Table II—Effect of Buffering *Eisenia* Extract on Carrageenan-Induced Rat Paw Edema

Treatment[a]	Mean Edema, $mm^3 \pm SE$ (6)[b]
Saline	1174 ± 99
Buffered[c] *Eisenia* extract	678 ± 176[d]

[a] Administered intraperitoneally 1 hr before carrageenan. [b] Number of animals. [c] Buffered at pH 7.4 with 0.2 *M* KH_2PO_4–Na_2PO_4. [d] $p < 0.05$.

multiple-range finding test. Comparison of the anti-inflammatory activity data utilized the Student two-tailed *t* test (7).

RESULTS AND DISCUSSION

The results of testing the activity of the *Eisenia* extract for ability to stabilize lysosomal membranes are presented in Table I. They have been corrected for any direct inhibition of the enzyme. Acid phosphatase was a poor indicator of lysosomal membrane condition, since the powerful stabilizing agent phenylbutazone, which was used as a positive control, inhibited enzyme release by only 7%. However, phenylbutazone (0.309 mg/ml) and the extract at a concentration of either 3.087 or 0.309 mg/ml offered significant inhibition of release of β-glucuronidase. At the lower extract concentration of 0.031 mg/ml, there was no protection.

Since some compounds have been shown to be anti-inflammatory because of their irritant properties (8), it was important to determine if the compound might be acting as an anti-inflammatory agent by virtue of its acidity; it normally was injected at pH 4.5. When the material was buffered at pH 7.4 and anti-inflammatory activity was assessed (Table II) using the carrageenan-induced rat paw edema model, there was only 42% inhibition of inflammation. This value is somewhat lower than the expected 88% (1), indicating that the protection afforded by the *Eisenia* extract is due in part to a nonspecific counterirritancy.

It appears that there are two mechanisms for the anti-inflammatory activity of the complex polymer isolated from *E. bicyclis* (Kjellman) Setchell. One is its ability to stabilize the membranes of lysosomes and inhibit the release of the destructive lysosomal enzymes, and the other is a counterirritancy effect.

REFERENCES

(1) D. M. Whitaker and G. P. Carlson, in "Food-Drugs from the Sea, Proceedings 1972," L. R. Worthen, Ed., Marine Technology Society, Washington, D.C., 1973, pp. 97–103.

(2) S. G. Zelenski and L. R. Worthen, *Bot. Mar.*, **17**, 191(1974).

(3) J. G. Houck, J. Johnston, and R. A. Jacob, in "Chemical Biology of Inflammation," J. C. Houck and B. K. Forscher, Eds., Pergamon, Oxford, England, 1968, pp. 19–26.

(4) L. J. Ignarro and J. Slywka, *Biochem. Pharmacol.*, **21**, 875(1972).

(5) L. J. Ignarro, *ibid.*, **20**, 2847(1971).

(6) C. G. Van Arman, A. J. Begany, L. M. Miller, and H. H. Pless, *J. Pharmacol. Exp. Ther.*, **150**, 328(1965).

(7) R. G. D. Steel and J. H. Torrie, "Principles and Procedures of Statistics," McGraw-Hill, New York, N.Y., 1960, pp. 73–107.

(8) S. Garattini, A. Jori, D. Bernardi, C. Carrara, S. Paglialunga, and D. Segre, in "Nonsteroidal Anti-inflammatory Drugs," S. Garattini and M. N. G. Dukes, Eds., "Excerpta Medica Foundation, Amsterdam, The Netherlands, 1965, pp. 151–161.

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Synthesis and Antimicrobial Activity of *N*-Substituted *N'*-Cyano-*S*-(triorganostannyl)isothioureas

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Abstract □ Six *N*-substituted *N'*-cyano-*S*-(trimethylstannyl)isothioureas were synthesized by the reaction of (trimethylstannyl)cyanamide with various organic isothiocyanates. The IR spectrum of each compound was obtained over the 4000–30-cm^{-1} range, and some bands were assigned. The six new compounds and five previously synthesized *N*-substituted *N'*-cyano-*S*-(triphenylstannyl)isothioureas were tested for and were found to exhibit antifungal activity. *N*-Phenyl-*N'*-cyano-*S*-(triphenylstannyl)isothiourea was also investigated for antibacterial activity and was observed to be especially inhibitory toward Gram-positive species. The antimicrobial activity of two compounds was compared to that of the oxygen analogs of these compounds.

Keyphrases □ *N'*-Cyano-*S*-(triorganostannyl)isothioureas, *N*-substituted—synthesis, IR spectra, antimicrobial activity □ Antifungal activity—synthesis of six and evaluation of 11 *N*-substituted *N'*-cyano-*S*-(triorganostannyl)isothioureas □ Antibacterial activity—evaluation of *N*-phenyl-*N'*-cyano-*S*-(triphenylstannyl)isothiourea

Recently, the synthesis of 13 *N*-substituted *N'*-cyano-*O*-(trimethylstannyl)isoureas (I*a*) and 12 *N*-substituted *N'*-cyano-*O*-(triphenylstannyl)isoureas (I*b*) was described (1). Six of these compounds were tested for and were found to exhibit antifungal activity; one (I*b*, R' = C_6H_5) was also investigated for an-

Table I—*N*-Substituted *N'*-Cyano-*S*-(trimethylstannyl)isothioureas

$RN{=}CSSn(CH_3)_3$
|
NHCN

Compound	R	Yield, %[a]	Melting Point[b]	Formula	Analysis, % Calc.	Found
1	CH_3	76	127–128°[c]	$C_6H_{13}N_3SSn$	C 25.93	26.01
					H 4.71	4.64
					N 15.12	15.18
					S 11.54	11.27
					Sn 42.70	42.83
2	cyclo-C_6H_{11}	66	149–150°[d]	$C_{11}H_{14}N_3SSn$	C 38.18	38.46
					H 6.12	6.09
					N 12.14	12.30
					S 9.27	9.52
					Sn 34.30	34.69
3	$C_6H_5CH_2$	76	106–107°[c]	$C_{12}H_{17}N_3SSn$	C 40.71	40.99
					H 4.84	4.84
					N 11.86	12.06
					S 9.05	9.01
					Sn 33.52	33.45
4	C_6H_5	61	125–126°[d]	$C_{11}H_{15}N_3SSn$	C 38.85	38.89
					H 4.45	4.21
					N 12.36	12.46
					S 9.43	9.38
					Sn 34.90	34.62
5	*p*-FC_6H_4	65	129–130°[d]	$C_{11}H_{14}FN_3SSn$	C 36.91	37.13
					H 3.94	4.08
					N 11.74	11.92
					S 8.96	8.78
					Sn 33.15	32.89
6	*p*-$O_2NC_6H_4$	68	140–141°[c]	$C_{11}H_{14}N_4O_2SSn$	C 34.31	34.20
					H 3.67	3.75
					N 14.55	14.41
					S 8.33	8.12
					Sn 30.82	30.68

[a] Based on material that melts within 5° of the analytical sample. [b] Analytical sample. [c] Recrystallized from ether. [d] Recrystallized from chloroform–*n*-pentane.

Table II—IR Spectra of *N*-Substituted *N'*-Cyano-*S*-(trimethylstannyl)isothioureas[a]

Compound	NH	C≡N	C=N	SnS	$Sn(CH_3)_3$ ν_{as}	ν_s
1	3322 (m)	2198 (s)	1515 (s)	390 (s)	541 (s)	472 (m)
2	3300 (m)	2179 (s)	1515 (s)	360 (m)[b]	541 (s)	481 (w)
3[c]	3289 (m)	2183 (s)	1536 (s)[d]	—[e]	541 (s)	513 (s)
4[f]	3236 (m)	2174 (s)	1520 (s)[d]	370 (m)[g]	541 (s)	488 (m)
5[h]	3236 (m)	2183 (s)	1517 (s)[d]	328 (s)[i]	535 (s)	500 (s)
6[j]	3390 (m)	2193 (s)	1538 (s)[d]	335 (m)[k]	541 (s)	490 (m)

[a] The SnS values were obtained using CsI pellets; the other values were obtained using KBr pellets. Values are expressed in centimeters^{-1}; s = strong, m = medium, and w = weak. [b] A band was present at 337 (s); in mineral oil, bands were present at 360 (w) and 335 (s). [c] A band was present at 556 (w). [d] This assignment is uncertain due to the presence of aromatic C=C bands in this region. [e] The only band observed in the 400–300-cm^{-1} region was at 306 (m) (303 m in mineral oil). [f] A band was present at 565 (w). [g] A band was present at 330 (w). [h] A band was present at 562 (w). [i] At 330 (s) in mineral oil. [j] A band was present at 553 (m), [k] A band was present at 315 (m). The same bands were present in mineral oil.

tibacterial activity and was found to be inhibitory toward Gram-positive species.

The purpose of the present study was to evaluate the antimicrobial activity of some *N*-substituted *N'*-cyano-*S*-(triorganostannyl)isothioureas (II*a* and II*b*), which are the sulfur analogs of I*a* and I*b*, respectively.

$R'N{=}COSnR_3$
|
NHCN

I*a*: R = CH_3
I*b*: R = C_6H_5

$R'N{=}CSSnR_3$
|
NHCN

II*a*: R = CH_3
II*b*: R = C_6H_5

EXPERIMENTAL[1]

***N*-Benzyl-*N'*-cyano-*S*-(trimethylstannyl)isothiourea (Compound 3)**—A mixture of (trimethylstannyl)cyanamide (2) (2.048 g, 0.01 mole), benzyl isothiocyanate (1.492 g, 0.01 mole), and ether (25 ml) was refluxed for 72 hr and filtered to give 2.7 g (76%) of Compound 3, mp 104–105°. Two recrystallizations from ether gave the analytical sample, mp 106–107°.

The other compounds in Table I were prepared in a similar manner.

Biological Methods—The organotin compounds were individually dissolved in tetrahydrofuran except for Compounds 7–10, which were solubilized in chloroform. The preparation of sterile

[1] Melting points were determined with a Mel-Temp capillary melting-point apparatus and are uncorrected. IR data were obtained using KBr pellets with a Beckman IR 8 spectrophotometer. The far IR data were obtained with a Perkin-Elmer model FIS-3 IR spectrophotometer (CsI pellets or mineral oil) and with a Perkin-Elmer model 21 double-beam IR spectrophotometer fitted with a cesium bromide prism and purged with nitrogen (KBr pellets). Elemental analyses were performed by Schwarzkopf Microanalytical Laboratory, Woodside, N.Y.

Table III—Antifungal Activity of *N*-Substituted *N'*-Cyano-*S*-(triorganostannyl)isothiourea Compounds

RN=C(SSnR'$_3$)–NHCN

Compound	R	R'	*Aspergillus niger* (ATCC 12845)			*Chaetomium globosum* (ATCC 6205)			*Cladosporium carpophilum* (ATCC 12117)			*Fusarium moniliforme* (ATCC 10052)			*Myrothecium verrucaria* (ATCC 9095)			*Penicillium notatum* (ATCC 9179)			*Rhizopus stolonifer* (ATCC 10404)			*Saccharomyces cerevisiae* (ATCC 9896)			*Trichoderma viride* (ATCC 8678)			*Trichophyton mentagrophytes* (ATCC 9129)		
			1[a]	10	100	1	10	100	1	10	100	1	10	100	1	10	100	1	10	100	1	10	100	1	10	100	1	10	100	1	10	100
1	CH_3	CH_3	–	–	2+	–	+	2+	–	2+	2+	–	+	+	–	+	2+	–	+	2+	–	–	–	–	+	+	–	–	–	2+	+	+	2+
2	CH_3	cyclo-C_6H_{11}	–	–	+	–	+	+	–	–	2+	–	+	+	–	+	2+	–	+	2+	–	–	–	–	+	+	–	–	–	2+	+	+	2+
3	CH_3	$C_6H_5CH_2$	–	–	2+	–	–	+	–	2+	2+	–	–	+	–	–	2+	–	–	+	–	–	–	–	+	+	–	–	–	+	+	+	+
4	CH_3	C_6H_5	–	–	+	–	+	+	–	2+	2+	–	+	+	–	+	2+	–	+	+	–	–	–	–	+	+	–	–	–	+	+	+	+
5	CH_3	*p*-FC_6H_4	–	–	+	–	–	+	–	2+	2+	–	–	+	–	+	+	–	+	+	–	–	–	–	+	+	–	–	–	+	+	+	+
6	CH_3	*p*-$O_2NC_6H_4$	–	–	2+	–	–	2+	–	+	2+	–	–	+	–	–	2+	–	+	+	–	–	–	–	+	+	–	–	–	+	+	+	+
7	C_2H_5	C_2H_5	–	–	+	–	+	+	–	+	+	–	–	+	–	–	+	–	+	+	–	+	+	–	+	+	–	–	–	+	+	+	+
8	C_2H_5	$C_6H_5CH_2$	–	–	+	–	–	+	–	+	+	–	–	+	–	–	+	–	+	+	–	+	+	–	+	+	–	–	–	+	+	+	+
9	C_6H_5	C_6H_5	–	+	+	–	+	+	–	–	–	–	+	+	–	+	+	–	+	+	–	2+	+	–	–	–	–	–	–	+	+	+	+
10	*p*-$C_2H_5OC_6H_4$	C_6H_5	+	+	+	–	–	+	–	–	–	–	–	+	–	+	+	–	+	2+	–	+	+	–	–	–	–	–	+	+	+	+	2+
11	*p*-$O_2NC_6H_4$	C_6H_5	+	+	+	–	+	+	–	–	–	+	+	+	+	+	+	–	–	–	–	+	+	–	–	–	–	–	+	+	+	+	+

[a] Indicates concentration of compounds employed in micrograms per milliliter; – indicates no inhibition of growth, + indicates partial inhibition of growth, and 2+ indicates complete inhibition of growth.

Table IV—Antibacterial Activity of *N*-Phenyl-*N'*-cyano-*S*-(triphenylstannyl)isothiourea (Compound 9)

Organism[a]	Concentration, μg/ml		
	1	10	100
Bacillus subtilis	2+[b]	2+	2+
Escherichia coli	–	–	+
Micrococcus agilis	2+	2+	2+
Staphylococcus aureus	2+	2+	2+

[a] Obtained from the culture collection of the Department of Biology, St. John's University. [b] – indicates no inhibition of growth, + indicates partial inhibition of growth, and 2+ indicates complete inhibition of growth.

solutions of the organotin compounds, the fungi employed, the antimicrobial testing procedures, and the determination of growth inhibition were reported previously (1).

One organotin compound, *N*-phenyl-*N'*-cyano-*S*-(triphenylstannyl)isothiourea (Compound 9), was also investigated for antibacterial activity according to the procedure reported earlier (1).

RESULTS AND DISCUSSION

Synthesis—The five *N*-substituted *N'*-cyano-*S*-(triphenylstannyl)isothioureas (II*b*) used in this study were described previously (3). The six *N*-substituted *N'*-cyano-*S*-(trimethylstannyl)-isothioureas (I*b*) are new compounds and were prepared by allowing (trimethylstannyl)cyanamide to react separately with six different organic isothiocyanates (1:1 mole ratio) in refluxing ether (Table I). The IR spectra of these compounds are summarized in Table II. The IR spectra contained both the ν_{as}(SnC) band and the ν_S(SnC) band, indicating that the trimethyltin group may be nonplanar in these compounds (4).

Biological Results—The 11 *N*-substituted *N'*-cyano-*S*-(triorganostannyl)isothiourea compounds were investigated for inhibition of growth of 10 fungal species. One compound was also investigated for antibacterial activity. Table III shows the antifungal activity of the compounds tested. The data presented indicate that the trimethyltin compounds (Compounds 1–6) were significantly more active than the triphenyltin compounds (Compounds 7–11). The incidence of total inhibition of fungal growth, for example, was six times greater with the trimethyltin compounds as compared to the triphenyltin compounds. In addition, the latter compounds did not exhibit total inhibition at a level of 10 μg/ml, whereas the trimethyltin compounds did in four instances.

Of the 11 compounds tested, the most effective antifungal activity, as indicated by total inhibition of growth, was exhibited by *N*-methyl-*N'*-cyano-*S*-(trimethylstannyl)isothiourea (Compound 1). From the data compiled, it was possible to compare the growth-inhibiting properties of specific trimethyltin compounds with their triphenyltin analogs. Thus, for example, *N*-benzyl-*N'*-cyano-*S*-(trimethylstannyl)isothiourea (Compound 3) completely inhibited four of 10 fungi, whereas the triphenyltin analog (Compound 8) displayed only partial inhibition of growth. The latter compound, however, did possess activity against *Rhizopus stolonifer*, which was lacking in the trimethyltin derivative. Similarly, *N*-phenyl-*N'*-cyano-*S*-(trimethylstannyl)isothiourea (Compound 4) displayed greater overall antifungal activity than its triphenyltin counterpart (Compound 9).

The superior antifungal activity of the trimethyltin compounds was even more evident when *N*-*p*-nitrophenyl-*N'*-cyano-*S*-(trimethylstannyl)isothiourea (Compound 6) was compared with its triphenyltin analog (Compound 11). The former compound completely inhibited five of the 10 fungi whereas the latter compound displayed total inhibition against only two of the fungi. Of interest was the fact that the trimethyltin compound partially inhibited *Saccharomyces cerevisiae*, an activity shared by only one other compound, Compound 1.

Further comparisons were possible between two of the organotin compounds reported in the present investigation and their oxygen analogs, the antimicrobial activities of which were described previously (1). For example, Compound 4 completely inhibited the growth of three of the 10 fungi, whereas *N*-phenyl-*N'*-cyano-*O*-(trimethylstannyl)isourea (1) totally inhibited only one fungus.

Secondly, *N*-phenyl-*N'*-cyano-*S*-(triphenylstannyl)isothiourea (Compound 9) and its oxygen analog (1) both exhibited total inhibition of two of the 10 fungi tested. The former compound completely inhibited *Aspergillus niger* and *R. stolonifer* at a concentration of 100 μg/ml. The oxygen analog, on the other hand, was most active against *Myrothecium verrucaria* and *S. cerevisiae* at a similar concentration. The latter activity is noteworthy in that none of the 11 compounds reported in the present investigation completely inhibited *S. cerevisiae*.

The antibacterial activity of Compound 9 is shown in Table IV. This compound was remarkably active against the three Gram-positive species, displaying complete inhibition at a concentration of 1 μg/ml. In addition, partial inhibition of the Gram-negative organism, *Escherichia coli*, was evident. This latter activity contrasts with that of the oxygen analog, *N*-phenyl-*N'*-cyano-*O*-(triphenylstannyl)isourea, which was previously reported to be ineffective against *E. coli* (1). In addition, among the four Gram-positive species tested, total inhibition by the oxygen analog was accomplished only against *Micrococcus agilis* (1).

REFERENCES

(1) E. J. Kupchik, M. A. Pisano, D. K. Parikh, and M. A. D'Amico, *J. Pharm. Sci.*, **63**, 261(1974).

(2) J. A. Feiccabrino and E. J. Kupchik, *J. Organometal. Chem.*, **56**, 167(1973).

(3) R. A. Cardona and E. J. Kupchik, *ibid*, **43**, 163(1972).

(4) R. Okawara and M. Wada, *Advan. Organometal. Chem.*, **5**, 137(1967).

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New Synthesis of *(RS)*-Carnitine Chloride

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Abstract □ A four-step synthesis of *(RS)*-carnitine chloride was developed using extremely mild reaction conditions and versatile intermediates. Crotyl chloride was converted to *tert*-butyl 3-butenoate using *tert*-butyl alcohol and triethylamine in ether. Treatment of *tert*-butyl 3-butenoate with *m*-chloroperbenzoic acid in chloroform afforded *tert*-butyl 3,4-epoxybutyrate. Reaction of this compound with trimethylamine hydrochloride in methanol, followed by mild acid hydrolysis of the *tert*-butyl ester, afforded *(RS)*-carnitine chloride.

Keyphrases □ *(RS)*-Carnitine chloride—four-step synthesis under mild reaction conditions from crotyl chloride □ Crotyl chloride—starting material in four-step synthesis of *(RS)*-carnitine chloride

The importance of *(R)*-(−)-carnitine chloride in the transport of fatty acids across membranes was demonstrated previously (1). Specifically, carnitine chloride catalyzes the oxidation of long chain fatty acids by participating in the enzyme-mediated transport of activated fatty acids from the cytoplasm to the β-oxidation sites within the mitochondria (2, 3). The objectives of this investigation were to develop a convenient and versatile synthetic route that could be readily adapted to the synthesis of not only carnitine chloride itself but also to carnitine analogs. The availability of carnitine analogs could provide valuable biochemical tools for investigating the structural requirements of carnitine chloride as a catalyst of fatty acid oxidation (4).

DISCUSSION

In 1966, Ozeki and Kusaka (5) described the synthesis of numerous 3-butenoate esters from crotyl chloride (I) and the appropriate alcohol in the presence of a variety of tertiary amines. It appeared that proper functionalization of the olefinic bond in one of the 3-butenoate esters would provide a convenient and relatively straightforward synthesis of *(RS)*-carnitine chloride (VI) and, thus, carnitine analogs. The *tert*-butyl ester was selected because of the extremely mild reaction conditions required for conversion to the free carboxylic acid (dilute hydrochloric acid at room temperature). Triethylamine was selected as the base because it provided the highest yield of the desired 3-butenoate ester, with only small amounts (3.1%) of the unwanted *cis*- and *trans*-2-butenoate esters (determined by GC).

Since the previous report (5) provided few experimental details and the reaction was not discussed from the standpoint of providing an extremely versatile synthetic intermediate, it was necessary to develop the appropriate reactions conditions to afford the desired *tert*-butyl 3-butenoate (II) in a yield that would be useful for completing the synthesis of VI. The small amount of the *cis*- and *trans*-2-butenoate esters produced during the preparation of II were of little concern because the following step in the synthetic sequence involved epoxidation of the olefinic bond. The mildest conditions possible were used that would eliminate the possibility of isomerization of the 3-butenoate ester to a mixture of the *cis*- and *trans*-2-butenoate esters during exposure to the *m*-chlorobenzoic acid.

It is also well known that α,β-unsaturated carbonyl compounds do not normally undergo epoxidation when subjected to the same reaction conditions as isolated olefinic bonds. The desired epoxy ester (III) was initially converted to the iodohydrin (IV) using the method of Cornforth (6). Compound IV was subsequently treated with trimethylamine in methanol to afford *tert*-butyl 4-dimethylamino-3-hydroxybutyrate methiodide (V*a*) which, when subjected to ion-exchange chromatography, afforded V*b*. The *tert*-butyl protecting group was then removed by the mild reaction conditions described to yield VI.

It was later found, after much experimental effort, that III could be directly converted into the ester V*b*, using trimethylamine hydrochloride in methanol, in a yield high enough to make the reaction synthetically feasible, thus eliminating one step in the synthetic sequence as well as the ion-exchange chromatography.