Benzothiazole compounds
XVIII. Preparation of benzothiazolium salts, their growth regulation effects, and antimicrobial activities

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Benzothiazolium salts showing stimulation and inhibitory effects on plant growth have been prepared by the reaction of benzothiazole and 2-methylbenzothiazole with alkyl halides or dimethyl sulfate. The highest stimulation effect was found with 3-alkoxycarbonylmethylbenzothiazolium bromides, 3-methylbenzothiazolium methyl sulfate, and 3-benzylbenzothiazolium bromide. Good antibacterial and antifungal activities were observed with 3-methylbenzothiazolium iodide, 3-ethylbenzothiazolium iodide, 3-methylbenzothiazolium methyl sulfate, 2-methyl-3-ethylbenzothiazolium iodide, and 3-allylbenzothiazolium bromide.

Реакцией бензотиазола и 2-метилбензотиазола с галогеналканами и диметилсульфатом были приготовлены бензотиазолиевые соли, проявляющие стимулирующее и ингибирующее воздействие на рост растений. Наивысший стимулирующий эффект был обнаружен у 3-алкоксикарбонилметилбензотиазолийбромидов, 3-метилбензотиазолийметилсульфата и 3-бензилбензотиазолийбромида. Хорошая антибактериальная и антигрибковая активность была обнаружена у 3-метилбензотиазолийиодида, 3-этилбензотиазолийиодида, 3-метилбензотиазолийметилсульфата, 2-метил-3-этилбензотиазолийиодида и 3-аллилбензотиазолийбромида.

Synthesis of benzothiazolium salts has been the object of long-term study from theoretical aspects [1—6] and practical application. A great number of works have

been devoted mainly to 2-alkylbenzothiazolium salts, where the methyl and methylene groups can enter condensation reactions with carbonyl compounds. The products of these reactions have found application in productions of dyes and additives to photographic emulsions as well as in the field of catalysis [7—11]. The aim of our work was to synthesize benzothiazolium salts not described so far and study their antimicrobial activities and stimulation effects on plant growth [12]. This problem has been given little or no attention at all thus far. With 2-phenyl-3-alkylbenzothiazolium salts tested it was found that the derivatives with lower alkyl were active [13]. 2-Methyl-3-ethylbenzothiazolium iodide with β-(ethylthio)acrolein diethyl acetal gives a dicarbocyanine dye which is used as a medicine against intestinal parasites under the name of dithiazine [9]. Growth stimulation with benzothiazolium salts has not been observed till now.

Synthesis of 3-substituted and 2,3-disubstituted benzothiazolium salts (Table 1) was accomplished by treatment of benzothiazole and 2-methylbenzothiazole with the appropriate alkyl halides or dimethyl sulfate. The most suitable reaction medium was proved to be the mixture of anhydrous dimethylformamide and acetone (2:1) at 55—60 °C. At higher temperatures the yields decreased and higher portions of a colour product, not identified yet, were formed. We assume in accordance with [14, 15] that this product was a dimer formed through an intermediate of the carbene type and subjected to further changes in the presence of aerial oxygen. In target plant growth regulations, synthetic phytohormones of the auxine type with the basic indole type structure of β-indolylacetic acid (IAA) are often utilized [16]. When nitrogen atom is replaced by carbon or oxygen, the formed compounds retain their phytohormonal properties and stimulate the growth of coleoptiles [17]. Replacement of the pyrrole ring with oxazole or thiazole rings results in compounds which also retain their phytohormonal activity analogous to auxine activity [16]. We have found that some of the synthesized derivatives (compounds *II*, *X*, *XIII*, *XV*, *XVIII*) are more effective growth stimulators than the standards, *i.e.* IAA and 2,4-dichlorophenoxyacetic acid (2,4-D). The results obtained are presented in Table 2. Since each benzothiazolium salt has its own characteristic curve for stimulation activity, those molar concentrations are presented at which the stimulation effect was the highest. The dependence of stimulation activity of some compounds (*X*, *XIII*, *XVIII*) on concentration is illustrated in Figs. 1 and 2 in comparison with the standards (IAA; 2,4-D). To express the activity of compounds at concentrations of maximum activity of the standard is not the best thing to do from the viewpoint of practical application. IAA shows the best activity +3.10 mm at 10^{-12} mol dm^{-3}, while the compound *V* only + 0.4 mm, *X* + 5.8 mm, *XV* + 5.55 mm, and *XVIII* + 3.0 mm at the same molarity. The standard 2,4-D showed the best activity + 4.95 mm on vetch at 10^{-9} mol dm^{-3}, while the compound *V* − 0.4 mm (inhibition), *X* + 6.3 mm, *XV* + 5 mm, and *XVIII* + 2 mm at the same molarity. We attempted to correlate the

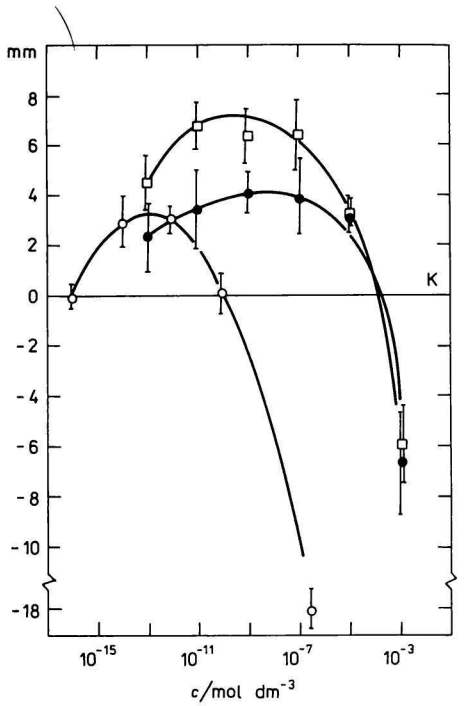


Fig. 1. Regulation effects of the compounds *X* and *XIII* on growth of vetch in comparison with the activity of β-indolylacetic acid (IAA).
○ IAA; □ *X*; ● *XIII.*

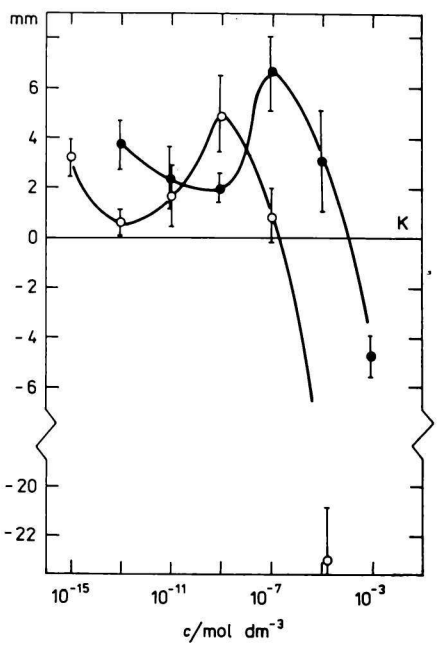
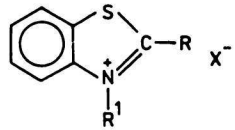


Fig. 2. Regulation effect of the compound *XVII* on growth of vetch in comparison with that of 2,4-dichlorophenoxyacetic acid (2,4-D).
○ 2,4-D; ● *XVII.*

Table 1

Characterization of the synthesized benzothiazolium salts



Compound	R	R¹	X⁻	Formula	*M*	Calculated/found % C	% H	% N	% S	Yield %	M.p. °C
I	H	CH_3	I	C_8H_8INS	277.13	34.67	2.90	5.05	11.57	73	213—215
						34.53	2.94	4.91	11.43		
II	H	CH_3	CH_3SO_4	$C_9H_{11}NO_4S_2$	261.32	41.36	4.24	5.35	24.54	78	104—105
						41.27	4.13	5.41	24.40		
III	H	C_2H_5	I	$C_9H_{10}INS$	291.16	37.12	3.46	4.81	11.01	63	139—142
						37.24	3.35	4.92	11.12		
IV	CH_3	C_2H_5	I	$C_{10}H_{12}INS$	305.18	39.35	3.96	4.58	10.50	69	197—198
						39.23	4.03	4.41	10.54		
V	H	$CH_2CH=CH_2$	Br	$C_{10}H_{10}BrNS$	256.17	46.88	3.93	5.46	12.51	69	147—149
						46.72	3.90	5.52	12.48		
VI	CH_3	$CH_2CH=CH_2$	Br	$C_{11}H_{12}BrNS$	270.20	48.89	4.47	5.18	11.86	60	216—217
						48.76	4.54	5.09	11.82		
VII	CH_3	$CH_2C\equiv CH$	Br	$C_{11}H_{10}BrNS$	268.18	49.26	3.75	5.22	11.95	64	244—246
						49.21	3.75	5.21	12.07		
VIII	H	CH_2COOH	Br	$C_9H_8BrNO_2S$	274.14	39.43	2.94	5.10	11.68	61	246—249
						39.57	2.80	5.19	11.62		
IX	CH_3	CH_2COOH	Br	$C_{10}H_{10}BrNO_2S$	288.17	41.68	3.49	4.86	11.12	68	224—225
						41.49	3.34	4.98	11.12		

Table 1 (Continued)

Compound	R	R¹	X⁻	Formula	*M*	Calculated/found				Yield %	M.p. °C
						% C	% H	% N	% S		
X	H	CH_2COOCH_3	Br	$C_{10}H_{10}BrNO_2S$	288.17	41.68	3.49	4.86	11.12	71	152
						41.52	3.39	4.56	10.97		Decomposition
XI	H	CH_2COOCH_3	Cl	$C_{10}H_{10}ClNO_2S$	243.71	49.28	4.13	5.74	13.15	15	195—198
						49.50	4.28	5.69	13.29		
XII	CH_3	CH_2COOCH_3	Br	$C_{11}H_{12}BrNO_2S$	302.19	43.72	4.00	4.63	10.61	59	214—217
						43.54	4.15	4.80	10.76		
XIII	H	$CH_2COOC_2H_5$	Br	$C_{11}H_{12}BrNO_2S$	302.19	43.72	4.00	4.63	10.61	75	174
						43.43	3.82	4.60	10.61		Decomposition
XIV	CH_3	$CH_2COOC_2H_5$	Br	$C_{12}H_{14}BrNO_2S$	316.22	45.58	4.46	4.42	10.13	58	189—190
						45.81	4.62	4.32	10.04		
XV	H	$CH_2COOC_3H_7$	Br	$C_{12}H_{14}BrNO_2S$	316.22	45.58	4.46	4.42	10.13	68	185
						45.27	4.31	4.28	10.20		Decomposition
XVI	CH_3	$CH_2COOC_3H_7$	Br	$C_{13}H_{16}BrNO_2S$	330.25	47.28	4.88	4.24	9.70	54	192—193
						47.42	4.68	4.10	9.57		Decomposition
XVII	H	$CH_2COO\text{-}i\text{-}C_3H_7$	Br	$C_{12}H_{14}BrNO_2S$	316.22	45.58	4.46	4.43	10.14	79	220
						45.39	4.35	4.33	10.20		Decomposition
XVIII	H	$CH_2C_6H_5$	Br	$C_{14}H_{12}BrNS$	306.22	54.91	3.94	4.57	10.47	74	201
						54.80	4.03	4.42	10.55		

Table 2

Stimulation and inhibitory effects of benzothiazolium salts on growth of vetch

Compound	Stimulation		Inhibition	
	+ mm	mol dm^{-3}	− mm	mol dm^{-3}
I	0.85	10^{-9}	8.8	10^{-3}
II	3.75	10^{-11}	5.5	10^{-3}
III	1.35	10^{-13}	12.4	10^{-3}
IV	4.25	10^{-7}	3.5	10^{-3}
V	2.6	10^{-13}	20.7	10^{-3}
VI	4.3	10^{-7}	8.15	10^{-3}
VII	0.9	10^{-9}	10.3	10^{-3}
VIII	0.95	10^{-11}	7.55	10^{-3}
IX	1.25	10^{-13}	1.70	10^{-11}
X	6.8	10^{-11}	6.0	10^{-3}
XI	5.0	10^{-5}	7.95	10^{-3}
XII	2.35	10^{-7}	6.2	10^{-3}
XIII	4.05	10^{-9}	6.75	10^{-3}
XIV	2.0	10^{-5}	10.9	10^{-3}
XV	5.55	10^{-13}	—	—
XVI	2.1	10^{-9}	4.2	10^{-3}
XVII	4.8	10^{-7}	5.35	10^{-3}
XVIII	6.7	10^{-7}	25.75	10^{-1}
IAA	3.10	10^{-12}	18.55	10^{-6}
2,4-D	4.95	10^{-9}	23.30	10^{-5}

stimulation effects of the derivatives mentioned above with partition coefficients determined spectrophotometrically for the system water—octanol or with the wavenumbers $\nu(C=N^+)$ and $\nu(C=C)$ (Table 3). However, we have not found a statistically significant correlation. During experiments, variabilities in biological heterogeneity were observed which could not be excluded from tests of such a nature. The dependence of stimulation activity on concentration of the stimulator is expressed in majority of cases by two- and three-phase curves, which is probably brought about by fluctuations of metabolism in degradation of the stimulator [18]. Practical application of compounds is simple since they are soluble in water. The concentrations at which the compounds became inhibitors are presented in Table 2.

The compounds synthesized were tested for antibacterial, antifungal (Table 4), and some derivatives also for antivermal activities. From the results obtained it follows that the compounds are antibacterially active fairly well, mainly against gram-positive bacteria. Good results were achieved also in the field of antifungal activity. It was found that the compounds at higher concentrations acted fungicidally and at lower concentrations fungistatically. The derivatives *I*, *V*, and *X* were

Table 3

IR and UV characteristics of the synthesized compounds

Compound	$\nu(C=N^+)$	$\nu(C=C)$	$\lambda_{max\ I}$/nm log ε_1	$\lambda_{max\ II}$/nm log ε_2	Compound	$\nu(C=N^+)$	$\nu(C=C)$	$\lambda_{max\ I}$/nm log ε_1	$\lambda_{max\ II}$/nm log ε_2
I	1580	1516	275 3.71		*X*	1578	1502	278 3.75	236 3.88
II	1584	1524	275 3.75	234 3.91	*XI*	1582	1502	280 3.78	236 3.92
III	1578	1504	276 3.72		*XII*	1573	1504	270 3.91	237 4.08
IV	1579	1514	274 3.79		*XIII*	1581	1504	280 3.76	236 3.89
V	1579	1499	278 3.74	235 3.93	*XIV*	1579	1512	279 3.79	239 3.87
VI	1579	1508	277 3.81	238 3.93	*XV*	1580	1498	280 3.76	236 3.89
VII	1570	1513	278 3.84	238 3.95	*XVI*	1578	1504	280 3.76	239 3.87
VIII	1581	1499	279 3.77	236 3.90	*XVII*	1579	1499	280 3.75	236 3.90
IX	1581	1518	278 3.80	238 3.88	*XVIII*	1581	1498	280 3.74	235 3.98

Table 4

Antimicrobial activity of the synthesized benzothiazolium salts

Compound	Minimum inhibitory concentration μg/disc				Fungicidal/statical concentration μg/ml	
	1	*2*	*3*	*4*	*5*	*6*
I	12.5	12.5	50	12.5	50/12.5	50/12.5
II	12.5	12.5	50	12.5	50/12.5	50/12.5
III	12.5	12.5	50	50	50/12.5	50/12.5
IV	12.5	12.5	50	50	200/50	200/12.5
V	12.5	12.5	50	12.5	50/12.5	50/12.5
VI	50.0	50.0	200	200	200/50	200/12.5
VII	50.0	50.0	200	200	200/50	200/12.5
IX	200.0	200.0	200	200	200/200	200/50
X	50.0	50.0	200	200	200/50	200/50
XII	200.0	200.0	>200	>200	>200/200	200/50
XIII	12.5	12.5	200	200	200/50	200/12.5
XIV	50.0	200.0	>200	>200	>200/200	200/50
XV	12.5	12.5	200	200	200/50	200/12.5
XVII	50.0	50.0	200	200	200/200	200/50

1. Staphylococcus aureus ATCC 6538; 2. Bacillus subtilis ATCC 6051; 3. Escherichia coli ATCC 9637; 4. Pseudomonas aeruginosa ATCC 10145; 5. Microsporum gypseum; 6. Trichophyton rubrum.

tested also for antivermal activity on *Pannagrellus redivivus* and *Rhabditis oxycerca*. The effect was followed in liquid medium in the presence of the appropriate compound by counting the motile individuals after 48 h cultivation. At higher concentrations (400—800 μg/ml) 100% lethal effect was observed. Of the three derivatives tested 3-allylbenzothiazolium bromide (*V*) was found to be most active.

Experimental

Benzothiazole was obtained by rectification (110—122 °C/1.6 kPa) of the technical product, conversion to benzothiazolium sulfate, and crystallization from ethanol. The base was liberated with NaOH solution, extracted with ether and after evaporation it was distilled at 116—118 °C/1.6 kPa. 2-Methylbenzothiazole was synthesized after [19].

Melting points, determined on a Kofler block, and analytical data of the synthesized compounds are presented in Table 1. Tetrahydrofuran containing 1—2% of methanol was used for crystallizations. The i.r. spectra of compounds in nujol suspensions were measured on a double-beam Perkin—Elmer 180 spectrophotometer in the region of 1700—1480 cm^{-1}. The apparatus was calibrated by polystyrene foil; $\nu(C=N^+)$ and

ν(C=C) were assigned according to [20]. Electronic spectra were measured on a double-beam Perkin—Elmer 450 spectrophotometer in distilled water. All the measurements were carried out at 25 °C.

Growth stimulation of roots was established after the modified method [11]. Tests were performed on a model object of vetch, which was chosen for its high homogeneity in germination of seeds and growth of sprouts. Stimulation activities of the synthesized compounds are presented in Table 2. Antibacterial activity was followed on bacterial strains of gram-positive *Staphylococcus aureus ATCC 6538* (*1*), *Bacillus subtilis ATCC 6051* (*2*) and gram-negative *Escherichia coli ATCC 9637* (*3*), and *Pseudomonas aeruginosa ATCC 10145* (*4*). The activity of the compounds at 200, 50, 3.1, and 0.8 μg/disc was established by the plate-diffusion method in a Mueller—Hinton agar adjusted for the appropriate strain [21]. In the results that concentration is presented which brought about a measurable zone of growth inhibition. Antifungal activity was followed by the test-tube dilution method [22] on strains isolated from pathogenic nest of *Microsporum gypseum* (*5*) and *Trichophyton rubrum* (*6*). The strains were obtained from the Czechoslovak State Collection of Type Cultures, Prague. In the case of the strain (*1*) also the quantitative dilution method in liquid culture medium was applied. The activity was evaluated spectrophotometrically on a Spekol-ZV spectrophotometer at 37 °C and 460 nm by following the bacterial growth at different concentrations of the compound. The results of antibacterial activities are presented in Table 4.

Benzothiazolium salts I—XVIII

Benzothiazole (2-methylbenzothiazole) (0.05 mol) and halogen derivative and dimethyl sulfate, respectively (0.06 mol) were dissolved in the anhydrous mixture (25—30 ml) of dimethylformamide and acetone (2:1). The reaction mixture was heated in a water bath at 55—60 °C. If the quaternary salt did not start to crystallize after 5 h, a small portion of the reaction mixture was washed with petroleum ether and acetone and crystallization was evoked by a glass rod. The crystalline substance was put back into the reaction mixture and heating was continued for further 4 h. After cooling the salt was sucked, washed with anhydrous acetone, and crystallized from anhydrous tetrahydrofuran containing 1—2% of methanol.

References

1. Elashvili, Z. M., Devadze, L. V., and Dzhapavidze, K. G., *Soobshch. Akad. Nauk Gruz. SSR 52*, 351 (1968).
2. Kroehnke, F. and Friedrich, W., *Chem. Ber. 96*, 1195 (1963).
3. Lochon, P., Meheux, P., and Neel, J., *Bull. Soc. Chim. Fr. 1968*, 1093.
4. Azzaro, M. and Metzger, J., *Bull. Soc. Chim. Fr. 1964*, 1575.
5. Deady, L. W., *Aust. J. Chem. 26*, 1949 (1973).
6. Deady, L. W., Davis, M., and Homfield, E., *Aust. J. Chem. 27*, 1917, 1221 (1974).

7. *Belg.* 591664; *Chem. Abstr. 55,* 9129 (1961).
8. *U.S.* 3113864; *Chem. Abstr. 60,* 6387 (1964).
9. Kendall, J. and Edwards, H. D., *U.S.* 2412815; *Chem. Abstr. 41,* 5041 (1947).
10. Ohi, R. and Arai, N., *Japan. Kokai* 7594918; *Chem. Abstr. 84,* 67805 (1976).
11. Bartsch, R. A., Huenig, S., and Quast, H., *J. Amer. Chem. Soc. 92,* 6007 (1970).
12. Sutoris, V., Sekerka, V., and Halgaš, J., *Czech. Appl.* 7634–80.
13. Garmaice, D. L., Paris, G. Y., Komlossy, J., Chambers, C. H., and McCrae, R. C., *J. Med. Chem. 12,* 30 (1969).
14. Wanzlik, H. W., Kleiner, H. J., Lasch, I., Fueldener, H. U., and Steinmaus, H., *Justus Liebigs Ann. Chem. 708,* 155 (1967).
15. Buza, D. and Krasushi, W., *Rocz. Chem. 49,* 2007 (1975).
16. Gamburg, K. Z., *Biokhimiya auksina i ego deistvie na kletki rasteniya,* p. 271. Nauka, Moscow, 1976.
17. Thimann, K. V., in *The Physiology of Plant Growth and Development.* P. 3—48. (Wilkins, M. B., Editor.) McGraw-Hill, London, 1969.
18. Basu, P., Kramer, P. F., and Feller, D. R., *J. Pharm. Pharmacol. 26,* 910 (1974).
19. Zubarovskii, V. M., *Zh. Obshch. Khim. 17,* 613 (1947).
20. Witko, P., *J. Amer. Chem. Soc. 76,* 5597 (1954).
21. Foltínová, P. and Blöckinger, G., *Biológia 25,* 175 (1970).
22. Foltínová, P., Sutoris, V., Blöckinger, G., and Ebringer, L., *Folia Microbiol.* (Prague) *23,* 225 (1978).

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