CHAPTER II

EXPERIMENT

2.1 Instruments

1. Infrared Spectrophotometer, Nicolet Impact 410
2. Nuclear Magnetic Resonance Spectometer, Bruker ACF200
3. CHNS/O Analyser, Perkin Elmer PE2400 SeriesII
4. X-Ray Fluorescent Spectrophotometer, Oxford ED2000
5. Refractometer, ATAGO NAR-1T

2.2 Reagents

1. Bis(tri-n-butyltin)oxide (AR. grade , Fluka)
2. Monoethanolamine (AR. grade , Fluka)
3. Diethanolamine (AR. grade , Fluka)
4. Ethylene glycol (Laboratory grade)
5. 1,3-propanediol (AR. grade , Fluka)
6. Triethylene glycol (AR. grade , Fluka)
7. Tetraethylene glycol (AR. grade , Fluka)
8. Toluene, dried with sodium, distilled at its boiling temperature and kept in closed containers.

2.3 Synthesis of Tributyltin Compounds

2.3.1 Synthesis of 2-[(Tributyltin) oxyl ethanamine.(cpd.1)

Monoethanolamine 1.00 g. (0.016 mole) , bis(tributyltin) oxide 4.88 g. (0.008 mole) and anhydrous toluene (80 ml.) were placed in a 100 ml. round - bottomed flask equipped with a magnetic stirrer, Dean-Stark equipment for water trapping and a condenser with a drying tube. The reaction mixture was stirred at reflux temperature (110 - 115 $^{\circ}$C) for three hours until the water was separated completely by formation of an azeotropic mixture with toluene. The toluene was then removed by distillation under reduced pressure using a prolonged pumping. The product was obtained as yellow viscous oil, after removal of the solvent. (5.56 g ; 99.28% Yield)

Elemental analysis (%) found : C 47.93, H 9.49, N 4.11, Sn 33.31, Calcd for $C_{14}H_{33}ONSn$: C 48.03, H 9.50, N 4.00, Sn 33.90.

FT - IR spectrum (neat) ν_{max} (cm^{-1}) 3350, 3295 (w), 2950-2800 (s) 1490 (m), 1080 (m), 680,710 (m), 490 (w) (Fig. 4)

^{1}H - NMR spectrum ($CDCl_3$) δ (ppm.) 0.70 - 1.75, 2.67, 3.65 (Fig. 5)

^{13}C - NMR spectrum ($CDCl_3$) δ (ppm.) 13.67, 14.82 (^{1}J(C-Sn) = 196.29 Hz) , 27.04, 28.02 (^{2}J(C-Sn) = 65.43 Hz) , 46.15, 68.25, (Fig. 6)

2.3.2 Synthesis of 2-[N-[(Tributyltin) oxylethyll amino] ethanol (cpd.2)

The synthesis was carried out by a procedure similar to that used for the synthesis of 2-[(Tributyltin) oxy] ethanamine ,but using 1.00 g. (0.0095 mole) diethanolamine and 2.83 g. (0.0047 mole) of bis(tributyltin) oxide to obtain 3.48 g. (98.86% Yield) of the title compound as light yellow vicous oil after removal of the solvent.

Elemental analysis (%) found : C 45.11, H 10.08, N 3.75, Sn 32.60, Calcd for $C_{14}H_{37}O_2NSn$: C 45.43, H 10.08 N 3.78, Sn 32.70.

FT - IR spectrum (neat) ν_{max} (cm^{-1}) 3280(br), 3303 (w), 2960-2800(s), 1502,1495 (s), 1090(m), 690,715 (s), 485 (w) (Fig. 7)

^{1}H - NMR spectrum ($CDCl_3$) δ (ppm.) 0.70 - 1.75, 2.68, 2.24, 3.61, 3.27 (Fig. 8)

^{13}C - NMR spectrum ($CDCl_3$) δ (ppm.) 13.63, 14.46 (^{1}J(C-Sn) = 251.65 Hz), 27.18, 27.98 (^{2}J(C-Sn) = 56.73Hz), 52.97, 54.19, 60.91, 65.22, (Fig. 9)

2.3.3 Synthesis of 2-[(Tributyltin) oxy] ethanol (cpd.3)

A similar procedure to that for the synthesis of 2-[(Tributyltin) oxy] ethanamine was used for this synthesis but using 1.00 g. (0.016 mole) of ethylene glycol and 4.80 g. (0.008 mole) of bis(tributytin) oxide to obtain 5.57 g. (99.11% yield) of the title compound as colorless viscous oil.

Elemental analysis (%) found : C 48.57, H 8.73, Sn 33.09, Calcd for $C_{14}H_{32}O_2Sn$: C 47.90, H 9.19, Sn 33.80.

FT - IR spectrum (neat) ν_{max} (cm^{-1}) 3250 (br), 2850-2800 (s) 1470(s), 1080 (m), 1035(m), 690,715 (s), 480(m) (Fig. 10)

^{1}H - NMR spectrum ($CDCl_3$) δ (ppm.) 0.70 - 1.75, 2.98, 3.55, 3.64, (Fig. 11)

^{13}C - NMR spectrum ($CDCl_3$) δ (ppm.) 13.85, 14.84 (^{1}J(C-Sn) = 166.09Hz), 27.02, 27.97 (^{2}J(C-Sn) = 70.46Hz), 65.03, 66.68, (Fig. 12)

2.3.4 Synthesis of 3-[(Tributyltin) oxyl propanol (cpd.4)

A similar procedure to that for the synthesis of 2-[(Tributyltin) oxyl ethanamine was used for this synthesis, but using 1.00 g. (0.013 mole) of propylene glycol and 3.92 g. (0.0065 mole) of bis(tributytin) oxide to obtain 4.68 g. (98.53% yield) of the title compound as colorless viscous oil.

Elemental analysis (%) found : C 49.98, H 9.79, Sn 31.94, Calcd for $C_{15}H_{34}O_2Sn$: C 49.40, H 9.39, Sn 32.51.

FT - IR spectrum (neat) ν_{max} (cm^{-1}) 3360(br), 2980-2790 (s), 1480 (s), 1050 (s), 1010(w), 695,710 (m), 485 (m) (Fig. 13)

^{1}H - NMR spectrum ($CDCl_3$) δ (ppm.) 0.70 - 1.75, 3.81 3.93, 4.39, (Fig. 14)

^{13}C - NMR spectrum ($CDCl_3$) δ (ppm.) 13.65, 14.48 (^{1}J(C-Sn) = 183.70 Hz), 27.10, 27.91 (^{2}J(C-Sn) = 62.91Hz), 35.32, 64.04, 67.45,(Fig. 15)

2.3.5 Synthesis of 2-[(Tributyltin)oxybis(ethyleneoxy)] ethanol (cpd.5)

The synthesis was carried out by a procedure similar to that used for the synthesis of 2-[(Tributyltin) oxy] ethanamine, but using 1.00 g. (0.007 mole) of triethylene glycol and 1.98 g. (0.0033mole) of bis(tributyltin) oxide and used refluxing time for five hours. This reaction obtained 3.03 g. (98.70% yield) of the title compound as colorless vicous oil after removal of the solvent.

Elemental analysis (%) found : C 48.87, H 9.72, Sn 26.45, Calcd for $C_{18}H_{40}O_4Sn$: C 49.26, H 9.12, Sn 27.04.

FT - IR spectrum (neat) ν_{max} (cm^{-1}) 3350(br), 2980-2820 (s), 1485(m), 1120 (m), 1070 (m), 1030 (w), 685,715 (m), 475(w) (Fig. 16)

^{1}H - NMR spectrum ($CDCl_3$) δ (ppm.) 0.70 - 1.75, 3.58, 3.64, 3.71 (Fig. 17)

^{13}C - NMR spectrum ($CDCl_3$) δ (ppm.) 13.91, 15.04 (^{1}J(C-Sn) = 177.16Hz), 27.21, 28.01 (^{2}J(C-Sn) = 64.42 Hz), 61.57, 65.49, 70.78, 74.95, (Fig. 18)

2.3.6 Synthesis of 2-[(Tributyltin)oxytris(ethyleneoxy)] ethanol (cpd.6)

The Synthesis was carried out by a procedure similar to that used for the synthesis of 2-[(Tributyltin)oxybis(ethyleneoxy)] ethanol, but using 1.00 g. (0.005 mole) of tetraethylene glycol and 1.53 g. (0.0025 mole) of bis (tributyltin) oxide to obtain 2.37 g. (98.34% Yield) of the title compound as colorless vicous oil after removal of the solvent.

Elemental analysis (%) found : C 49.17, H 9.03, Sn 24.30, Calcd for $C_{20}H_{44}O_5Sn$: C 49.75, H 9.11, Sn 24.58.

FT - IR spectrum (neat) ν_{max} (cm^{-1}) 3365(br), 2970-2800 (s), 1495(m), 1100 (m), 1075 (m), 1025 (m), 690,710 (w), 470(w) (Fig. 19)

^1H - NMR spectrum ($CDCl_3$) δ (ppm.) 0.70 - 1.75, 3.45, 3.52 , 3.73, 4.62 (Fig. 20)

^{13}C - NMR spectrum ($CDCl_3$) δ (ppm.) 13.55, 14.83 (^1J(C-Sn) = 193.77 Hz), 27.07, 27.96 (^2J(C-Sn) = 60.90 Hz), 61.14, 65.08, 69.98, 70.37, 72.90, 75.01, (Fig. 21)

2.4 Preparation and Analysis of Aqueous Solutions of Tributyltin Compound. [6]

Solutions of the tributyltin compounds which were synthesized in 2.3, were prepared by stirring the tin compounds in water as described below.

Tributyltin compound 2.50 g. and 50 ml. distilled water were placed in 250 ml. erlenmeyer flask and stirred with a magnetic stirrer for 1 hour at room temperature. The resulting mixture was allowed to settle overnight (24 hr.) and aqueous supernatant solution was then removed. The aqueous organotin concentration was determined by X-Ray Fluorescent technique.

2.5 The Fungitoxicity Testing of Tributyltin Compounds by the Agar Dilution Method.

2.5.1 Materials

Tributyltin compound solutions : diluted by 2 fold dilution method.

Disks : No. 1 Whatman filter paper was used for preparing the disks with 6 mm. diameter and sterilized. Each of the dissolved tributyltin compounds was loaded onto the disks at various concentration. When dyed, the disks were stored at $4^{\circ}C$.

Fungi : *Aspergillus* sp., and *Penicillum* sp. : From the department of Microbiology, Faculty of Science, Chulalongkorn University.

Trichoderma Reesei. : From Thailand Institute of Scientific and Technological Research.

2.5.2 Method [9]

The spore of test fungi were picked by loop and suspended into 10.0 ml. sterile water which placed in a test tube. The number of spore was counted for about 3×10^{7} spore/ml. by using a haemacytometer. A suspended spore of fungi was spread on petri - plates (100 mm.) containing

Potato Dextrose Agar (PDA) and allowed to dry for 15 min. The disks were then applied and the plates were incubated at room temperature for 7 and 30 days. The diameters of the clear zones around the disks were measured with a ruler. Two readings were taken for each disk at right angles, and two disks on separate plates were used for each tributyltin compounds and averaged to determine the minimal inhibitory concentration (MIC).