APPLIED MICROBIOLOGY, July 1967, p. 710–717
Vol. 15, No. 4

Printed in U.S.A.

Antifungal Activity of Isothiocyanates and Related Compounds

II. Mononuclear Aromatic Isothiocyanates

Ľ. DROBNICA, M. ZEMANOVÁ, P. NEMEC, P. KRISTIÁN, K. ANTOŠ, AND A. HULKA

Department of Microbiology and Biochemistry and Department of Organic Chemistry, Faculty of Chemistry, Slovak Polytechnical University, and Department of Microbiology, Faculty of Natural Sciences, Komenský University, Bratislava, ČSSR

Received for publication 9 November 1966

Antifungal activity on *Aspergillus niger*, *Penicillium cyclopium*, and *Rhizopus oryzae*, as well as on additional saprophytic and parasitic fungi, was determined in 57 substituted derivatives of phenylisothiocyanate. Most of the investigated compounds displayed rather equal activity against the three mentioned fungi, in contradistinction to the analogues of natural benzyl- and β-phenylethylisothiocyanate with their characteristic low activity against *R. oryzae*. Differences occurred in the type of activity of compounds in which the —NCS group is directly bound on the aromatic moiety, as compared with those compounds in which this group is bound to the aliphatic radical or to the aromatic moiety indirectly by means of the methyl group or by a longer aliphatic chain. The results obtained confirm the negative influence of ionized substituents on the aromatic moiety, i.e., of —COOH, —CH_2—COOH, and —SO_3H groups, as well as of substituents which cause an extreme increase in reactivity of the —NCS group resulting in a high instability of the entire isothiocyanate molecule.

The preceding paper (7) dealt with the antifungal activity of a number of natural isothiocyanates and their analogues. The most active of them were the benzyl- and β-phenylethylisothiocyanates and their synthetic analogues, the latter being sometimes more active than all investigated natural compounds.

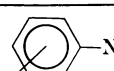
The present paper presents data on the antifungal activity of 57 mononuclear aromatic isothiocyanates, i.e., substituted phenylisothiocyanates. It must be stressed that phenylisothiocyanate itself is different chemically from the natural compounds mentioned in the previous paper (7). This is due primarily to the way in which the —NCS group is bound to the radical, since naturally occurring compounds in which the —NCS group is directly bound to the aromatic radical have not been detected. In elucidating the mode of biological activity of various isothiocyanates (Drobnica et al., *unpublished data*), it is important to stress differences between (i) natural isothiocyanates and their analogues and (ii) mono- and polynuclear aromatic isothiocyanates. These differences will be the subject of further communications.

MATERIALS AND METHODS

Compounds. All isothiocyanates studied are listed in Tables 1 to 4. They were all synthetized in our laboratories with the use of procedures described previously (2, 3, 8). The compounds designated as XXIII, XXV, XXXIV–XXXVIII, XL, XLI, XLV–XLVIII, LIII, and LV–LVII were originally synthesized in our laboratories (1, 8–12). Usually, freshly prepared ethyl alcohol solutions were used for testing the antifungal activity. With compounds XXVI to XXVIII and XXXIX to XLI, higher concentrations in media were obtained by adding homogeneous suspensions in ethyl alcohol or diethyleneglycol. The final concentration of the solvent in medium never exceeded 1%.

Determination of antifungal activity. Antifungal activity was determined as described previously (7), except *Aspergillus fumigatus* was replaced by a *Penicillium chrysogenum* strain 10/12 from the Type Culture Collection of the Department of Microbiology and Biochemistry, Slovak Polytechnical University, Bratislava. For strains of *A. niger*, *P. cyclopium*, and *Rhizopus oryzae*, each compound was tested in three parallel series; usually, six to eight various concentrations were employed and incubation was at 28 C. At 6 days after inoculation, or 4 days with *A. niger*, the dry weight of the grown fungal mass was determined. Dry-weight values were plotted against logarithms of corresponding molar concentrations of compounds

TABLE 1. *Activity of phenylisothiocyanates substituted with halogen or the methyl group on the growth of Aspergillus niger, Penicillium cyclopium, and Rhizopus oryzae*

No.	 R =	Mol wt	ED_{50} and ED_{100} values (moles per liter) and activity after 14 days of incubation[a]						RE[b]
			A. niger		*P. cyclopium*		*R. oryzae*		
I	H—	135.178	17×10^{-6}		18×10^{-6}		52×10^{-6}		
			11×10^{-5}	B	20×10^{-5}	B	32×10^{-5}	C	1.0
II	4-Cl—	169.627	98×10^{-7}		77×10^{-7}		11×10^{-6}		
			28×10^{-6}	D	63×10^{-6}	D	32×10^{-6}	E	3.9
III	3-Cl—	169.627	38×10^{-6}				29×10^{-6}		
			73×10^{-6}	D	—	—	73×10^{-6}	D	1.5
IV	4-Br—	214.086	79×10^{-7}		25×10^{-6}		20×10^{-6}		
			25×10^{-6}	D	63×10^{-6}	D	63×10^{-6}	D	4.4
V	3-Br—	214.086	63×10^{-7}		14×10^{-6}		11×10^{-6}		
			28×10^{-6}	D	63×10^{-6}	D	32×10^{-6}	E	3.9
VI	4-I—	261.090	50×10^{-7}		17×10^{-6}		11×10^{-6}		
			20×10^{-6}	E	78×10^{-6}	C	32×10^{-6}	E	5.5
VII	3-I—	261.090	18×10^{-6}				56×10^{-6}		
			28×10^{-6}	E	—	—	11×10^{-5}	D	3.9
VIII	4-CH_3—	149.204	—	D	—	D	—	D	—
IX	3-CH_3—	149.204	28×10^{-6}		63×10^{-6}		59×10^{-6}		
			11×10^{-5}	C	32×10^{-5}	C	36×10^{-5}	C	1.0

[a] For each compound, a corresponding ED_{50} value is given followed below by the ED_{100} value. The ED_{50} and ED_{100} values for *A. niger* were assessed after 4 days, and in an additional two fungi after 6 days, of incubation. Block letters designate the lowest of the tested compound concentrations still capable of suppressing fungal growth entirely after 14 days (in moles per liter, $\times 10^{-5}$): A, 300; B, 150; C, 75; D, 37.5; E, 7.5; F, 3.75; G, 0.75.

[b] Relative effectivity compared with phenylisothiocyanate (*A. niger*; ED_{100}).

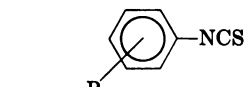
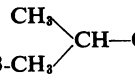
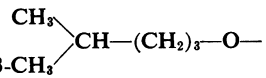
in the culture medium, and a graphic procedure was used to calculate ED_{50} and ED_{100} values. The third series was observed at 14 days, and those concentrations of isothiocyanates still able to suppress growth completely were noted. With other strains of fungi, the activity of a given compound was simply expressed as the lowest concentration of compound capable of complete inhibition of growth after a given time interval (Table 5). Details are presented in the preceding paper (7).

RESULTS ANS DISCUSSION

Most of the compounds tested were 3- and 4-substituted phenylisothiocyanates. Table 1 presents the data obtained with monosubstituted halogen or methyl derivatives. The most active were 4-halogen substituted compounds, including 4-iodophenyl- and 4-bromophenylisothiocyanate. Table 2 presents results obtained with 32 derivatives of phenylisothiocyanate monosubstituted with hydroxyl, carboxyl, alkoxyl, carbalkoxyl or other related substituents. It is of interest to stress the relatively low activity of carboxyphenylisothiocyanates and isothiocyanate derivatives of phenylacetic acid, i.e., of compounds in which the substituent will occur in the ionized form in medium with a *p*H over 6.5 and pK_a below 5 (9). These ionized forms are much more soluble than the corresponding nonionized ones, when the latter, in fact, are the effective forms. It is just this fact which may explain the low antifungal activity of isothiocyanates with a carboxyl group. Esterification of these substituents resulted in a considerable increase of activity, e.g., 4-carbmethoxy- and 4-carbethoxyphenylisothiocyanates and analogue 3-substituted derivatives (XXIX to XXXII). Prolongation of the aliphatic chain in carbalkoxyphenylisothiocyanates (XXXIII to XXXVIII) is accompanied by a decrease in antifungal activity. The same phenomenon applies also for the group of alkoxyphenylisothiocyanates in which again the methoxy- and ethoxyphenylisothiocyanates display the highest activity.

Nitrogen- and sulfur-containing derivatives are shown in Table 3. In addition to the 4-isothiocyanatophenylmethylsulfide, both dimethylaminophenylisothiocyanates are very active because their dimethylaminogroup may convert into the ionized state, which, however, does not occur in culture media (9). The reverse is true for 4-isothiocyanatobenzenesulfonic acid (Na salt), which consequently also shows a low activity. The low activity of nitrophenylisothiocyanates (XLII, XLIII) may be explained by

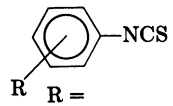
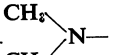
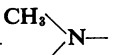
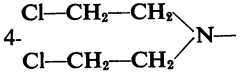
TABLE 2. *Antifungal activity of hydroxy-, carboxy-, alkoxy-, carbalkoxy-, and further related phenylisothiocyanates on Aspergillus niger, Penicillium cyclopium, and Rhizopus oryzae*

No.	 R =	Mol wt	ED_{50} and ED_{100} values (moles per liter) and activity after 14 days of incubation[a]						RE[b]
			A. niger		*P. cyclopium*		*R. oryzae*		
X	4-HO—	151.178	16×10^{-6}				38×10^{-6}		
			27×10^{-6}	E	—	F	81×10^{-6}	D	4.0
XI	3-HO—	151.178	11×10^{-6}				18×10^{-6}		
			27×10^{-6}	F	—	—	39×10^{-6}	E	4.0
XII	4-CH_3—O—	165.204	—	G	—	F	—	F	—
XIII	3-CH_3—O—	165.204	13×10^{-6}				37×10^{-6}		
			24×10^{-6}	E	—	—	80×10^{-6}	D	4.5
XIV	4-CH_3—CH_2—O—	179.230	31×10^{-7}		28×10^{-6}		12×10^{-6}		
			18×10^{-6}	E	14×10^{-5}	D	56×10^{-6}	D	6.1
XV	3-CH_3—CH_2—O—	179.230	13×10^{-6}				56×10^{-6}		
			22×10^{-6}	E	—	—	10×10^{-5}	D	5.0
XVI		193.256	15×10^{-6}				55×10^{-6}		
			26×10^{-6}	E	—	—	10×10^{-5}	D	4.2
XVII	4-CH_3—$(CH_2)_4$—O—	221.308	—	C	—	—	—	—	—
XVIII	3-CH_3—$(CH_2)_4$—O—	221.308	69×10^{-6}				47×10^{-6}		
			15×10^{-5}	C	—	—	14×10^{-5}	D	0.7
XIX	3-CH_3—$(CH_2)_5$—O—	235.334	15×10^{-6}				25×10^{-6}		
			27×10^{-6}	E	–	D	10×10^{-5}	D	4.0
XX		235.334	$>75 \times 10^{-5}$		$>75 \times 10^{-5}$		11×10^{-5}		
			$>75 \times 10^{-5}$	> C	$>75 \times 10^{-5}$	> C	25×10^{-5}	C	<0.1
XXI	4-CH_3—$(CH_2)_7$—O—	263.386	—	E	—	—	—	—	—
XXII	3-CH_3—CO—O—	193.214	16×10^{-6}				55×10^{-6}		
			29×10^{-6}	E	—	—	10×10^{-5}	D	3.7
XXIII	3-CH_3—$(CH_2)_2$—CO—O—	221.266	74×10^{-6}				54×10^{-6}		
			12×10^{-5}	D	—	—	12×10^{-5}	D	0.9
XXIV	4-CH_3—CO—	177.214	38×10^{-6}		40×10^{-6}		17×10^{-5}		
			11×10^{-5}	C	10×10^{-5}	D	36×10^{-5}	C	1.0
XXV	3-CH_3—CO—	177.214	37×10^{-6}		35×10^{-6}		63×10^{-6}		
			11×10^{-5}	D	99×10^{-6}	D	19×10^{-5}	D	1.0
XXVI	4-HOOC—	179.188	$>15 \times 10^{-4}$		$>75 \times 10^{-5}$		$>75 \times 10^{-5}$		
			$>15 \times 10^{-4}$	> B	$>75 \times 10^{-5}$	> C	$>75 \times 10^{-5}$	> C	<0.07
XXVII	3-HOOC—	179.188	$>75 \times 10^{-5}$		$>75 \times 10^{-5}$		$>75 \times 10^{-5}$		
			$>75 \times 10^{-5}$	> C	$>75 \times 10^{-5}$	> C	$>75 \times 10^{-5}$	> C	<0.1
XXVIII	4-NaOOC—	201.177	$>15 \times 10^{-4}$		$>15 \times 10^{-4}$		$>15 \times 10^{-4}$		
			$>15 \times 10^{-4}$	> B	$>15 \times 10^{-4}$	> B	$>15 \times 10^{-4}$	> B	<0.07

XXIX	4-CH_3—O—OC—	193.214	20×10^{-6}		12×10^{-6}		42×10^{-6}		
			32×10^{-6}	D	27×10^{-6}	E	80×10^{-6}	D	3.4
XXX	3-CH_3—O—OC—	193.214	25×10^{-6}				70×10^{-6}		
			50×10^{-6}	D	—	—	91×10^{-6}	D	2.2
XXXI	4-CH_3—CH_2—O—OC—	207.240	11×10^{-6}		31×10^{-6}		32×10^{-6}		
			28×10^{-6}	D	64×10^{-6}	D	64×10^{-6}	D	3.9
XXXII	3-CH_3—CH_2—O—OC—	207.240	28×10^{-6}				37×10^{-6}		
			50×10^{-6}	D	—	D	80×10^{-6}	D	2.2
XXXIII	4-CH_3—$(CH_2)_4$—O—OC—	249.318	$>75 \times 10^{-5}$				$>75 \times 10^{-5}$		
			$>75 \times 10^{-5}$	> C	—	C	$>75 \times 10^{-5}$	> C	<0.1
XXXIV	3-CH_3—$(CH_2)_4$—O—OC—	249.318	$>75 \times 10^{-5}$				$>75 \times 10^{-5}$		
			$>75 \times 10^{-5}$	> C	—	—	$>75 \times 10^{-5}$	> C	<0.1
XXXV	4-CH_3—$(CH_2)_5$—O—OC—	263.344	$>75 \times 10^{-5}$				$>75 \times 10^{-5}$		
			$>75 \times 10^{-5}$	> C	—	> C	$>75 \times 10^{-5}$	> C	<0.1
XXXVI	3-CH_3—$(CH_2)_5$—O—OC—	263.344	$>75 \times 10^{-5}$		$>75 \times 10^{-5}$		$>75 \times 10^{-5}$		
			$>75 \times 10^{-5}$	> C	$>75 \times 10^{-5}$	> C	$>75 \times 10^{-5}$	> C	<0.1
XXXVII	4-CH_3—$(CH_2)_7$—O—OC—	291.396	$>75 \times 10^{-5}$		$>75 \times 10^{-5}$		$>75 \times 10^{-5}$		
			$>75 \times 10^{-5}$	> C	$>75 \times 10^{-5}$	> C	$>75 \times 10^{-5}$	> C	<0.1
XXXVIII	3-CH_3—$(CH_2)_7$—O—OC—	291.396	$>75 \times 10^{-5}$		$>75 \times 10^{-5}$		$>75 \times 10^{-5}$		
			$>75 \times 10^{-5}$	> C	$>75 \times 10^{-5}$	> C	$>75 \times 10^{-5}$	> C	<0.1
XXXIX	4-HOOC—CH_2—	193.214	$>75 \times 10^{-5}$		$>75 \times 10^{-5}$		ca. 40×10^{-5}		
			$>75 \times 10^{-5}$	> C	$>75 \times 10^{-5}$	> C	ca. 80×10^{-5}	> C	<0.1
XL	4-HOOC—CH_2—O—	209.214	$>75 \times 10^{-5}$		$>75 \times 10^{-5}$		$>75 \times 10^{-5}$		
			$>75 \times 10^{-5}$	> C	$>75 \times 10^{-5}$	> C	$>75 \times 10^{-5}$	> C	<0.1
XLI	3-HOOC—CH_2—O—	209.214	$>75 \times 10^{-5}$		$>75 \times 10^{-5}$		$>75 \times 10^{-5}$		
			$>75 \times 10^{-5}$	> C	$>75 \times 10^{-5}$	> C	$>75 \times 10^{-5}$	> C	<0.1

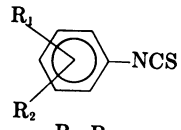
[a] See footnote *a*, Table 1.
[b] See footnote *b*, Table 1.

TABLE 3. *Antifungal activity of substituted phenylisothiocyanates containing nitrogen or sulfur in the substituents on Aspergillus niger, Penicillium cyclopium, and Rhizopus oryzae*

No.	 R =	Mol wt	ED_{50} and ED_{100} values (moles per liter) and activity after 14 days of incubation[a]			RE[b]
			A. niger	*P. cyclopium*	*R. oryzae*	
XLII	4-NO_2—	180.178	ca. 75×10^{-5} $>75 \times 10^{-5}$ > C	ca. 75×10^{-5} $>75 \times 10^{-5}$ > C	ca. 75×10^{-5} $>75 \times 10^{-5}$ > C	<0.1
XLIII	3-NO_2—	180.178	ca. 10×10^{-5} $>75 \times 10^{-5}$ > C	— —	19×10^{-5} 66×10^{-5} C	<0.1
XLIV	4- 	178.246	91×10^{-7} 24×10^{-6} E	26×10^{-6} 64×10^{-6} C	16×10^{-6} 32×10^{-6} E	4.5
XLV	3- 	178.246	14×10^{-6} 24×10^{-6} E	— —	36×10^{-6} 73×10^{-6} D	4.5
XLVI	4- 	275.196	$>75 \times 10^{-5}$ $>75 \times 10^{-5}$ > C	$>75 \times 10^{-5}$ $>75 \times 10^{-5}$ > C	$>75 \times 10^{-5}$ $>75 \times 10^{-5}$ > C	<0.1
XLVII	3-CH_3—CO—NH—	192.230	14×10^{-6} 25×10^{-6} E	— —	50×10^{-6} 91×10^{-6} D	4.4
XLVIII	3-CN—	160.188	13×10^{-5} 50×10^{-5} > C	— —	19×10^{-5} 38×10^{-5} C	0.2
IL	4-CH_3—S—	181.264	10×10^{-6} 19×10^{-6} E	32×10^{-6} 88×10^{-6} D	13×10^{-6} 34×10^{-6} D	5.7
L	4-NaO_3S—	237.227	40×10^{-5} 14×10^{-4} > B	— —	— —	0.07
LI	3-NaO_3S—	237.227	$>75 \times 10^{-5}$ $>75 \times 10^{-5}$ > C	$>75 \times 10^{-5}$ $>75 \times 10^{-5}$ > C	$>75 \times 10^{-5}$ $>75 \times 10^{-5}$ > C	<0.1

[a] See footnote *a*, Table 1.
[b] See footnote *b*, Table 1.

TABLE 4. *Antifungal activity of disubstituted phenylisothiocyanates on Aspergillus niger, Penicillium cyclopium, and Rhizopus oryzae*

No.	 $R_1, R_2 =$	Mol wt	ED_{50} and ED_{100} values (moles per liter) and activity after 14 days of incubation[a]			RE
			A. niger	*P. cyclopium*	*R. oryzae*	
LII	2-Cl—, 4-Cl—	204.076	$>15 \times 10^{-4}$	ca. 10×10^{-4}	ca. 10×10^{-4}	
			$>15 \times 10^{-4}$ > B	$>15 \times 10^{-4}$ > B	$>15 \times 10^{-4}$ > B	<0.07
LIII	2-HOOC—, 4-I—	305.100	ca. 40×10^{-5}		$>75 \times 10^{-5}$	
			ca. 13×10^{-4} > B	— B	$>75 \times 10^{-5}$ > C	ca. 0.08
LIV	3-HO—, 4-HOOC—	195.188	$>75 \times 10^{-5}$	$>75 \times 10^{-5}$	$>75 \times 10^{-5}$	
			$>75 \times 10^{-5}$ > C	$>75 \times 10^{-5}$ > C	$>75 \times 10^{-5}$ > C	<0.1
LV	3-CH_3—CO—NH—, 4-HOOC—	236.240	$>15 \times 10^{-4}$	$>15 \times 10^{-4}$	$>15 \times 10^{-4}$	
			$>15 \times 10^{-4}$ > B	$>15 \times 10^{-4}$ > B	$>15 \times 10^{-4}$ > B	<0.07
LVI	2-HOOC—, 5-CH_3—CO—NH—	236.240	$>15 \times 10^{-4}$	$>15 \times 10^{-4}$	$>15 \times 10^{-4}$	
			$>15 \times 10^{-4}$ > B	$>15 \times 10^{-4}$ > B	$>15 \times 10^{-4}$ > B	<0.07
LVII	2-NO_2—, 4-CH_3—	194.204	— B	— B	— C	—

[a] See footnote *a*, Table 1.
[b] See footnote *b*, Table 1.

TABLE 5. *Activity of several phenylisothiocyanates in 16 fungal strains*

Isothiocyanate	Tested for[a]	ED_{100} in moles per liter ($\times 10^{-5}$)				
		<1	1–10	10–50	50–100	>100
Phenyl-	1–5, 7–12	—	10, 11	1–3, 5, 7, 9	4, 8, 12	—
4-Chlorophenyl-	1–5, 7–12	11	1–5, 7–10	12	—	—
4-Bromophenyl-	1–5, 7–16	16	1–5, 7–9, 11, 12, 14	10, 15	—	13
3-Bromophenyl-	1–5, 7–11, 13–16	16	1–5, 7–11, 15	14	13	—
4-Iodophenyl-	1–5, 7–12	11	1–5, 7–9	10, 12	—	—
4-Dimethylaminophenyl-	1–5, 7–11, 13–16	16	1–3, 5, 7–11, 15	4, 13, 14	—	—
4-Acetophenyl-	1, 3–5, 7–11	—	—	1, 3, 7–11	—	4, 5
4-Carboxyphenyl-(Na salt)	1, 2, 4, 5, 7–12	—	—	—	—	1, 2, 4, 5, 7–12
4-Carbethoxyphenyl-	1, 2, 4, 5, 7–11	11	1, 2, 4, 5, 7–10	—	—	—
2,4-Dichlorophenyl-	1–5, 7–11	—	—	—	5, 8, 10, 11	1–4, 7, 9

[a] Individual strains are numbered as follows (numbers in parentheses indicate days of incubation): 1, *Aspergillus niger* (4); 2, *Penicillium cyclopium* (6); 3, *Rhizopus oryzae* (6); 4, *A. flavus* (6); 5, *A. oryzae* (5); 6, *P. chrysogenum* (5); 7, *P. brevi-compactum* (5); 8, *Cladosporium herbarum* (6); 9, *Trichoderma viride* (7); 10, *Alternaria tenuis* (7); 11, *Monilia sitophila* (7); 12, *Trichophyton gypseum* var. *asteroides* (30); 13, *Cytospora* sp. (14); 14, *Schizophyllum commune* (14); 15, *Fusarium* sp. (14); 16, *Cephalothecium roseum* (14).

TABLE 6. *Relative effectivity of several natural isothiocyanates and their synthetic analogues and of phenylisothiocyanates* (ED_{100}; *Aspergillus niger*)

Compound	Relative effectivity compared with	
	Allyliso-thiocyanate	Phenyliso-thiocyanate
Natural isothiocyanates and their analogues		
Allyl-	1.0	0.17
Benzyl-	3.7	0.61
β-Phenylethyl-	32	5.2
4-Iodobenzyl-	130	22
β-(4-Methylphenyl)-ethyl-	66	11
Phenylisothiocyanates		
Phenyl-	6.0	1.0
4-Iodophenyl-	33	5.5
4-Ethoxyphenyl-	37	6.1
4-Phenylmethylsulfide-	35	5.8
4-Dimethylaminophenyl-	25	4.6

extremely high reactivity and the resulting instability of these compounds (4, 5). The nitrogen mustard analogue (XLVI), though being chemically an exclusive isothiocyanate, belongs to the group of practically ineffective derivatives. A relatively low activity was also detected in the group of disubstituted phenylisothiocyanates (Table 4).

Table 5 presents a survey on the activity of several aforementioned isothiocyanates on an additional 13 fungi representing saprophytes, phytopathogenic fungi, and dermatophytes. Table 6 compares the activity of some natural isothiocyanates and their analogues with the activity of phenylisothiocyanate on *A. niger*. The relative effectivity (RE) of several isothiocyanates, compared with the effect of allyl- or phenylisothiocyanate, leads to the conclusion that even the most active substituted phenylisothiocyanates do not achieve the activity of natural benzyl- and β-phenylethylisothiocyanate analogues, i.e., of 4-iodobenzyl- and β-(4-methylphenyl)-ethylisothiocyanate. This conclusion will be reached, of course, only by evaluating the ED_{100} values for *A. niger* on the 4th day of cultivation. Much higher compound concentrations are necessary for long-term growth inhibition of fungi, particularly with natural isothiocyanates and their mentioned analogues. Thus, total growth inhibition after 14 days of incubation will be achieved in the presence of more than 15×10^{-4} moles of allylisothiocyanate per liter, whereas 6×10^{-4} moles per liter will be sufficient in this respect after 4 days of incubation. This difference cannot be explained only by the typical volatility of allylisothiocyanate, since a similar phenomenon was observed also in other chemically related derivatives of low volatility. Benzyl- and β-phenylethylisothiocyanates also show a low activity against *R. oryzae* (7), whereas no significant difference in activity on *R. oryzae*, *A. niger*, and *P. cyclopium* is registered with substituted phenylisothiocyanates. For these reasons, several phenylisothiocyanate derivatives, as well as the most active natural isothiocyanate analogues, represent remarkable antifungal compounds. There are, however, some differences in the mode of antifungal action (6, 13).

LITERATURE CITED

1. ANTOŠ, K., A. HULKA, P. KRISTIÁN, Ľ. DROBNICA, AND P. NEMEC. 1959. Isothiocyanates. I. Synthesis of some carboxyphenylisothiocyanates with biological activity. Chem. Zvesti **13**:27–31.
2. ANTOŠ, K., A. HULKA, P. KRISTIÁN, P. NEMEC, AND Ľ. DROBNICA. 1961. Synthesis of isothiocyanates derived from biologically active substances. Acta Chem. Univ. Polytech. Slovakiensis, Bratislava, p. 35–39.
3. BAČÍKOVÁ, D., P. NEMEC, Ľ. DROBNICA, K. ANTOŠ, P. KRISTIÁN, AND A. HULKA. 1965. Antiworm activity of some natural and synthetic compounds. I. Effect of aliphatic and mononuclear aromatic isothiocyanates on Turbatrix aceti. J. Antibiotics (Tokyo) Ser. A **17**:162–170.
4. DROBNICA, Ľ., AND J. AUGUSTÍN. 1965. Reactions of isothiocyanates with amino acids, peptides and proteins. I. Kinetics of the reaction of aromatic isothiocyanates with glycine. Collection Czech. Chem. Commun. **30**:99–104.
5. DROBNICA, Ľ., AND J. AUGUSTÍN. 1965. Reactions of isothiocyanates with amino acids, peptides and proteins. III. Kinetics and mechanism of the reaction of aromatic isothiocyanates with thioglycolic acid. Collection Czech. Chem. Commun. **30**:1618–1625.
6. DROBNICA, Ľ., P. NEMEC, K. ANTOŠ, P. KRISTIÁN, AND A. HULKA. 1964. Reaktionen von Isothiocyansäureestern auf Molekül- und Zellniveau, p. 61–69. *In* J. Podoba and P. Langer [ed.], Naturally occurring goitrogens and thyroid function. Slovak Academy of Sciences, Bratislava.
7. DROBNICA, Ľ., M. ZEMANOVÁ, P. NEMEC, K. ANTOŠ, P. KRISTIÁN, A. ŠTULLEROVÁ, V. KNOPPOVÁ, AND P. NEMEC, JR. 1967. Antifungal activity of isothiocyanates and related compounds. I. Naturally occurring isothiocyantes and their analogues. Appl. Microbiol. **15**:701–709.
8. KRISTIÁN, P., K. ANTOŠ, Ľ. DROBNICA, AND M. VARGOVÁ. 1966. Synthesis of isothiocyanatophenoxyacetic and isothiocyanatophenylacetic

acid. Acta Chem. Univ. Polytech. Slovakiensis, Bratislava, p. 26–32.

9. KRISTIÁN, P., K. ANTOŠ, D. VLACHOVÁ, AND R. ZAHRADNÍK. 1963. Electronic nature of the isothiocyanato group and its effect on the basicity of acridines. Collection Czech. Chem. Commun. **28**:1651–1655.
10. KRISTIÁN, P., A. HULKA, K. ANTOŠ, P. NEMEC, AND Ľ. DROBNICA. 1959. Isothiocyanates. II. Synthesis of isothiocyanates derived from cancerogenic and cytostatic compounds. Chem. Zvesti **13**:103–107.
11. KRISTIÁN, P., Š. KOVÁČ, AND K. ANTOŠ. 1964. Preparation and infrared spectra of substituted phenylisothiocyanates. Collection Czech. Chem. Commun. **29**:2507–2512.
12. KRISTIÁN, P., Š. KOVÁČ, K. ANTOŠ, AND B. TOMÁNEK. 1964. Isothiocyanates. XIII. Synthesis and infrared absorption spectra of meta-carbalkoxyphenylisothiocyanates and para-carbalkoxyphenylisothiocyanates. Chem. Zvesti **18**:81–89.
13. NEMEC, P., Ľ. DROBNICA, K. ANTOŠ, P. KRISTIÁN, AND A. HULKA. 1962. Biologische Wirksamkeit synthetischer Isothiocyansäureester. III. Einige Derivate mutagener and karzinogener Stoffe. Biologické práce, VIII/2. Slovak Academy of Sciences, Bratislava, p. 84.