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Resolution and Biological Activity of the Optical Isomers of 3-*tert*-Butylphenyl *sec*-Butylcarbamate

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There have been several reports on the difference in biological activities of optical isomers of pesticides molecules with asymmetric carbon. Fukuto et al. (1964) reported the effect of chirality on the biological activities of carbamate insecticides and the (-)-isomer of 2-sec-butylphenyl methylcarbamate was 3 to 8 times more toxic to houseflies and mosquito larvae than (+)-isomer. This result was directly related to the inhibition of acetylcholinesterase activity. Moreover, (-)-miotine (2-dimethylaminoethylphenyl methylcarbamate), not pesticide, was 2 to 10 times more toxic to a variety of test animals than the (+)-isomer (White and Stedman, 1937).

It is generally found that N-methyl or N,N-dimethylcarbamate are better inhibitors than the carbamates with longer N-alkyl chain. Yu et al. (1972) reported that the cause of decrease in inhibitory potence for longer N-alkyl compounds was different for the acetylcholinesterase from different animals. It was also found that substituted phenyl propylcarbamate was a good inhibitor of acetylcholinesterase obtained from the carbamate resistant strain of green rice leafhopper (Yamamoto et al. 1977).

This report is concerned with the synthesis and examination of the biological activities of 3-tert-butylphenyl sec-butylcarbamate (TBPSBC) with an asymmetric carbon in N-alkyl group.

MATERIALS AND METHODS

NMR spectra were obtained a JEOL FX-90 Q spectrometer at 90 MHz in deuteriochloroform solution with tetramethylsilane as the internal standard. Chemical ionization mass spectra (CI-MS) were obtained with a Shimadzu LKB-9000 B mass spectrometer with isobutane as the reagent gas. Optical rotations were measured at 20°C in toluene or methanol with a cell path length of 100 mm by using a Jasco DIP-140 polarimeter at the sodium line (589 nm). Thin-layer chromatography (TLC) was performed on silica gel 60 GF_{254} chromatoplates (E. Merck, Germany) and visualized by spraying 0.1 % (w/v) methanolic p-nitrobenzenediazonium fluoborate solution after exposing to 0.1 N aqueous potassium hydroxide.

Optical isomers of 3-tert-butylphenyl sec-butylcarbamate (TBPSBC) were synthesized by using the resolved (+)- or (-)-sec-butylamine which were obtained as a salt of tartaric acid by the method of Bruck et al. (1956). Namely, after racemic sec-butylamine was treated with an equivalent of (+)- or (-)-tartaric acid, each diastereomer was recrystallized from water and the resolved sec-butylamine was obtained by treatment with sodium hydroxide. $[\alpha]_D$ (c=1.35, in toluene, 20°C) of the isomers were + 7.20 and - 7.05, respectively.

3-tert-Butylphenol and dimethylaniline were added dropwise to a cold (0-5°C) toluene solution of phosgene which was generated by mixing trichloromethylchloroformate (supplied from Hodogaya Chemical Co., LTD) with charcoal at 40°C. After the removal of an excess of phosgene, (+)- or (-)-sec-butylamine resolved was added slowly to hemi-equi-molar amount of 3-tert-butylphenyl chloroformate in cold toluene (-10°C), and stirred for 4 hour at room temperature. The resultant carbamates were purified by preparative TLC with a mixture of n-hexane-acetone (5:1, Rf=0.45). $[\alpha]_D$ (c= 1.50, in methanol, 20°C) of carbamates synthesized were + 8.34 and - 8.15 for (+)- and (-)-isomer, respectively. Mass spectra (m/z) of both isomers were 250 (M + 1), 151 ($C_4H_9C_6H_4OH$ + 1, base peak) and 100 ($CONHC_4H_9$).

Insecticidal activity was determined with adzuki bean weevil (Callosobruchus chinensis L.) and carbamate susceptible and resistant straines of brown rice planthopper (Nilaparvata lugens Stål). For adzuki bean weevil, test tube method designed by Suwanai (1957) was utilized with a slight modification. A series of half-concentrations of insecticide was prepared by dissolving the test compound into acetone. Hundred µl acetone solution was poured into a test tube (17.6 x 0.7 cm) and acetone was evaporated spontaneously. After drying up, twenty 2-day old insects were put into each test tube. Test tubes were plugged with cotton and held at 25°C. For 3- to 4-day adult of brown rice planthopper, 0.5 µl of acetone solution containing carbamate was applied topically on the dorsal part of insects. Ten insects were used at each dose level and held at 25°C. Mortality was determined 48 hrs. after the treatment and LD_{50} values were estimated by the computer system designed for probit analysis from the result of three or four replications.

Fish toxicity was determined with topmouth gudgeon (Pseudorasbora parva). The test fish was kept in a laboratory for one month and food was given once a day until 48 hrs. before the experiment was started. The fish was 2.5 to 3.0 cm in length and 0.5 to 0.8 g in weight. Two hundred µl of acetone solution containing test compound was added into water in a 10 liters glass aquarium tank and stirred well. The temperature of the water was held at 22 ± 2°C during the experiment and mortality was determined 48 hrs. after the treatment. Four replications of 10 fishes were used each dose level.

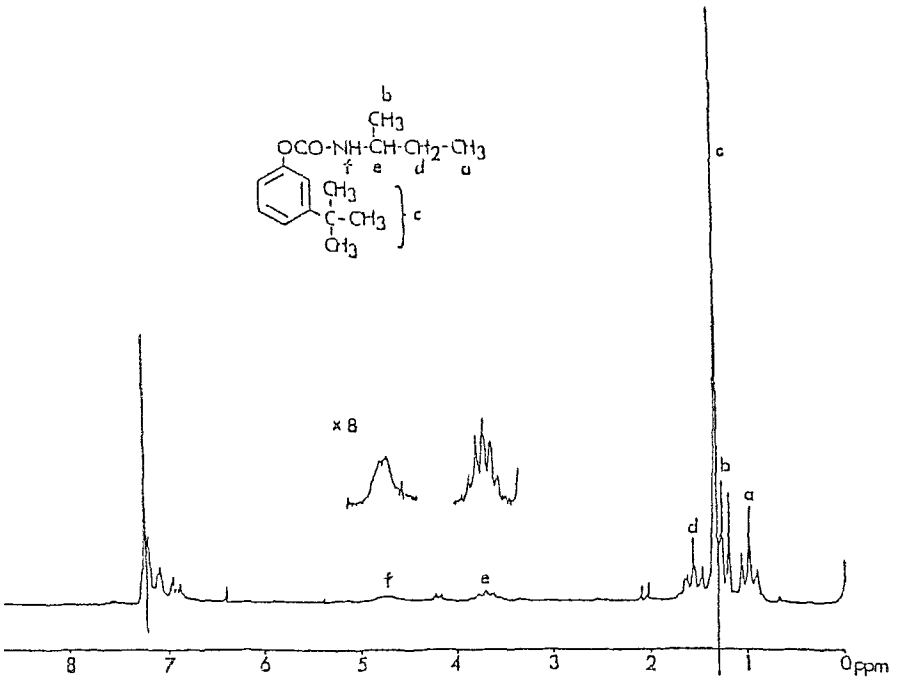

Figure 1. Proton NMR of racemate of 3-tert-butylphenyl sec-butylcarbamate

The molar concentration for 50 % inhibition (I_{50}) was determined for acetylcholinesterase of adzuki bean weevil. Adults of adzuki bean weevil 48 hrs. after emergence were homogenized with ten times volume of 0.1 M phosphate buffer (pH 7.4) and filtered through three fold gauze. The filtrate was used as an enzyme source. AChE activity was determined by the radiometric method of Siakotos et al. (1969), using [1-^{14}C]acetylcholine chloride (55.3 mCi/m mol) which was purchased from Amersham, England. The reaction mixture consisted of 0.3 ml of 0.1 M phosphate buffer, 0.3 ml of the enzyme solution, 0.3 ml of inhibitor solution dissolved in acetone-distilled water (1:10), 0.3 ml of 2.5 x 10^{-2}M [1-^{14}C]acetylcholine chloride and 0.3 ml of distilled water. After incubation at 37°C for 30 min, the reaction was stopped by addition of Amberlite CG-120 resin suspended in dioxane. Aliquots of dioxane were taken into vials for radioassay. After adding scintillation solution, radio activity was determined by Aloka LSC-673 liquid scintillation spectrometer.

RESULTS AND DISCUSSION

Proton NMR spectrum of TBPSBC synthesized was shown in Figure 1. The proton NMR signals were almost similar between racemate and two enantiomers of the compound. For assessment of the optical purity of the resolved compounds, NMR method is carried out by using the optically active europium chemicals for the shift reagent such as tris-[3-(heptafluoropropylhydroxymethylene)-(+)-cam-

Table 1. Proton NMR of methine of 3-tert-butylphenyl sec-butylcarbamate in the presence of shift reagent [Eu(Ⅲ)-TFMC]

Eu(Ⅲ)-TFMC (mg)		0	17.86	35.72	53.58
Molar ratio of Eu(Ⅲ)-TFMC to TBPSBC(%)		0	50.0	100.0	150.0
Chemical shift (ppm)	(+)-TBPSBC	3.77	4.20	4.52	4.71
	(-)-TBPSBC	3.77	4.15	4.45	4.64
Difference (ppm)		0	0.05	0.07	0.07

Table 2. Biological activity of optical isomers of 3-tert-butylphenyl sec-butylcarbamate (TBPSBC) and 3-tert-butylphenyl methylcarbamate (TBPMC)

		TBPSBC (+)	TBPSBC (-)	TBPSBC (±)	TBPMC
LD_{50} to adzuki bean weevil (μg/test tube)		430.1	911.9	548.7	5.6
LD_{50} to brown rice planthopper (μg/g insect)	susceptible	3.3	3.1	3.3	0.35
	resistant	10.9	10.1	11.1	0.70
TLm to topmouth gudgeon (ppm)		7.6	8.1	7.9	2.0
I_{50} for acetylcholinesterase of adzuki bean weevil (molar concen.)		7.16×10^{-3}	$>10^{-2}$	$>10^{-2}$	1.46×10^{-6}

phorate] europium (III) and tris-(heptafluorobutanoylpivaloylmetanate)europium. NMR signals of TBPSBC synthesized were shifted to a lower magnetic field in the presence of the shift reagent tris-[3-(trifluoromethylhydroxymethylene)-(+)-camphorate]europium(III) [Eu(III)-TFMC]. As shown in Table 1, the chemical shift differences of the methine carbon connected with the amino group were obtained between enantiomers, but the differences were small and the NMR analysis for measuring optical purity was less satisfactory. In the presence of 0.5 molar equivalent of Eu(III)-TFMC, the enantiomers of alanine methyl ester showed readily observable shift difference, and the shift difference of the methine carbon was 0.24 ppm (Ajisaka et al. 1972).

Table 2 shows the toxicity of the optical isomers to insects and fish. Although the (+)-isomer showed slightly more toxic than the (-)-isomer at LD_{50} to adzuki bean weevil, the difference of LD_{50} to brown rice planthopper and TLm to topmouth gudgeon between the enantiomers was not significant. Compared with the biological activity of 3-tert-butylphenyl methylcarbamate(TBPMC) which was good toxicant to sheep blowflies and mice (Fraser et al. 1967), the activity of TBPSBC is lower than that of TBPMC. Moreover, TBPSBC was considerably poor inhibitor of acetylcholinesterase. I_{50} values of racemate and (-)-isomer were above 10^{-2} M, because the I_{50} values could not determine for the insolubility of TBPSBC synthesized. While, I_{50} value of TBPMC was 1.46×10^{-6} M. It may be concluded that the biological activities of TBPSBC was weak inherently.

On the toxicological properties of organophosphorus esters containing asymmetric carbons, Hassan et al. (1968) reported that the (+)-carbon isomers of both malathion and malaoxon were only 2 fold more toxic to mice and houseflies. Wustner et al. (1973) reported that, of the two asymmetric centers in O-2-(ethylthio)ethyl ethylphosphonothioate, the (-)-phosphorus isomer was much more as toxicants to houseflies, mosquito larvae and mice than the (+)-compound, but toxicity of optical isomers of carbon was not largely different between enantiomers. The optical isomer of O,O-dimethyl S-(α-ethoxycarbonylbenzyl)phosphorothioate which contain an asymmetric carbon showed a marked selectivity in the degree of toxicity to various insects and mice (Ohkawa et al. 1976). Further study is needed for the biological effect of optical isomer synthesized to different animals, insects and plants.

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