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Synthesis and Antimicrobial Activity of Triorganotin 5-Nitro-2-furoates

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Received June 29, 1981, from the **Department of Chemistry and the ‡Department of Biological Sciences, St. John's University, New York, NY 11439.* Accepted for publication July 27, 1981.

Abstract □ Five triorganotin 5-nitro-2-furoates were synthesized by reacting 5-nitro-2-furoic acid with either the corresponding bis(triorganotin) oxide or the corresponding triorganotin hydroxide. The IR spectrum of each compound was obtained over the 4000–200-cm^{-1} range, and some of the bands were assigned. One compound, tri-*n*-butyltin 5-nitro-2-furoate, was an excellent antifungal agent, completely inhibiting the growth of six of ten test fungi at a concentration of 1 μg/ml. The new compounds were also investigated for antibacterial activity and were especially inhibitory toward Gram-positive species. Two of the compounds completely inhibited the Gram-negative bacterium *Escherichia coli* at a concentration of 100 μg/ml.

Keyphrases □ Organometallics—triorganotin compounds, synthesis, tested for antifungal and antibacterial activity □ Tin—triorganotin compounds, synthesis and evaluation as antifungal and antibacterial agents □ Antifungal agents, potential—triorganotin compounds, synthesis □ Antibacterials, potential—triorganotin compounds, synthesis

Many biocidal applications have been found or suggested for organotin compounds (1, 2). The specific organotin compounds currently used in agriculture were reviewed recently (3–5). Their use in agriculture as fungicides and pesticides is of special interest because they degrade to nontoxic inorganic compounds and, therefore, appear to pose little threat to the environment (6–10). Recently, a series of diorganotin dihalide complexes was shown to exhibit antitumor activity (11).

It was reported previously that *N*-substituted *N*-(triphenylstannyl)cyanamides (I) are better antifungal agents than *N*-substituted *N'*-cyano-*S*-(triphenylstannyl)isothioureas (II) and *N*-substituted *N'*-cyano-*O*-(triphenylstannyl)isoureas (III) (12). The I compounds were similar in activity to ethyl *N*-aryl-*S*-(triphenylstannyl)isothiocarbamates (IV). Triethylammonium (organocyanoamino)chlorotriphenylstannates (V), which are the triethylammonium chloride complexes of the I compounds, reportedly exhibit higher antifungal activity than the I compounds (12). Although all of the compounds mentioned inhibited Gram-positive bacteria, they showed little inhibitory activity toward Gram-negative bacteria. In this respect, they resemble numerous other organotin compounds (13–16). One purpose of the present study was to synthesize some organotin compounds that might inhibit both Gram-positive and Gram-negative bacteria. Since 5-nitro-2-substituted furans are known to inhibit both Gram-positive and Gram-negative bacteria (17–19), the antibacterial activity of some triorganotin 5-nitro-2-furoates (VI) was studied. The antifungal activity of these

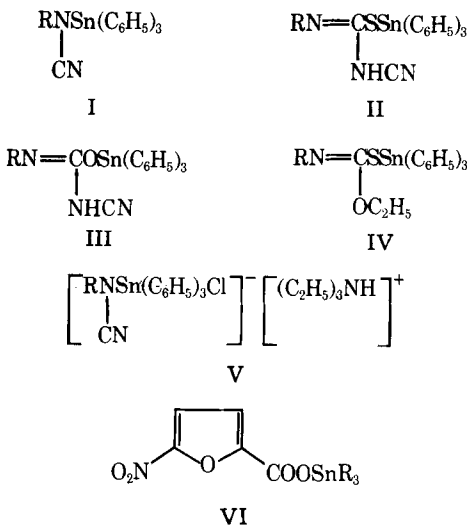

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Table I—Triorganotin 5-Nitro-2-furoates

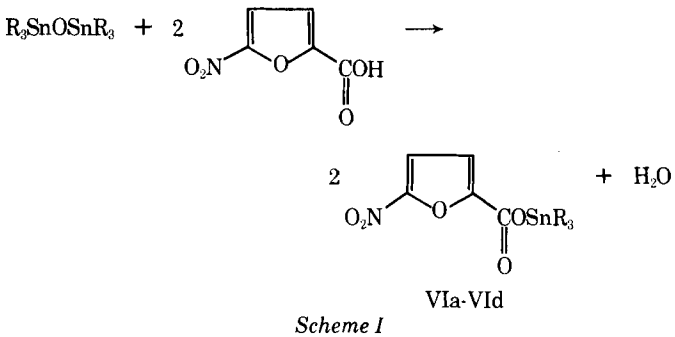
Compound	R	Yield, %[a]	Melting Point[b]	Formula	Analysis, % Calc.	Found
VI*a*	C_6H_5	90	111.8–112.5°	$C_{23}H_{17}NO_5Sn$	C 54.59	54.51
					H 3.39	3.59
					N 2.77	2.90
					Sn 23.45	23.09
VI*b*	n-C_4H_9	97	68–69.7°	$C_{17}H_{29}NO_5Sn$	C 45.77	45.60
					H 6.55	6.63
					N 3.14	3.28
					Sn 26.61	26.67
VI*c*	cyclo-C_6H_{11}	97	154.8–156°	$C_{23}H_{35}NO_5Sn$	C 52.70	52.92
					H 6.73	6.98
					N 2.67	2.82
					Sn 22.64	22.57
VI*d*	$CH_2C(CH_3)_2C_6H_5$	93	79.3–80°	$C_{35}H_{41}NO_5Sn$	C 62.33	62.56
					H 6.13	6.26
					N 2.08	2.13
					Sn 17.60	17.84
VI*e*	CH_3	94	190–192°	$C_8H_{11}NO_5Sn$	C 30.04	30.15
					H 3.47	3.74
					N 4.38	4.43
					Sn 37.12	36.89

[a] Based on material melting within 5° of the analytical sample. [b] Refers to the analytical sample.

Table II—IR Spectra of Triorganotin 5-Nitro-2-furoates[a]

Compound	C=O[b]	C—O	SnR_3[c] ν_{as}	ν_s
VI*a*	1613s	1355s	268s[d]	234s
VI*b*	1610s	1350s	605w	510w
VI*c*	1613s	1353s	610w	510w
VI*d*	1613s	1353s	610m	510w
VI*e*	1615s[e]	1345s	558m	510w

[a] Values are expressed in centimeters^{-1}; s = strong, m = medium, and w = weak. The data for 4000–400 cm^{-1} were obtained using potassim bromide pellets. The data for 400–200 cm^{-1} were obtained using mineral oil. [b] Refs. 22 and 23. [c] Refs. 24 and 25. [d] A strong band was present at 280 cm^{-1}. [e] A strong band was present at 1630 cm^{-1}.



Scheme I

compounds was compared also to that of the I–V compounds.

RESULTS AND DISCUSSION

Synthesis—Compounds VI*a*–VI*e* (Table I) were prepared by reacting 5-nitro-2-furoic acid with either the corresponding bis(triorganotin) oxide (VI*a*–VI*d*) (Scheme I) or the corresponding triorganotin hydroxide (VI*e*) (Scheme II). The compounds were identified by elemental analysis (Table I) and IR (Table II). Their IR spectra are considerably different from those of organic esters. Whereas the C=O stretching vibration of normal saturated organic esters occurs in the range 1750–1730 cm^{-1} (20), the C=O stretching vibration of the organotin esters in Table II occurs in the range 1630–1610 cm^{-1}. This shift to lower frequency may be due to intermolecular or intramolecular coordination between the carbonyl oxygen and the tin atom. Interestingly, this shift occurred even for compound VI*d*, which has bulky neophyl groups around the tin atom. The organotin esters contained both the ν_{as}(SnC) and the ν_a(SnC) bands indicating that the triorganotin groups may be nonplanar in these compounds (21).

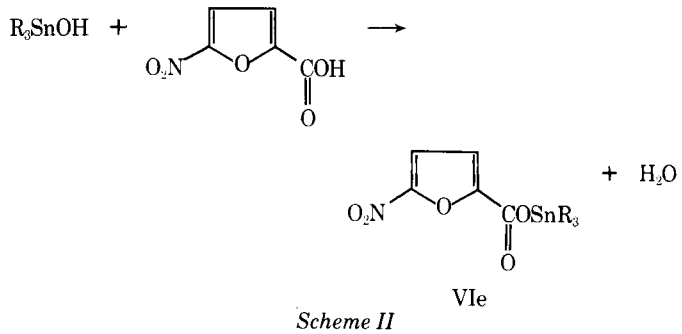
Biological Results—Compound VI*b* was the best antifungal agent of the VI compounds (Table III), completely inhibiting the growth of six of the ten test fungi at 1 μg/ml and all of the test fungi at 10 μg/ml. Compound VI*b* was the best antifungal agent of the I–VI compound series. Compound VI*a* was the second best antifungal agent of the VI

Table III—Antifungal Activity of Triorganotin 5-Nitro-2-furoates[a]

Compound	*Aspergillus niger* (ATCC 12845) 1[b]	10	100	*Chaetomium globosum* (ATCC 6205) 1	10	100	*Cladosporium carpophilum* (ATCC 12117) 1	10	100	*Fusarium moniliforme* (ATCC 10052) 1	10	100	*Myrothecium verrucaria* (ATCC 9095) 1	10	100
VI*a*	+	2+	2+	+	+	+	2+	2+	2+	+	+	+	+	2+	2+
VI*b*	2+	2+	2+	2+	2+	2+	2+	2+	2+	+	2+	2+	2+	2+	2+
VI*c*	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+
VI*d*	−	−	−	−	+	+	−	−	−	−	−	−	−	+	+
VI*e*	−	+	+	−	+	+	+	2+	2+	−	+	+	+	+	2+

Compound	*Penicillium notatum* (ATCC 9179) 1	10	100	*Rhizopus stolonifer* (ATCC 10404) 1	10	100	*Saccharomyces cerevisiae* (ATCC 9896) 1	10	100	*Trichoderma viride* (ATCC 8678) 1	10	100	*Trichophyton mentagrophytes* (ATCC 9129) 1	10	100
VI*a*	+	2+	2+	+	+	+	−	+	+	+	+	+	+	2+	2+
VI*b*	+	2+	2+	+	2+	2+	2+	2+	2+	2+	2+	2+	+	2+	2+
VI*c*	+	+	2+	−	+	+	−	−	−	+	+	+	+	+	+
VI*d*	−	+	+	−	−	−	−	−	−	−	−	−	+	+	+
VI*e*	−	+	2+	+	+	+	−	−	−	+	+	+	+	+	+

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



VIe

Scheme II

compounds, partially inhibiting the growth of all of the test fungi, except *Saccharomyces cerevisiae*, at 1 μg/ml and completely inhibiting the growth of five of the ten test fungi at 10 μg/ml. Compound VI*e* was a generally better antifungal agent than compound VI*c*. Compound VI*e* completely inhibited the growth of *Cladosporium carpophilum* at 10 μg/ml and *Penicillium notatum* at 100 μg/ml. Compound V*c*, on the other hand, inhibited completely the growth of only one fungus (*Penicillium notatum*) at 100 μg/ml. Compounds VI*e* and VI*c* were inactive toward *Saccharomyces cerevisiae* at all concentrations. The poorest antifungal agent of the VI compound series was VI*d*, which was completely inactive toward six of the ten test fungi at all concentrations. The inactivity of VI*d* may be due to the bulky neophyl groups attached to the tin atom in this compound.

The antibacterial activity of the VI compounds is shown in Table IV. Compounds VI*a* and VI*b* both completely inhibited the Gram-positive bacteria *Bacillus megaterium* and *Staphylococcus aureus* at the minimum concentration of organotin compound (1 μg/ml). The activity of compounds VI*a* and VI*b* towards *S. aureus* was identical to that previously observed for the II, IV, and V compounds. The one previously tested III compound, on the other hand, could only completely inhibit this bacterium at 100 μg/ml. Compound VI*e* was the least active of the VI compounds toward the two Gram-positive bacteria. Compound VI*e* was the least active of the II–VI compounds toward *S. aureus*. Compounds VI*b* and VI*e* were the only series VI compounds to show activity toward the Gram-negative bacteria *Pseudomonas aerusginosa* and *Escherichia coli*. Compounds VI*b* and VI*e* both completely inhibited *E. coli* at 100 μg/ml. The one previously tested III compound was inactive toward this bacterium at 100 μg/ml. The II, IV, and V compounds only inhibited growth of this bacterium partially at 100 μg/ml. Compound VI*e* was able to completely inhibit *P. aeruginosa* at 100 μg/ml, while compound VI*b* could only partially inhibit this bacterium at 100 μg/ml.

EXPERIMENTAL[1]

Triphenyltin 5-Nitro-2-furoate (VI*a*)—A mixture of bis(triphenyltin) oxide (11.38 g, 0.01589 mole), 5-nitro-2-furoic acid (5.00 g, 0.0318 mole), and benzene (100 ml) was refluxed for 19 hr. The mixture was filtered, and the benzene was evaporated from the filtrate to give 14.41 g (90%) of VI*a*, mp 111–132°. Four recrystallizations from heptane–benzene (7:5) gave the analytical sample, mp 111.8–112.5°.

Tri-*n*-butyltin 5-Nitro-2-furoate (VI*b*)—A mixture of bis(tri-*n*-butyltin) oxide (10.43 g, 0.01750 mole), 5-nitro-2-furoic acid (5.50 g, 0.0350 mole), and benzene (100 ml) was refluxed for 19 hr. The mixture was filtered, and the benzene was evaporated from the filtrate to give 15.18 g (97%) of VI*b*, mp 62.0–67.0°. Four recrystallizations from heptane gave the analytical sample, mp 68–69.7°.

Tricyclohexyltin 5-Nitro-2-furoate (VI*c*)—A mixture of bis(tricyclohexyltin) oxide (26) (11.96 g, 0.1590 mole), 5-nitro-2-furoic acid (5.00 g, 0.0318 mole), and benzene (100 ml) was refluxed for 16 hr. The mixture was filtered, and the benzene was evaporated from the filtrate to give 16.09 g (97%) of VI*c*, mp 147–155.5°. Four recrystallizations from heptane gave the analytical sample, mp 154.8–156°.

Trineophyltin 5-Nitro-2-furoate (VI*d*)—A mixture of bis(trineophyltin) oxide (27) (2.63 g, 0.00250 mole), 5-nitro-2-furoic acid (0.78 g, 0.0050 mole), and benzene (25 ml) was refluxed for 26.5 hr. The mixture was filtered, and the benzene was evaporated from the filtrate to give 3.14 g (93%) of VI*d*, mp 74–78°. Three recrystallizations from heptane gave the analytical sample, mp 79.3–80°.

Trimethyltin 5-Nitro-2-furoate (VI*e*)—A mixture of trimethyltin hydroxide (9.20 g, 0.0509 mole), 5-nitro-2-furoic acid (8.00 g, 0.0509 mole), and benzene (75 ml) was refluxed for 19 hr. The precipitate was collected on a filter paper and dried to give 15.30 g (94%) of VI*e*, mp 188.2–195.5°. Three recrystallizations from benzene gave the analytical sample, mp 190–192°.

Biological Methods—The compounds were individually dissolved in tetrahydrofuran. The preparation of sterile solutions of the compounds, the fungi employed, the antifungal testing procedures, and the determination of growth inhibition were reported previously (28).

The compounds were also investigated for antibacterial activity according to the procedure reported earlier (28).

[1] Melting points were determined with a Mel-Temp capillary melting point apparatus and are uncorrected. IR data were obtained with a Perkin-Elmer model 283 spectrophotometer. The far IR data were obtained with a Perkin-Elmer model FIS-3 spectrophotometer. The benzene used as a solvent in the synthesis of the organotin esters was dried over sodium ribbon. The water produced in the reactions was removed with the aid of a Dean-Stark trap. Elemental analyses were performed by Schwarzkopf Microanalytical Laboratory, Woodside, N.Y.

Table IV—Antibacterial Activity of Triorganotin 5-Nitro-2-furoates

Compound	*Pseudomonas aeruginosa*[a]			*Escherichia coli*			*Bacillus megaterium*			*Staphylococcus aureus*		
	1[b]	10	100	1	10	100	1	10	100	1	10	100
VI*a*	–	–	–	–	–	–	2+	2+	2+	2+	2+	2+
VI*b*	–	–	+	–	–	2+	2+	2+	2+	2+	2+	2+
VI*c*	–	–	–	–	–	–	+	2+	2+	+	2+	2+
VI*d*	–	–	–	–	–	–	+	2+	2+	–	2+	2+
VI*e*	–	–	2+	–	–	2+	+	+	+	–	–	+

[a] Bacteria were obtained from the culture collection of the Department of Biological Sciences, St. John's University. [b] 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.

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Morphine Pharmacokinetics: GLC Assay *versus* Radioimmunoassay

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Abstract □ The validity of a radioimmunoassay (RIA) for research on the pharmacokinetics of morphine has been questioned because of the possible measurement of cross-reactive metabolites. An RIA using antiserum derived from the 3-*O*-carboxymethylmorphine hapten was compared with a specific GLC assay in the measurement of plasma morphine concentrations in humans. The ratio of values for morphine concentrations measured using RIA and those measured using GLC was determined. The RIA values resulted in a 27% overestimation of this ratio. This overestimation did not significantly affect the values for terminal elimination half-life, volume of distribution at steady state, or total body clearance that were derived using results from each assay and model-independent pharmacokinetic techniques.

Keyphrases □ Morphine—pharmacokinetic determination from radioimmunoassay and GLC assay compared □ Pharmacokinetics—morphine, determination from radioimmunoassay and GLC assay compared □ Radioimmunoassay—morphine, comparison with GLC assay, pharmacokinetics □ GLC—morphine, comparison with radioimmunoassay, pharmacokinetics

The radioimmunoassay (RIA) for morphine, first described by Spector *et al.* (1, 2), has been used to characterize the pharmacokinetic profile of morphine (3, 4). A major concern in using any immunoassay for pharmacokinetic research is its accuracy in measuring the true drug concentration. For antibody generated in one laboratory (at morphine concentrations of 20 ng/ml), at least eight times more morphine-3-glucuronide than morphine was required to produce equivalent displacement of the labeled dihydromorphine (5). At 40 ng/ml, more than 32 times more morphine-3-glucuronide was required for an equivalent displacement. Similar results were obtained when relatively high morphine concentrations (1.8–3.1 μg/ml) were measured in rats using the RIA and a specific fluorometric assay (6). This concentration range markedly exceeds that occurring after therapeutic doses in humans. Catlin (7) questioned the validity of RIA for pharmacokinetic analysis, demonstrating a variability in the specificity of the antibody and interference from morphine metabolites that can result in discrepant interpretations.

Because of the limited sensitivity of currently available analytical methods for measuring morphine, it previously has not been possible to validate the accuracy of RIA for morphine in humans because of the low plasma concentrations (1–50 ng/ml) of morphine attained following pharmacological doses of morphine (0.15 mg/kg). A new specific and sensitive GLC assay was used to reevaluate plasma samples of morphine obtained in a previous pharmacokinetic study that were originally analyzed by RIA. The morphine concentrations measured by the different assays were then compared.

EXPERIMENTAL

Plasma Analysis—Plasma samples that were analyzed in a previous study on morphine pharmacokinetics using RIA were reevaluated using a specific GLC morphine assay. The analysis was undertaken on five of the six subjects who had received 10 mg of intravenous morphine sulfate and on four of the five subjects who had received 10 mg of intramuscular morphine sulfate. All samples had remained frozen at −30° until the time of assay. The GLC assays were performed ~2 years after the RIA measurements. There was no evidence of sample deterioration during this time. Demographic characteristics of the patient population and the drug administration protocol were described earlier (4).

Morphine concentrations in plasma were quantitated using RIA and rabbit antimorphine antisera[1] as described previously (4). The detection limit of this assay was 1 ng/ml, and the pooled coefficient of variation was 8.3% for a series of two to three identical samples containing known concentrations of morphine ranging from 1.0 to 45 ng/ml. Although the specificity of the antisera was not assessed, previous reports of antisera from the same source described its relative affinity for morphine, morphine metabolites, and other opiate alkaloids (5). Morphine concentrations in plasma were also determined using the GLC method described by Edlund (8). The detection limit of the assay was 1 ng/ml. The coefficient of variation of the assay was 4 and 10% at 62 and 0.8 ng/ml, respectively.

Data Analysis—Three analyses were performed on the data to determine differences between the two assays and the consequences of these differences on the derived pharmacokinetic values for morphine. The first analysis used linear regression through the origin (9) to compare the morphine concentrations measured with the two assays. Only plasma morphine concentrations <60 ng/ml were used, which included 118 of 138 possible data pairs. The distribution of the 20 data points in the 70–350-ng/ml concentration range was not sufficiently uniform for accurate regression analysis. The excluded data points represented the high morphine concentrations that occurred immediately after the 2-min rapid intravenous infusion. To determine the possible influence of metabolites on the RIA at low concentrations, linear regression through the origin was performed on only those concentrations measured 1 hr after drug administration. In both regression analyses, the 95% confidence interval of the slope was computed to determine if the slope differed significantly from 1.

In the second data analysis, the relative precision and bias of the RIA, as compared with the GLC morphine assay, were determined using approaches suggested previously (10). Relative precision measures the deviation or prediction error of the morphine concentration determined using the RIA compared to the value measured by GLC. This prediction error may have a systematic component called relative bias. The relative bias is the degree to which the typical RIA prediction is either too high

[1] Obtained from Dr. Sidney Spector, Roche Institute of Molecular Biology, Nutley, N.J.

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