3) and ethanol (20 cm 3) at reflux temperature. After adding alkyl halide (0.036 mol) the reaction mixture was kept at reflux temperature for 3 h and afterwards for 16 h at 5 °C. The products were recrystallized from suitable solvents: ethanol (XIV), ethanol—water (VIII, X), benzene (VII, XI), benzene—cyclohexane (IX), ethyl acetate (XII, XIII) using charcoal.

The yield of compounds VII (methyl derivative), VIII, and XII (ethyl and hydroxyethyl derivatives),

known from literature [10—12], was 80.9 %, 59.4 %, and 84.0 %, respectively.

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Benzothiazole Compounds XLI. Synthesis of 3-Alkoxycarbonyloxymethyl-2-benzothiazolinones

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The reaction of 3-hydroxymethyl-2-benzothiazolinone with the esters of chloroformic acid afforded 3-alkoxycarbonyloxymethyl-2-benzothiazolinones. When tested on *Vicia sativa* L., cv. Solarka and *Triticum aestivum*, several derivatives showed growth-stimulating effects.

Some derivatives of 3-substituted 2-benzothiazolinones, prepared by alkylation of 2-hydroxybenzothiazole, have evidenced good growth-regulating activity [1, 2]. It was the aim of our work to synthesize 3-alkoxycarbonyloxymethyl-2-benzothiazolinones which we expected to have analogous effects. The starting compound, 3-hydroxymethyl-2-benzothiazolinone was prepared from 2-hydroxybenzothiazole and formaldehyde in ethanol [3]. 3-Hydroxymethyl-2-benzothiazolinone in acetone reacted with the esters of chloroformic acid in the presence of triethylamine affording 3-alkoxycarbonyloxymethyl-2-benzothiazolinones.

These compounds dissolved in methanol showed low absorption in the UV spectra at $\lambda = 280$ and 286 nm ($\log \epsilon \approx 2.4 \text{ m}^2 \text{ mol}^{-1}$) and much higher absorption at $\lambda = 216$ nm ($\log \epsilon \approx 3.4 \text{ m}^2 \text{ mol}^{-1}$). The position and intensity of the absorption bands are very little influenced by changing the alkyl in ester group.

The growth-regulating activity of the synthesized compounds (Table 1) was studied on *Vicia sativa* L., cv. Solarka and on *Triticum aestivum*. It was found that compounds 3-methoxy- (I), 3-ethoxy- (II), 3-(2-chloroethoxy)- (III), and 3-propargyloxycarbonyloxymethyl-2-benzothiazolinone (IV) showed