Synthesis and Antimicrobial Activity of *N*-Substituted *N'*-Cyano-*O*-(triorganostannyl)isoureas

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Abstract □ Twenty-five *N*-substituted *N'*-cyano-*O*-(triorganostannyl)isoureas were synthesized by the reaction of (triphenylstannyl)cyanamide or (trimethylstannyl)cyanamide with various organic isocyanates. The IR spectrum of each compound was obtained over the 4000–30 cm^{-1} range, and some bands were assigned. Six compounds were tested for, and found to exhibit, antifungal activity. One of these compounds was also investigated for antibacterial activity and was observed to be inhibitory toward Gram-positive species.

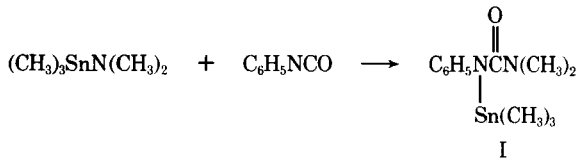
Keyphrases □ *N'*-Cyano-*O*-(triorganostannyl)isoureas, *N*-substituted—synthesis, IR spectra, antimicrobial activity □ Antifungal activity—synthesis and evaluation of six *N*-substituted *N'*-cyano-*O*-(triorganostannyl)isoureas □ Antibacterial activity—synthesis and evaluation of *N*-phenyl-*N'*-cyano-*O*-(triphenylstannyl)isourea

The reaction of dimethylaminotrimethylstannane with phenyl isocyanate was reported (1) to afford a 1:1 addition compound, which was assigned, on the basis of its IR spectrum, Structure I (Scheme I). The IR spectrum of I contained a band at 1660 cm^{-1}, which was assigned to a carbonyl stretching mode of a fully substituted urea. Recently, (triphenylstannyl)-cyanamide was reported (2) to form a 1:1 addition compound with phenyl isocyanate, but no suggestion was made as to its structure. As in I, the tin in this compound could be bonded to nitrogen (Structure II); another possibility, however, is a structure in which the tin is bonded to oxygen (Structure III).

It was considered of interest to synthesize a number of (triorganostannyl)cyanamide–organic isocyanate addition compounds and to study their IR spectra to determine whether the tin is bonded to nitrogen or to oxygen. Since some organotin compounds were previously found to exhibit antifungal and antibacterial activity (3), it was also of interest to determine the antifungal and antibacterial activity of some of these compounds.

RESULTS AND DISCUSSION

Synthesis—Thirteen (trimethylstannyl)cyanamide–organic isocyanate addition compounds were prepared by allowing (trimethylstannyl)cyanamide to react separately with 13 different organic isocyanates (1:1 mole ratio) in acetonitrile at room temperature (Table I). In addition, 12 (triphenylstannyl)cyanamide–organic isocyanate addition compounds were prepared in a similar manner by allowing (triphenylstannyl)cyanamide to react separately with 12 different organic isocyanates (Table II). The IR spectra of these compounds are summarized in Tables III and IV. The assignments are based largely on the work of others (4–10). None of the compounds exhibited a band near 1660 cm^{-1} due to the carbonyl group of a urea (11); this band appears at 1667 cm^{-1} in 1-cyano-3-phenylurea (12). The absence of carbonyl absorption and the presence of a band that can be assigned to the C=N group (13), especially in the cases of Compounds 1–6 (Table III) which do not contain interfering phenyl absorptions, suggest that the compounds are *N*-substituted *N'*-cyano-*O*-(triorganostannyl)isoureas rather than 1-cyano-3-phenyl-3-(triorganostannyl)ureas. The trimethylstannyl compounds (Table III) contained both the ν_{as} (SnC) band and the ν_s (SnC) band, indicating that the trimethyltin group is nonplanar in these compounds (17).



Scheme I

Unlike the tin–oxygen bond in many organotin alkoxides (14), the tin–oxygen bond in these compounds was found to be stable in water. For example, 88% of *N*-phenyl-*N'*-cyano-*O*-(triphenylstannyl)isourea (III) was recovered unchanged after being stirred in water–tetrahydrofuran (2:50)(pH 5.5) for 24 hr at 26°. The hydrolytic stability of III may be due to intramolecular or intermolecular coordination between tin and the nitrogen of the CN group. Compound III was unstable in 6 *N* hydrochloric acid at 26°, with one of the products formed being triphenyltin chloride.

Biological Results—Six of the *N*-substituted *N'*-cyano-*O*-(triorganostannyl)isourea compounds were investigated for the inhibition of growth of 10 fungus species. One compound was also investigated for antibacterial activity. Table V lists the antifungal activity of the compounds tested. From the data presented, it can be seen that the *N*-substituted *N'*-cyano-*O*-(triphenylstannyl)isoureas (Compounds 18 and 26) were more active than the trimethylstannyl compounds (Compounds 1, 7, 9, and 10). Complete inhibition of *Aspergillus niger* and *Trichophyton mentagrophytes* occurred in the presence of 100 μg/ml of Compound 18, *N*-cyclohexyl-*N'*-cyano-*O*-(triphenylstannyl)isourea. At a similar concentration, Compound 26, *N*-phenyl-*N'*-cyano-*O*-(triphenylstannyl)isourea (III), totally inhibited both *Saccharomyces cerevisiae* and *T. mentagrophytes*. The latter compound was also investigated for antibacterial activity (Table VI). The data show that Compound 26 was active against Gram-positive but not Gram-negative species. The Gram-positive bacteria displayed several degrees of sensitivity, with *Micrococcus agilis* being completely inhibited at 1 μg/ml, whereas for *Bacillus subtilis* 10 μg/ml was required for a similar effect. *Staphylococcus aureus* was the least sensitive of the Gram-positive species tested, 100 μg/ml of Compound 26 being required for complete inhibition of growth.

EXPERIMENTAL[1]

***N*-Ethyl-*N'*-cyano-*O*-(trimethylstannyl)isourea (Compound 1)**—A mixture of (trimethylstannyl)cyanamide (15) (0.819 g, 0.004 mole), ethyl isocyanate (0.33 ml, 0.0042 mole), and acetonitrile (25 ml) was stirred at 26° for 24 hr and filtered to give 0.82 g (72%) of Compound 1, mp 158–162°. Two recrystallizations from tetrahydrofuran–*n*-pentane gave the analytical sample, mp 160–161°.

[1] Melting points were determined with a Mel-Temp capillary melting-point apparatus and are uncorrected. The 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 (polyethylene pellets) 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 I—*N*-Substituted *N'*-Cyano-*O*-(trimethylstannyl)isoureas[a]

$$RN{=}C(OSn(CH_3)_3)NHCN$$

Compound	R	Yield[b], %	Melting Point[c]	Formula	Analysis, %	Calc.	Found
1	C_2H_5	72	160–161°	$C_7H_{15}N_3OSn$	C	30.47	30.15
					H	5.48	5.60
					N	15.23	15.02
					Sn	43.02	43.14
2	n-C_3H_7	85	148–150°	$C_8H_{17}N_3OSn$	C	33.14	33.69
					H	5.91	5.96
					N	14.49	14.04
					Sn	40.93	40.62
3	n-C_4H_9	73	148–150°	$C_9H_{19}N_3OSn$	C	35.56	35.24
					H	6.30	6.34
					N	13.82	13.82
					Sn	39.05	38.87
4	$(CH_3)_3C$	86	154–156°	$C_9H_{19}N_3OSn$	C	35.56	35.70
					H	6.30	6.43
					N	13.82	14.08
					Sn	39.05	38.77
5	cyclo-C_6H_{11}	89	187–188°	$C_{11}H_{21}N_3OSn$	C	40.04	39.94
					H	6.41	6.63
					N	12.73	12.42
					Sn	35.97	35.91
6	$CH_2{=}CHCH_2$	88	140–141°	$C_8H_{15}N_3OSn$	C	33.37	33.71
					H	5.25	5.41
					N	14.59	14.78
					Sn	41.23	40.89
7	C_6H_5	70	168°	$C_{11}H_{15}N_3OSn$	C	40.78	40.83
					H	4.67	4.80
					N	12.97	12.69
					Sn	36.63	36.77
8	m-$CH_3C_6H_4$	75	151–152°[d]	$C_{12}H_{17}N_3OSn$	C	42.65	42.88
					H	5.07	5.28
					N	12.43	12.63
					Sn	35.12	35.31
9	p-$CH_3C_6H_4$	88	173–176°	$C_{12}H_{17}N_3OSn$	C	42.65	42.73
					H	5.07	5.24
					N	12.43	12.20
					Sn	35.12	35.56
10	p-$C_2H_5OC_6H_4$	73	170–171°	$C_{13}H_{19}N_3O_2Sn$	C	42.43	42.22
					H	5.21	5.28
					N	11.42	11.66
					Sn	32.25	32.14
11	p-FC_6H_4	89	174–175°	$C_{11}H_{11}FN_3OSn$	C	38.98	38.78
					H	3.27	4.08
					N	12.45	12.40
					Sn	35.02	35.03
12	p-ClC_6H_4	82	176–178°	$C_{11}H_{14}ClN_3OSn$	C	36.86	36.68
					H	3.94	3.94
					N	11.72	11.77
					Sn	33.11	32.90
13	m-$O_2NC_6H_4$	35	165–166°	$C_{11}H_{14}N_4O_3Sn$	C	35.81	35.71
					H	3.82	3.77
					N	15.18	14.95
					Sn	32.17	32.17

[a] Unless otherwise indicated, the recrystallization solvent was tetrahydrofuran–*n*-pentane. [b] Based on material that melts within 5° of the analytical sample. [c] Analytical sample. [d] Recrystallized from acetonitrile.

Compounds 2–13 were prepared in a similar manner.

***N*-Ethyl-*N'*-cyano-*O*-(triphenylstannyl)isourea (Compound 14)**—A mixture of (triphenylstannyl)cyanamide (16) (1.56 g, 0.004 mole), ethyl isocyanate (0.33 ml, 0.0042 mole), and acetonitrile (25 ml) was stirred at 26° for 24 hr and filtered to give 2.63 g (72%) of Compound 14, mp 173–174°. Recrystallization from tetrahydrofuran–*n*-pentane gave the analytical sample, mp 174°.

Compounds 15–25 were prepared in a similar manner.

Biological Methods—The organotin compounds were individually dissolved in tetrahydrofuran, and 1% solutions of each were sterilized by filtration (Seitz) (0.1-μm porosity). Dilutions of the sterile solutions were prepared and added to liquefied potato dextrose agar[2] to yield final concentrations of 100, 10, and 1 μg/ml. The agar, containing the appropriate organotin compound, was poured into sterile petri dishes and allowed to solidify overnight. The agar surface was inoculated centrally with a 2-mm² portion of the growth from a 10-day-old culture of each fungus[3] with the exception of *S. cerevisiae* in which case 2-day-old cultures were employed. Inoculated plates were incubated for 14 days, and observations of growth inhibition were made by measuring the diameters of the colonies present on the agar surface.

One organotin compound, *N*-phenyl-*N'*-cyano-*O*-(triphenylstannyl)isourea (III), was also investigated for antibacterial activity. It was incorporated into sterile tryptic soy agar[2] at levels of 100, 10, and 1 μg/ml. The agar was also seeded with a 1:100 dilu-

$$C_6H_5NC(=O)NHCN \quad \text{with } Sn(C_6H_5)_3 \text{ on N}$$

II

$$C_6H_5N{=}C(OSn(C_6H_5)_3)NHCN$$

III

[2] Difco.

[3] All fungi were obtained from the American Type Culture Collection (ATCC), Rockville, Md.

Table II—*N*-Substituted *N'*-Cyano-*O*-(triphenylstannyl)isoureas[a]

$$RN{=}C(OSn(C_6H_5)_3)NHCN$$

Compound	R	Yield[b], %	Melting Point[c]	Formula	Analysis, %	
					Calc.	Found
14	C_2H_5	72	174°	$C_{22}H_{21}N_3OSn$	C 57.18	57.09
					H 4.58	4.76
					N 9.09	9.35
					Sn 25.68	25.76
15	*n*-C_3H_7	61	175–177°	$C_{23}H_{23}N_3OSn$	C 58.02	58.26
					H 4.87	5.11
					N 8.82	8.65
					Sn 24.93	24.71
16	*n*-C_4H_9	73	157–158°	$C_{24}H_{25}N_3OSn$	C 58.81	58.94
					H 5.14	5.00
					N 8.57	8.47
					Sn 24.21	24.09
17	$(CH_3)_3C$	84	160–161°	$C_{24}H_{25}N_3OSn$	C 58.81	59.29
					H 5.14	4.99
					N 8.57	8.36
					Sn 24.21	23.96
18	cyclo-C_6H_{11}	89	181–183°	$C_{26}H_{27}N_3OSn$	C 60.50	60.50
					H 5.27	5.45
					N 8.14	7.83
					Sn 22.99	23.09
19	$CH_2{=}CHCH_2$	86	167–170°[d]	$C_{23}H_{21}N_3OSn$	C 58.26	58.18
					H 4.46	4.44
					N 8.86	9.09
					Sn 25.03	25.19
20	*m*-$CH_3C_6H_4$	91	162–163°[e]	$C_{27}H_{23}N_3OSn$	C 61.87	61.76
					H 4.42	4.54
					N 8.02	7.84
					Sn 22.64	22.83
21	*p*-$CH_3C_6H_4$	80	158–159°[e]	$C_{27}H_{23}N_3OSn$	C 61.87	61.67
					H 4.42	4.53
					N 8.02	7.91
					Sn 22.64	22.91
22	*p*-$C_2H_5OC_6H_4$	81	165–168°[d]	$C_{28}H_{25}N_3O_2Sn$	C 60.68	60.85
					H 4.55	4.55
					N 7.58	7.28
					Sn 21.42	21.28
23	*p*-FC_6H_4	89	156–158°[d]	$C_{26}H_{20}FN_3OSn$	C 59.13	59.11
					H 3.81	3.79
					N 7.95	7.87
					Sn 22.47	22.59
24	*p*-ClC_6H_4	80	130–132°[d]	$C_{26}H_{20}ClN_3OSn$	C 57.40	57.38
					H 3.70	3.77
					N 7.71	7.93
					Sn 21.79	21.92
25	*m*-$O_2NC_6H_4$	52	160–162°[e]	$C_{26}H_{20}N_4O_3Sn$	C 56.25	56.48
					H 3.63	3.86
					N 10.09	10.13
					Sn 21.38	21.59

[a] Unless otherwise indicated, the recrystallization solvent was tetrahydrofuran–*n*-pentane. [b] Based on material that melts within 5° of the analytical sample. [c] Analytical sample. [d] Washed with ether. [e] Recrystallized from acetonitrile.

Table III—IR Spectra of *N*-Substituted *N'*-Cyano-*O*-(trimethylstannyl)isoureas[a]

Compound	NH	C≡N	C=N	$Sn(CH_3)_3$	
				ν_{as}	ν_s
1	3268 s, 3106 m	2165 s	1550 s	550 s	500 s
2	3268 m, 3106 m	2179 s	1555 s	553 s	516 m
3[b]	3279 m, 3049 m	2183 s	1560 s	546 s	521 m
4	3322 m, 3086 w	2174 s	1541 s	553 s	513 s
5[c]	3268 s, 3067 w	2174 s	1541 s	553 s	493 m
6[d]	3268 s	2179 s	1538 s	544 s	518 w
7[e]	3257 m	2198 s	1524 s[f]	556 s	503 s
8[g]	3268 m	2179 s	1531 s[f]	544 s, br	—[h]
9	3257 w	2188 s	1515 s[f]	553 s	503 s
10	3257 m	2198 s, 2179 s	1527[f]	553 s	516 s
11[k]	3257 m	2198 s, 2169 s, sh	1538[f]	559 s	510 s
12[l]	3247 w	2188 s, 2169 s, sh	1515[f]	541 s	495 s
13[m]	3257 w	2188 s	1504[f]	550 s, br	—[h]

[a] Values are expressed in centimeters^{-1}; s = strong, m = medium, w = weak, br = broad, and sh = shoulder. The data were obtained using KBr pellets. [b] A band at 479 m was present. [c] Bands were present at 614 s, 571 s, 529 w, 474 w, and 453 w. [d] Bands were present at 3106 m, 599 w, 559 m, and 454 s. [e] Bands were present at 613 s, 568 s, and 535 m. [f] This assignment is uncertain due to the presence of aromatic C=C bands in this region. [g] A band was present at 614 s. [h] The band may be overlapped by the broad ν_{as} band. [k] A band was present at 524 s. [l] A band was present at 463 s. [m] Bands were present at 614 s and 568 s.

Table IV—IR Spectra of *N*-Substituted *N'*-Cyano-*O*-(triphenylstannyl)isoureas[a]

Compound	NH	C≡N	C=N[b]	C_6H_5 Ring Vibration	$Sn(C_6H_5)_3$ ν_{as}	$Sn(C_6H_5)_3$ ν_s
14	3436 m	2188 s	1560 s	451 s	276 s	225 m
15	3401 m	2165 s	1560 s	455 s	277 s	229 m
16	3401 m	2174 s	1558 s	455 s	277 s	227 m
17	3401 m	2183 s, 2160 s	1546 s	455 s	277 s	230 s
18	3436 m, 3413 m	2174 s	1538 s	444 s	279 s	224 s
19	3413 m	2179 s	1538 s	448 s	275 s	227 s
20	3367 m	2179 s	1558 s	453 s	272 s	225 m
21	3390 m	2198 s	1555 s	455 s	276 s	230 m
22	3367 s	2193 s	1550 s	453 s	279 s	223 m
23	3367 m	2188 s	1553 s	453 s	276 s	228 m
24	3367 w	2193 s	1555 s	444 s	278 s	230 m
25	3401 m	2208 s, 2160 s	1515 s	461 s	270 s	217 s

[a] Values are expressed in centimeters^{-1}; s = strong, m = medium, and w = weak. The $Sn(C_6H_5)_3$ values were obtained using polyethylene pellets; the other data were obtained using KBr pellets. [b] This assignment is uncertain due to the presence of aromatic C=C bands in this region.

Table V—Antifungal Activity of *N*-Substituted *N'*-Cyano-*O*-(triorganostannyl)isourea Compounds

Compound[a]	*Aspergillus niger* (12845)[b] 1[c]	10	100	*Chaetomium globosum* (6205) 1	10	100	*Cladosporium carpophilum* (12117) 1	10	100	*Fusarium moniliforme* (10052) 1	10	100	*Myrothecium verrucaria* (9095) 1	10	100
1	−	−	+	−	−	+	−	−	2+	−	−	+	−	+	+
7	−	−	+	−	−	+	−	−	2+	−	−	+	−	+	+
9	−	−	+	−	−	+	−	−	2+	−	−	+	−	+	+
10	−	−	+	−	−	+	−	+	2+	−	−	+	−	−	+
18	−	+	2+	−	+	+	+	+	+	−	+	+	−	+	+
26	−	+	+	−	+	+	−	+	+	−	+	+	+	+	+

Compound[a]	*Penicillium notatum* (9179) 1	10	100	*Rhizopus stolonifer* (10404) 1	10	100	*Saccharomyces cerevisiae* (9896) 1	10	100	*Trichoderma viride* (8678) 1	10	100	*Trichophyton mentagrophytes* (9129) 1	10	100
1	−	−	+	−	−	−	−	−	−	−	−	−	−	+	+
7	−	−	+	−	−	−	−	−	−	−	−	−	−	+	+
9	−	−	+	−	−	−	−	−	−	−	−	−	−	+	+
10	−	−	+	−	−	−	−	−	−	−	−	−	−	+	+
18	−	+	+	−	+	+	−	−	+	−	−	+	+	+	2+
26	−	+	+	−	+	+	−	−	2+	−	−	+	+	+	2+

[a] Except for Compound 26, the numbers correspond to the compounds in Tables I and II; Compound 26 is *N*-phenyl-*N'*-cyano-*O*-(triphenylstannyl)isourea (III). [b] Numbers in parenthesis indicate ATCC designation of each fungus. [c] 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.

tion of an 18-hr-old culture of the desired bacterial species[4]. The plates were incubated for 24 hr at 37°, except for *Micrococcus agilis* which was maintained for 72 hr at 25°. Observations of bacterial growth were made following the appropriate incubation period.

A solvent control was included in all experiments, as were growth controls for all fungal and bacterial species. The latter were employed for comparative purposes to determine the extent of inhibition exhibited by the organotin compounds.

Table VI—Antibacterial Activity of *N*-Phenyl-*N'*-cyano-*O*-(triphenylstannyl)isourea (III)

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

[a] − indicates no inhibition of growth, + indicates partial inhibition of growth, and 2+ indicates complete inhibition of growth.

[4] Obtained from the culture collection of the Department of Biology, St. John's University.

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PHARMACEUTICAL ANALYSIS

Determination of Calcium Pantothenate in Chewable Multivitamin Tablets

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Abstract □ A hybrid procedure was developed for the determination of calcium pantothenate in a chewable multivitamin tablet. Applicable operations and techniques were adopted from different published sources. The resulting procedure incorporates solvent extraction, chromatography, hydrolysis, and color development. This method proved to be more accurate and precise than any published method investigated.

Keyphrases □ Calcium pantothenate in chewable multivitamin tablets—analysis, procedure using various analytical techniques □ Multivitamin tablets, chewable—analysis of calcium pantothenate, procedure using various analytical techniques □ Pantothenate, calcium, in chewable multivitamin tablets—analysis, procedure using various analytical techniques

In this laboratory, the determination of calcium pantothenate by wet methods has led to many difficulties. It has been necessary to modify and revise existing procedures continually to obtain reliable results on various dosage forms, as well as on similar dosage forms containing different pharmaceutic aids.

Most often the ninhydrin method (1) or an adaptation proved to be suitable. This method, however, could not be made applicable to certain chewable multivitamin tablet formulations containing relatively high levels of natural sweeteners. The most serious objection to this method was the pronounced variability. A statistical analysis showed the coefficient of variation to be over 6%. Analysis of variance studies indicated significant differences in the variability between replicates, days, and operators. Therefore, this method was considered unapplicable.

The iodine method (2, 3) was the next to be investigated. The lack of precision was also a problem with this procedure (3). Some developmental work was done to improve the precision. Subsequently, a statistical evaluation was undertaken which yielded a high coefficient of variation of 8.4%.

Finally, the naphthoquinone method (4) was tried. In this case, the chromatographic separation achieved by Panalaks and Campbell could not be duplicated. The pantothenate was partially eluted with the boric acid wash and tailed badly during the elution step. Consequently, poor results were obtained.

A search of the literature did not reveal any alternative procedures that were considered compatible with techniques and equipment available in this laboratory. However, during this in-depth study of available methods, it became evident that certain techniques involving sample cleanup and detection employed by various authors were very effective. The possibility that a hybrid procedure possessing combined advantages could be developed seemed promising. Such a procedure did, in fact, evolve from the combination of carefully selected existing steps and techniques.

This procedure, when applied to the product mentioned, supplied results significantly more accurate and precise than those provided by any published method tried. No special apparatus is required. Puri-