THE ANTIWORM ACTIVITY OF SOME NATURAL AND SYNTHETIC COMPOUNDS. II

THE EFFECT OF ARYLALKYL- AND ARYLISOTHIOCYANATES ON *TURBATRIX ACETI*

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Data are presented on the antiworm activity of 74 natural and synthetic isothiocyanates. They were characterized by the values of ED_{100} *i. e.* molar concentrations of the compounds in culture medium causing irreversible arrest of the motility of *Turbatrix aceti* at the end of 20 hours' action. Some data are discussed with respect to the relationships between the structure and the biological activity of isothiocyanates. Benzyl-, arylalkyl-, benzohydryl-, cinamoyl-, diphenyl-, derivatives of isothiocyanates and of polycondensed aromatic isothiocyanates were studied. Most of the described isothiocyanates were new compounds synthesized for the first time.

In a previous paper[1] we dealt with the effect of mononuclear aromatic isothiocyanates, mostly phenylisothiocyanate derivatives, on *Turbatrix aceti*. Of 52 compounds 4-diphenylisothiocyanate and 4-bromobenzylisocyanate were the most noteworthy ones. In this connection, it appeared interesting to investigate the activity of a series of new substituted 4-diphenylisothiocyanates and substituted benzylisothiocyanates. Isothiocyanate derivatives of benzohydryl and other arylalkylisothiocyanates, *i. e.* compounds in which the functional NCS-group is bound to the aromatic nucleus by means of an aliphatic chain, resemble structurally to the afore-mentioned substituted benzylisothiocyanates.

The results presented in this communication concern isothiocyanates of the above groups as well as several derivatives of polynuclear hydrocarbons. Some of the compounds described in the present report are characterized by noteworthy effects on other biological systems[2~6]. The mechanism of antimicrobial and cytotoxic effect of some mononuclear aromatic isothiocyanates and of benzyl type isothiocyanates was also studied in detail[7~10]. Especially in the large series of phenylisothiocyanate derivatives, studied also with regard to their physico-chemical properties, new knowledge has been gained relating structure, physico-chemical properties, and the biological activity of these compounds[11,12]. Also known from professional and patent

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literature is the antiworm effect of isothiocyanates, *e.g.* of methylisothiocyanate[13], chloralkylisothiocyanates[14], and phenylenediisothiocyanates[15].

Experimental Procedure

Compounds studied:

In Tables 1~6 are presented the structural formulas of the isothiocyanates studied and references to papers describing their preparation and physico-chemical properties. All compounds under discussion were prepared in our laboratories. Most of them are new compounds synthesized for the first time.

Material and methods:

To test antiworm activity, we used the strain *Turbatrix aceti*, obtained from Fring's vinegar generator and cultivated on a reciprocal shaker at 27°C in 500 ml flasks containing 100 ml yeast extract with addition of 3 % of ethanol and 5 % of acetic acid. A culture containing 1,000 to 1,200 individuals per ml was added to the culture medium. The antiworm activity was determined as described previously[1] except that the test substances were added to the culture in dimethylsulfoxide to give a concentration was 1 % dimethylsulfoxide. We found that dimethylsulfoxide at a concentration of 2.5 % failed to exert any effect on the motility of *T. aceti* over a period of several days. Isothiocyanates were added in all instances as fresh solutions.

Results and Discussion

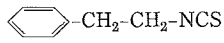
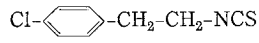
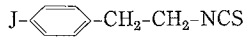
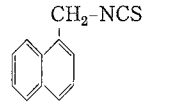
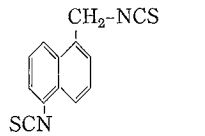
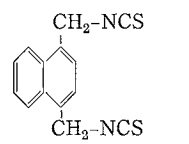
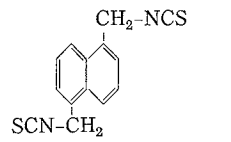
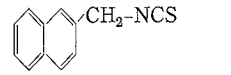
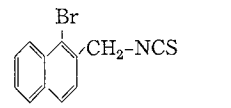
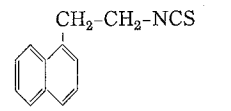
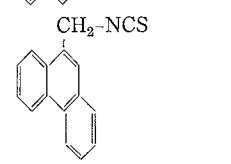
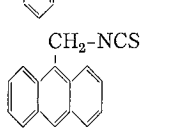
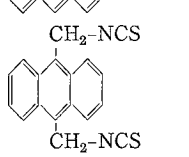
Tables 1~6 show the activities of the studied isothiocyanates against *Turbatrix aceti*. They are characterized by the value of ED_{100}, *i.e.* the minimal inhibition concentration inducing irreversible arrest of movement at the end of 20 hours of action at laboratory temperature. Values are given in μg/ml and moles/liter. In order to compare the efficacy, the effect of each derivative is expressed relative to that of phenylisothiocyanate. For isothiocyanates derived by substitution of the hydrogen

Table 1. Activity of benzylisothiocyanate analogues against *Turbatrix aceti*

R =	Mol. wt.	References	ED_{100} μg/ml	ED_{100} mole/liter $\cdot 10^{-5}$	RE_{PhITC} *	RE_{BITC} **
H-	149.20	16	6.6	4.5	1.1	1.0
4-Cl-	183.65	16	1.0	0.54	9.3	8.3
4-Br-	228.11	16	1.1	0.48	10	9.4
4-J-	275.12	16	1.7	0.62	8.1	7.3
$4\text{-}CH_3$-	163.23	16	2.5	1.5	3.3	3.0
$4\text{-}CH_3\text{-}O$-	179.23	16	6.0	3.3	1.5	1.4
4-CN-	174.21	16	18	10	0.5	0.45
$4\text{-}NO_2$-	194.20	16	18	9.3	0.54	0.42
3-Cl-	183.65	16	1.5	0.82	6.1	3.7
3-Br-	228.11	16	2.5	1.1	4.6	4.1
3-J-	275.12	16	3.0	1.1	4.6	4.1
$3\text{-}CH_3$-	163.23	16	3.7	2.3	2.2	2.0
4-Br-C$_6$H$_4$-	303.20	17	25	8.2	0.61	0.55
$4\text{-}O_2N$-C$_6$H$_4$-	269.29	17	5.0	1.9	2.6	2.4

* relative effectivity compared with that of phenylisothiocyanate (molar concentrations)
** relative effectivity compared with that of benzylisothiocyanate (molar concentrations)

Table 2. Activity of arylalkylisothiocyanates against *Turbatrix aceti*

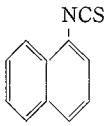
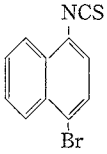
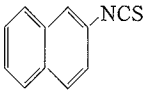
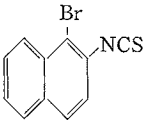
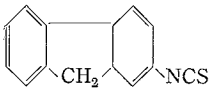
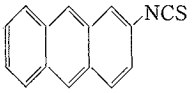
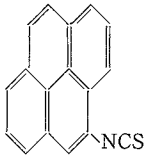
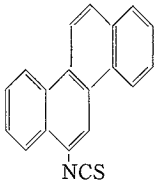
Structural formula	Mol. wt.	References	ED_{100}		RE_{PhITC} *	RE_{BITC} **
			μg/ml	mole/liter $\cdot 10^{-5}$		
	163.23	19	4.5	2.8	1.8	1.6
	197.68	19	1.3	0.66	7.6	8.0
	289.14	19	1.7	0.59	8.5	7.6
	199.20	18	6.5	3.3	1.5	1.4
	256.34	20	>100	>39	<0.13	<0.12
	270.37	20	>100	>37	<0.14	<0.12
	270.37	17	>100	>37	0.14	<0.12
	199.20	17	3.0	1.5	3.3	3.0
	278.10	17	53	19	0.26	0.24
	213.23	17	100	47	0.11	0.09
	249.32	20	>100	>40	<0.13	<0.11
	249.32	20	>100	>40	<0.13	<0.11
	320.25	17	>100	>31	<0.16	<0.15

*, ** See footnotes * and ** in Table 1.

atom in the aromatic ring by another atom or group the efficacy is expressed also relative to the basic unsubstituted isothiocyanate. Table 1 summarizes the data on the antiworm activity of isothiocyanates of the benzyl type. These compounds possess a noteworthy antiworm activity. By substitution of hydrogen of the aromatic ring in position 3- or 4- with halogens, methoxyl group and methyl group, the efficacy increases. Likewise, the introduction of another aromatic ring in the molecule increases antiworm activity.

Table 2 represents isothiocyanates derived from aromatic hydrocarbons in which the functional NCS-group is bound by means of the methylene or ethylene group.

Table 3. Activity of polynuclear aromatic isothiocyanates against *Turbatrix aceti*

Structural formula	Mol. wt.	References	ED_{100}		RE_{PhITC} *
			μg/ml	mole/liter $\cdot 10^{-5}$	
	185.18	5	100	54	0.09
	268.15	17	>100	>37	<0.14
	185.18	5	1	0.54	9.3
	268.15	17	>100	>37	<0.14
	223.29	21	>100**	>45	<0.11
	235.30	22	>100	>42	<0.12
	259.32	22	>100	>39	<0.13
	285.29	22	>100	>35	<0.14

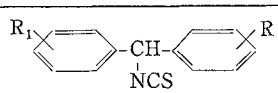
* See footnote * in Table 1.

** ED_{100} 1 μg/ml after 48 hours of action.

Of all compounds with condensed aromatic cycles only 1-naphtylmethyl- and 2-naphtylmethyl-isothiocyanates are efficacious against *T. aceti*. The introduction of another substituent or the increase in the size of the molecule by another aromatic nucleus results in loss of activity. Compounds of the β-phenylethylisocyanate type are as active as substituted benzylisothiocyanates.

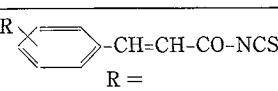
Polynuclear aromatic isothiocyanates in which the NCS-group is bound directly to the nucleus are ineffective towards *T. aceti* (Table 3), except 2-naphtylisothiocyanate, which possesses high activity (compare Tables 2 and 3). It is rather interesting to

Table 4. Activity of benzhydrylisothiocyanate analogues against *Turbatrix aceti*

		Mol. wt.	References	ED_{100}		RE_{PhITC} *
				μg/ml	mole/liter $\cdot 10^{-5}$	
R_1=	R =					
H-	H-	225.30	23	>100	>44	<0.11
H-	2-Cl-	259.75	24	>100	>38	<0.13
H-	3-Cl-	259.75	27	>100	>38	<0.13
4-Cl-	4-Cl-	294.20	25	>100	>34	<0.15
H-	2-Br-	304.21	27	>100	>33	<0.15
H-	3-Br-	304.21	27	>100	>33	<0.15
H-	4-Br-	304.21	27	>100	>33	<0.15
4-Br-	4-Br-	383.12	27	>100	>26	<0.19
H-	2-J-	351.21	27	>100	>28	<0.18
H-	3-J-	351.21	27	>100	>28	<0.18
H-	4-J-	351.21	27	>100	>28	<0.18
H-	3-CH_3-	239.33	27	>100	>42	<0.12
H-	4-CH_3-	239.33	26	>100	>42	<0.12
4-CH_3-	4-CH_3-	253.35	25	>100	>39	<0.13
H-	2-CH_3-O-	255.33	27	>100	>39	<0.13
H-	4-CH_3-O-	255.33	27	>100	>39	<0.13
4-CH_3-O-	4-CH_3-O-	283.34	27	>100	>35	<0.14
H-	3-NO_2-	270.30	27	>100	>37	<0.14
H-	4-NO_2-	270.30	26	>100	>37	<0.14
H-	4-SCN-	282.38	27	>100	>35	<0.14

* See footnote * in Table 1.

Table 5. Activity of cynamoylisothiocyanate analogues against *Turbatrix aceti*

	Mol. wt.	References	ED_{100}		RE *
			μg/ml	mole/liter $\cdot 10^{-5}$	
R =					
H-	189.23	28	>100	>53	<0.094
4-CH_3-	203.26	29	>100	>50	<0.10
4-CH_3-O-	219.26	29	>100	>46	<0.11
3-Br-	268.15	29	>100	>34	<0.15
4-Br-	268.15	29	>100	>34	<0.15
2-NO_2-	234.24	29	>100	>43	<0.12
3-NO_2-	234.24	29	>100	>43	<0.12
4-NO_2-	234.24	29	>100	>43	<0.12

* See footnote * in Table 1.

Table 6. Activity of *p*-substituted derivatives of diphenylisothiocyanate against *Turbatrix aceti*

R-⟨⟩-⟨⟩-NCS R =	Mol. wt.	References	ED_{100} *		RE_{PhITC} **	$RE_{PiPhITC}$ ***
			μg/ml	mole/liter $\cdot 10^{-5}$		
H-	211.28	30	0.5	0.24	21	1
Cl-	245.73	31	1.0	0.41	12	0.59
Br-	290.18	31	1.6	0.55	10	0.44
J-	337.19	31	6.4	1.9	2.6	0.13
HO-	227.28	31	2.6	1.1	4.6	0.22
NC-	236.29	31	0.2	0.085	59	2.8
O_2N-	256.27	31	1.0	0.39	13	0.62
$(CH_3)_2N$-	254.33	31	35	14	0.36	0.017
CH_3-	225.30	31	0.6	0.27	19	0.89
CH_3-CO-NH-	268.33	31	23	8.6	0.58	0.028
SCN-	268.35	32	50	19	0.26	0.013

* After 72 hours of action ED_{100} of each derivative is ≤ 1 μg/ml.
** Relative effectivity compared with that of phenylisothiocyanate (molar concentration).
*** Relative effectivity compared with that of 4-diphenylisothiocyanate (molar concentration).

note that its structual isomer 1-naphtylisothiocyanate possesses very little activity. The same relationships between these naphthylisothiocyanates have been found also in other biological tests[5]. Fluorenyl-4-isothiocyanate exhibits high activity, but after a prolonged period of action only. It resembles the diphenyl derivatives (Table 6).

Table 4 represents twenty benzhydrylisothiocyanates, all of which are ineffective against *T. aceti*. If we consider these compounds as derivatives of benzylisothiocyanate, then substitution of the hydrogen of the methylene- group by an aromatic nucleus leads to the loss of their antiworm activity. This may be due to steric effects or to reduced reactivity of the NCS-group. However, this group of compounds is very interesting by virtue of their antibacterial activity[4,9].

Arylalkylisothiocyanates derived from cinamoylisothiocyanate are ineffective against *T. aceti* (Table 5). Of all isothiocyanates studied, 4-diphenylylisothiocyanates show the highest antiworm activity (Table 6). Depending on the type of substitution in the position 4 of the aromatic ring, they exhibit their activity after longer or shorter period of action, but at the end of 72 hours of action concentrations of 1 μg/ml of all diphenyl derivatives were active.

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