Original Articles

Stereospecificity in Oxidation of the Optical Isomers of *O*-Ethyl *O*-2-Nitro-5-Methylphenyl *N*-Isopropyl Phosphoramidothioate (S-2571) by Liver Mixed Function Oxidase and UV Light

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It was confirmed that the enantiomers in racemic S-2571 (*O*-ethyl *O*-2-nitro-5-methylphenyl *N*-isopropyl phosphoramidothioate) were stereoselectively metabolized by rabbit liver microsomal mixed function oxidase to give dextrorotatory products of parent compound, the oxon analog and derivatives hydroxylated at the 5-methyl group. The corresponding products obtained from (+)-S-2571 were all dextrorotatory and those from (–)-S-2571 were levorotatory. Oxidation of the optical isomers of S-2571 to the corresponding oxon analogs by UV light was also stereospecific. Comparison with the results from *m*-chloroperoxybenzoic acid oxidation suggests that oxidation of P=S to P=O by the mixed function oxidase and UV light may proceed with retention of configuration at phosphorus.

INTRODUCTION

The mixed function oxidase system plays an important role in metabolism of organophosphorus compounds in living organisms.^{1,2)} The enzyme system is located in the microsomal fraction of animal tissue. The reactions mediated by the enzyme system include: (1) oxidation of thiophosphoryl (P=S) compounds to the corresponding phosphoryl (P=O) compounds, (2) cleavage of P–O–aryl linkage and (3) hydroxylation at alkyl and aryl groups. The oxidation of P=S to P=O also occurs by UV light.³⁾

Mechanism of the oxidation of P=S to P=O compounds by enzymatic and model oxidation systems were extensively studied.^{4,5)} Stereochemical studies showed that the oxidation of P=S to P=O with *m*-chloroperoxybenzoic acid proceeds with a high degree of retention of configuration at phosphorus.⁶⁾ In addition, Lee *et al.*⁷⁾ reported that the oxidation of

fonofos to fonofos oxon by mouse liver microsomal mixed function oxidase proceeds with retention at phosphorus. A preliminary report described that racemic S-2571 (*O*-ethyl *O*-2-nitro-5-methylphenyl *N*-isopropyl phosphoramidothioate) was stereoselectively metabolized by rabbit liver mixed function oxidase to give dextrorotatory products of the parent compound, the oxon analog and hydroxylation derivatives at the 5-methyl group.⁸⁾

This paper is concerned with additional studies made to define the stereospecificity in oxidations of the resolved isomers of S-2571 by rabbit liver mixed function oxidase as well as UV light.

MATERIALS AND METHODS

1. Spectroscopy

Nuclear magnetic resonance spectra (nmr) were determined in deuteriochloroform using tetramethylsilane as internal standard, on a Hitachi R-20B spectrometer at 60 MHz, Elec-

tron impact mass spectra (ms-ei) were obtained with a Shimadzu-LKB 9000 mass spectrometer. Samples were analyzed at 12 and 70 eV by means of a direct insertion probe. Chemical ionization mass spectra (ms-ci) were also obtained with the same spectrometer equipped with a chemical ionization source using isobutane as the reactant gas. Infrared spectra (ir) were recorded on a Hitachi EPI-G3 spectrometer. Samples were analyzed as a thin film on a NaCl crystal. Optical rotation were measured with a Perkin-Elmer 141 polarimeter in chloroform (1 dm cell). Circular dichroism (CD) and optical rotatory dispersion (ORD) were recorded with a model UV-5 optical rotatory dispersion recorder (Japan spectroscopic Co., Ltd., Tokyo, Japan) as a 0.1% (W/V) ethanol solution.

2. Chromatography

Thin-layer chromatography (tlc) utilized precoated silica gel 60F-254 chromatoplates (with fluorescent indicator, 20×20 cm, 0.25 mm gel thickness, E. Merck) for analytical and preparative purposes and silica gel 60 chromatoplates (without fluorescent indicator, 20×20 cm, 0.25 mm gel thickness, E. Merck) for photooxidation study. Solvent systems used in TLC and R_f values for a variety of S-2571 derivatives are given in Table 1. Radioactive spots on TLC plates were detected by radio-

autography and TLC radioscanning with a model TRM-1B TLC radioscanner (Aloka, Tokyo, Japan). Percent distribution of radioactive spots was determined by scraping gel regions from the chromatoplates into scintillation vials and counting them with a liquid scintillation spectrometer. Unlabeled reference compounds were visualized as follows; (1) ultraviolet fluorescence quenching, (2) spraying 10% KOH in methanol (nitrophenols) and (3) spraying 1% palladium chloride in 1N HCl (sulfur compounds).

The amount of S-2571 was also determined by gas-liquid chromatography (GLC) using a Shimadzu GC-3AF gas chromatograph equipped with a flame ionization detector. The chemical was analyzed on a glass column (3.2 feet×0.5 cm i.d.) packed with 5% DC 200 and 10% QF-1 on Chromosorb W (60–80 mesh). The GLC operating conditions were; column temperature, 235°C; inlet pressure of 1.05 kg/cm², 0.04 kg/cm² and 0.04 kg/cm² for N₂, H₂ and air, respectively. Internal standard for this system was Cremart (*O*-ethyl *O*-2-nitro-5-methylphenyl *N*-*sec*-butyl phosphoramidothioate). The R_f values for S-2571 and Cremart were 3.8 min and 4.5 min, respectively.

3. Radioanalysis

The radioactivity of organosoluble fractions from extraction of incubation mixtures and gel regions from TLC plates was determined with a Packard Tri-carb Liquid Scintillation Spectrometer model 3385 using 15 ml of a dioxane scintillation fluid (5 g of 2,5-diphenyloxazole and 100 g of naphthalene in 1 liter of dioxane). Industrial X-ray 150 films (Fuji Photo Film Co., Ltd., Tokyo, Japan) were used for radioautography. The developed TLC plates were exposed to films and held at 0° to 4°C for about one month.

4. Chemicals

The racemic form of S-2571 (*O*-ethyl *O*-2-nitro-5-methylphenyl *N*-isopropyl phosphoramidothioate) labeled with ³H at phenyl protons was synthesized by Makari *et al.*⁹⁾ This preparation had a specific activity of 14.1 mCi/mmol and a radiochemical purity of more than 99% based on TLC analysis.

The (+)- and (–)-isomers of S-2571 were

Table 1 TLC solvent systems and R_f values for S-2571 and its degradation products.

Product	R_f values with indicated solvent systems ^{a)}		
	A	B	C
I S-2571	0.55	0.65	
II S-2571-oxon	0.30	0.41	
III S-2571-CH ₂ OH	0.15	0.32	
IV S-2571-oxon-CH ₂ OH	0.08	0.15	0.20
V S-2571-CHO	0.47	0.53	
VI 2-Nitro-5-methylphenol	0.10	0.70	
VII 2-Nitro-5-hydroxy-methylphenol	0.60	0.25	0.78

^{a)} The TLC solvent systems are as follows; A, *n*-hexane-acetone (5/2); B, *n*-hexane-toluene-acetic acid (3/15/2); C, ethyl acetate.

prepared by the methods reported previously.⁸⁾ The enantiomers of *O*-ethyl *O*-hydrogen *O*-2-nitro-5-methylphenyl phosphorothioic acid were resolved by recrystallization of the diastereomeric brucine salts. Each of the resolved phosphorothioic acids was chlorinated with phosphorus pentachloride, followed by treating with isopropylamine, to give optically active S-2571; (+)-S-2571, $[\alpha]_D^{20} +54.5^\circ$ (c 6.0), (–)-S-2571, $[\alpha]_D^{20} -54.7^\circ$ (c 8.2).

S-2571 contains nitro and phenyl chromophore absorbings at ca, 260 and 330 nm, respectively in the molecule. The ORD curve of (+)-S-2571 showed a peak ($\Phi = -2 \times 10^3$) at 385 nm and a trough ($\Phi = 4.8 \times 10^3$) at 240 nm. (–)-S-2571 showed the opposite curve. The CD curve of (+)-S-2571 was characterized by a positive Cotton effect ($\theta = 4.5 \times 10^3$) at 330 nm and a negative Cotton effect ($\theta = -5.5 \times 10^3$) at 260 nm. (–)-S-2571 showed the opposite Cotton effects at 260 and 330 nm, respectively.

The enantiomers of the oxon analog (S-2571-oxon) were each prepared from the corresponding S-2571 isomers by oxidation with *m*-chloroperoxybenzoic acid according to the method of Herriot,⁶⁾ as described below. *m*-Chloroperoxybenzoic acid (Wako Purechemical Industries, Osaka, Japan, 213 mg) was added to a stirring solution of (+)-S-2571 or (–)-S-2571 (300 mg) in 5 ml of dichloromethane over 30 min period at 0°C. The reaction mixture was concentrated to about 20% of the original volume and then cooled to partially precipitate *m*-chlorobenzoic acid. The dichloromethane soluble material was purified by preparative tlc in *n*-hexane-acetone (5/2). The structure of the products was confirmed by nmr, ir and ms. Nmr, δ 1.20 [9H, m, CH₃, CH(CH₃)₂], 2.38 (3H, s, CH₃), 3.30 (1H, m, NH), 3.50 (1H, m, CH) and 7.00–7.75 ppm (3H, m, aromatic); ir, 3230 (NH), 1600 and 1490 (aromatic ring), 1520 and 1360 (NO₂), 1270 (P=O), 1240 (P–O-aryl), and 1040 cm^{–1} (P–O-alkyl); ms-ei, *m/e* 287 (M–CH₃), 244 (M–NHCH(CH₃)₂, base peak), 216, 176 and 136. No impurities were detected by nmr and tlc.

O-Ethyl *O*-2-nitro-5-hydroxymethylphenyl *N*-isopropyl phosphoramidothioate (S-2571–CH₂OH) was synthesized in the following. *O*-Ethyl phosphorothionylchloride (1.79 g) was

dropwisely added to a mixture of 1.69 g of 2-nitro-5-hydroxyphenol and 1.2 g of triethylamine in 20 ml ether at 0°C. Stirring was continued for 3 hr and then 1 N HCl was added to the reaction mixture to neutralize excess triethylamine. The reaction mixture was concentrated and dropwisely added to a solution of 3 g of isopropylamine in ether at 0°C. After 3 hr stirring, excess isopropylamine was neutralized by adding 1 N HCl and the ether layer was dried over anhydrous Na₂SO₄. After concentration, the product was purified by preparative tlc in *n*-hexane-acetone (5/2). The yield was 1.2 g. The structure was confirmed by nmr, ir and ms. Nmr, δ 1.0–1.5 [9H, m, CH₃, CH(CH₃)₂], 3.3–3.9 (3H, m, OH, CH, NH), 4.15 (2H, q, CH₂), 4.70 (2H, s, CH₂–OH), and 7.2–7.8 ppm (3H, m, aromatic); ir, 3400 (OH, NH), 1600 and 1490 (aromatic ring), 1530 and 1350 (NO₂), 1250 (P–O-aryl), and 1030 cm^{–1} (P–O-alkyl); ms-ci, *m/e* 355 (M+1).

O-Ethyl *O*-2-nitro-5-hydroxymethylphenyl *N*-isopropyl phosphoramidate (S-2571-oxon–CH₂OH) was prepared from S-2571–CH₂OH by oxidation with *m*-chloroperoxybenzoic acid, as with S-2571-oxon. The product was purified by preparative tlc in ethylacetate. Nmr, δ 1.0–1.45 [9H, m, CH₃, CH(CH₃)₂], 3.0–3.8 (3H, m, OH, NH, CH), 4.20 (2H, m, CH₂), 4.70 (2H, s, CH₂OH) and 7.2–7.9 ppm (3H, m, aromatic); ir, 3350 (NH, OH), 1600 and 1590 (aromatic ring), 1520 and 1350 (NO₂), 1250 (P=O), 1230 (P–O-aryl) and 1030 cm^{–1} (P–O-alkyl); ms-ci, *m/e* 303 (M–CH₃), 260 [M–NHCH(CH₃)₂, base peak], 232, 192, and 152.

O-Ethyl *O*-2-nitro-5-formylphenyl *N*-isopropyl phosphoroamidothioate (S-2571–CHO) was prepared by reaction of *O*-ethyl phosphorothionylchloride with 3-hydroxy-4-nitrobenzaldehyde, followed by reaction with isopropylamine, as described for S-2571–CH₂OH. The obtained product was purified by preparative tlc in *n*-hexane-acetone (5/2). Nmr, δ 1.1–1.5 [9H, m, CH₃, CH(CH₃)₂], 3.4–3.9 (2H, m, CH, NH), 4.2 (2H, q, CH₂), 7.6–8.2 (3H, m, aromatic), and 10.0 ppm (1H, s, CHO); ir, 3350 (NH), 1705 (CHO), 1600 and 1500 (aromatic ring), 1590 and 1350 (NO₂), 1250 (P–O-aryl), 1030 cm^{–1} (P–O-alkyl); ms-ci, *m/e* 333 (M+1).

5. Photodecomposition

Each S-2571 isomer (120 mg) dissolved in 20 ml ether was evenly applied to 2 tlc plates (20×20 cm, without fluorescent indicator) by a brush. After evaporation of the ether, the plates were exposed to ultraviolet (UV) light for 2 hr at a distance of 6 cm below a 172.5 watt high pressure UV lamp (Taika Kogyo, Osaka, Japan). After exposure, the silica gel was scraped free from the plates, and the products were extracted with acetone. After concentration of the acetone solution, individual products were separated by preparative tlc in *n*-hexane-acetone (5/2) and then the appropriate gel regions were extracted with acetone. The remaining parent compound was analyzed by glc. Each product was identified by tlc co-chromatography with the authentic standards and optical rotations were determined.

6. Metabolism by Rabbit Liver Mixed Function Oxidase

The liver microsomal fraction was prepared from male rabbit (Albino Native Japanese strain, 2 kg). A 20% (W/V) rabbit liver homogenate in 1.15% KCl was centrifuged at 9,000 g for 20 min to obtain the 9,000 g supernatant fraction, which was in turn centrifuged at 105,000 g for 60 min to sediment microsomes. The microsomal pellet was washed once by resuspension in the same solution and recentrifugation. The incubation mixtures in 5 ml of 0.05 M *tris*-HCl buffer, pH 7.5, contained microsomes equivalent to 400 mg fresh liver weight, 5.5 μ mol reduced nicotinamide adenine dinucleotide phosphate (NADPH, Oriental Yeast Co., Ltd., Tokyo, Japan) and 0.5 μ mole substrate added last in 50 μ l acetone. The mixtures were incubated with shaking at 37°C for 1 hr in 50 ml open Erlenmeyer flasks. A total of 200 to 400 separate incubations was carried out for metabolite preparation. After incubation, each mixture was adjusted to pH 1–2 by addition of 1 N HCl and 1 g of (NH₄)₂SO₄ was added. The mixtures were extracted three times with chloroform. The combined extracts were concentrated and analyzed by tlc in *n*-hexane-acetone (5/2). Each isolated metabolite was identified by tlc cochromatography with authentic standards and by ms-ci. The optical rotations were also determined.

For the comparison of metabolic rate of racemic, (+)- and (–)-S-2571, each chloroform extract was subjected to preparative tlc and developed in solvent system B. The remaining unchanged S-2571 was extracted from the appropriate gel regions with acetone. The acetone solution was concentrated, the residue was dissolved in a small volume of acetone, and aliquots of the solution were subjected to glc.

RESULTS

1. Oxidation of the Optical Isomers of S-2571 with *m*-Chloroperoxybenzoic Acid

Each of the S-2571 isomers was quantitatively and stereospecifically converted to the corresponding S-2571-oxon isomers by *m*-chloroperoxybenzoic acid oxidation. (+)-S-2571 produced dextrorotatory S-2571-oxon in a high yield and (–)-S-2571 gave the levorotatory oxon analog. Although the absolute configuration of the optical isomers of S-2571 and S-2571-oxon were not determined, oxidation of the S-2571 isomers to the corresponding oxon analogs with *m*-chloroperoxybenzoic acid appears likely to proceed with a high degree of retention at phosphorus, as reported by Herriot.⁶⁾

2. Photooxidation of the Racemic, (+)- and (–)-Forms of S-2571

When deposits of racemic ³H-S-2571 on silica gel chromatoplates at the rate of 0.25 mg/cm² were irradiated with UV light, the radioactivity readily decreased from the treated chromatoplates. After 120 min, 53% of the applied radioactivity remained on the tlc plate. Of these, 28.3, 18.6, 1.3 and 4.8% of the applied accounted for S-2571, S-2571-oxon, 2-nitro-5-methylphenol and others, respectively. These photoproducts were identified by tlc cochromatography with the authentic standards in solvent systems A and B.

Subsequently, each S-2571 isomer (120 mg) was applied to 2 chromatoplates and then irradiated with UV light for 2 hr. The optical rotation and approximate yield of the recovered S-2571 and S-2571-oxon are presented in Table 2. S-2571 and S-2571-oxon recovered from racemic S-2571 were optically inactive. On the other hand, the corresponding products

Table 2 The specific rotation and optical purity of S-2571 and its photoproducts recovered after irradiation with UV light on silica gel tlc plates.

Starting material	Photoproduct	Yield (mg)	$[\alpha]_D^{25}$	Optical purity (%)
(±)-S-2571	S-2571	8	0° (c 0.8)	0
	S-2571-oxon	58	-0.9° (c 4.5)	0
	2-Nitro-5-methylphenol	2	—	—
(+)—S-2571	S-2571	4	+50.0° (c 0.4)	94
	S-2571-oxon	64	+46.8° (c 6.0)	92
	2-Nitro-5-methylphenol	3	—	—
(—)-S-2571	S-2571	7	-51.0° (c 0.7)	96
	S-2571-oxon	60	-47.5° (c 4.0)	92
	2-Nitro-5-methylphenol	3	—	—

from (+)-S-2571 were dextrorotatory, and these from (—)-S-2571 were levorotatory. The optical purity of the oxon analogs was more than 94% relative to those of standard chemicals. These data indicate that the optical isomers of S-2571 were stereospecifically oxidized to the corresponding oxon analogs by UV irradiation. These findings suggest that photo-oxidation of the optical isomers of S-2571 may follow the same stereochemical process as that of *m*-chloroperoxybenzoic acid oxidation.

The rate of photodegradation of racemic, (+)- and (—)-S-2571 was determined by monitoring quantitatively the remaining unchanged compounds by glc. The rate for the three forms of S-2571 was virtually identical, as was the extent of degradation (72%) after 120 min. There seems to be no difference in the photodegradation rate of the S-2571 isomers.

3. Metabolism of the Racemic, (+)- and (—)-Forms of S-2571 by Rabbit Liver Mixed Function Oxidase

When the racemic form of ³H-S-2571 was incubated with rabbit liver microsomes plus NADPH system, more than 7 spots were detected on tlc in solvent systems A and B. Of these, 6 metabolites were identified on the basis of the following criteria: S-2571-oxon (II) [tlc cochromatography with the authentic standard in solvent systems A and B, and ms-ci, *m/e* 303 (M+1)]; S-2571-CH₂OH (III) [cochromatography in solvent systems A and B, and ms-ci, *m/e* 335 (M+1)]; S-2571-oxon-CH₂OH (IV) (cochromatography in solvent

Table 3 Amounts of metabolites of racemic S-2571 formed in rabbit liver microsomes plus NADPH system.

Metabolite	% of administered radioactivity	
	Microsome	Microsome + NADPH
I S-2571	98.1	21.3
II S-2571-oxon	0.5	22.0
III S-2571-CH ₂ OH	0.2	11.0
IV S-2571-oxon-CH ₂ OH	0	12.7
V S-2571-CHO	0	3.8
VI 2-Nitro-5-methylphenol	0	2.8
VII 2-Nitro-5-hydroxymethylphenol	0	9.5
Others	0.3	16.9
Total	99.1	99.0

systems B and C), S-2571-CHO (V) [cochromatography in solvent systems A and B, and ms-ci, *m/e* 333 (M+1)], 2-nitro-5-methylphenol (VI) (cochromatography in solvent systems B and C) and 2-nitro-5-hydroxymethylphenol (VII) (cochromatography in solvent systems B and C). The percentage of these metabolites is presented in Table 3. The major metabolites II, III, and IV were oxidation products retaining the phosphorus triester linkage. The hydrolysis products such as 2-nitro-5-methylphenol and 2-nitro-5-hydroxymethylphenol were found in lesser amounts. Thus, it was suggested that racemic S-2571 predominantly underwent oxidation of P=S to P=O, hydroxylation at the 5-methyl group and combination of both reactions by the microsomes plus NADPH system. For the metabolite preparation, 45 mg of racemic S-2571 per one experi-

Table 4 The specific rotation and optical purity of S-2571 and its metabolites recovered after incubation of the racemic, (+)- and (-)-forms of S-2571 with the microsomes plus NADPH system.

Exp. No.	Substrate	Product	Yield (mg)	$[\alpha]_D^{20}$	Optical purity
1	(±)-S-2571	S-2571	5.5	+16.6° (c 0.8)	31%
		S-2571-oxon	4.7	+20.8° (c 0.7)	39%
		S-2571-CH ₂ OH	4.8	+8.8° (c 0.7)	—
		S-2571-oxon-CH ₂ OH	5.0	+7.0° (c 0.8)	—
2	(±)-S-2571	S-2571	4.1	+22.8° (c 0.6)	43%
		S-2571-oxon	4.0	+17.2° (c 0.6)	33%
		S-2571-CH ₂ OH	3.5	+10.8° (c 0.5)	—
		S-2571-oxon-CH ₂ OH	4.0	+7.0° (c 0.6)	—
3	(+)—S-2571	S-2571	2.5	+48.0° (c 0.4)	88%
		S-2571-oxon	4.5	+47.5° (c 0.8)	89%
		S-2571-CH ₂ OH	3.0	+40.0° (c 0.5)	—
		S-2571-oxon-CH ₂ OH	3.0	+34.0° (c 0.5)	—
4	(—)-S-2571	S-2571	3.0	—48.9° (c 0.5)	89%
		S-2571-oxon	3.0	—46.5° (c 0.5)	88%
		S-2571-CH ₂ OH	5.0	—39.4° (c 0.8)	—
		S-2571-oxon-CH ₂ OH	3.0	—32.0° (c 0.5)	—

ment was incubated with the microsomes plus NADPH system, and the remaining parent compound (I) and three major metabolites (II, III, IV) were isolated. This experiment was repeated two times for confirmation of the results presented. The optical rotation and approximate yield of these products are shown in Table 4. The products obtained from racemic S-2571 were all dextrorotatory, indicating that the enantiomers in racemic S-2571 underwent different metabolic transformations by the rabbit liver microsome enzyme system used.

The optical purity of the recovered S-2571 and S-2571-oxon was estimated owing to differences in the specific rotations of the synthesized isomers of S-2571 and S-2571-oxon. The data indicate that the recovered S-2571 and S-2571-oxon contain 65–71% and 66–69%, respectively, of the (+)-isomers. In the separate experiments, 31 mg of (+)- or (—)-S-2571 was metabolized under the same conditions. Both isomers gave the same types of metabolites as obtained with racemic S-2571. The remaining S-2571 and three metabolites (II, III, IV) from (+)-S-2571 were all dextrorotatory. On the contrary, these from (—)-S-2571 were levorotatory. The optical purity of S-2571 and S-2571-oxon was 88–89% and 88–89%, respectively. These data

indicate that the optical isomers of S-2571 were stereospecifically oxidized to the corresponding oxon analogs by the microsomes plus NADPH system.

For the comparison of the metabolic rate of racemic, (+)- and (—)-S-2571, the remaining starting materials were determined by glc. After incubation, the remaining parent compounds were 21.4, 8.3 and 9.1% of the applied for racemic, (+)- and (—)-S-2571, respectively. (+)- and (—)-S-2571 were metabolized at almost equal rates, whereas racemic S-2571 had a tend to be metabolized more slowly than both isomers. From these data, it was not clear whether there is a difference in the rate of metabolism of the optical isomers of S-2571 by the microsomes plus NADPH system.

DISCUSSION

Figure 1 gives proposed metabolic pathways for S-2571 in rabbit liver mixed function oxidase. The racemic, (+)- and (—)-forms of S-2571 predominantly underwent oxidation of P=S to P=O, hydroxylation at the 5-methyl group and combination of both reactions in rabbit liver microsomal mixed function oxidase. Cleavage of P–O–aryl linkage by the mixed function oxidase occurred to a lesser extent relative to the oxidation reactions. Enzymatic oxidation of racemic S-2571 yielded dextro-

Fig. 1 Proposed metabolic pathways for S-2571 in the rabbit liver microsomes plus NADPH system and UV light.

→ rabbit liver microsomes + NADPH, ----→ UV light.

rotatory products of the parent compound, S-2571-oxon, S-2571-CH₂OH and S-2571-oxon-CH₂OH. It may be reasonable to presume that the (–)-isomer in racemic S-2571 is more rapidly metabolized by the mixed function oxidase than the (+)-isomer, and the (–)-isomers of the oxidation products are also more easily cleaved by the mixed function oxidase and/or microsomal arylesterase-type enzyme. The latter enzyme is known to cleave the P–O–aryl linkage of the oxon analogs^{10,11}. It was not clear whether differential metabolism of the enantiomers is mainly due to either oxidation of the parent compound by the mixed function oxidase, or hydrolysis of the oxidation products by arylesterase-type enzyme. However, quantitation of the unchanged compounds by glc could not reveal a difference in the rate of the initial metabolism of the resolved isomers of S-2571 by the mixed function oxidase, although the resolved isomers were more rapidly metabolized than the racemic compound. For assessment of the process of differential metabolism, amounts of the metabolites from (+)- and (–)-S-2571 should be quantitatively determined using radiolabeled enantiomers.

Studies with (+)- and (–)-S-2571 indicate that both oxidation of P=S to P=O by the mixed function oxidase proceeded stereo-

specifically. Similarly, the resolved isomers of S-2571 were stereospecifically oxidized to the corresponding oxon analogs by UV light irradiation. However, there was no difference in the rate of photodecomposition of the resolved isomers. The degree of stereospecificity in the oxidations by the mixed function oxidase and UV light was extremely high and was similar to that in *m*-chloroperoxybenzoic acid oxidation. Since the oxon analogs produced by the mixed function oxidase and UV irradiation were equal in rotation sign to those obtained by *m*-chloroperoxybenzoic acid oxidation, they must have identical configuration at phosphorus. This may be explainable by assuming retention during the oxidation reaction. Formation of the derivatives hydroxylated at the 5-methyl group appears not to affect the asymmetric center in the molecule. Therefore, hydroxylation at the 5-methyl group may proceed with retention. However, the absolute structures of the resolved S-2571 and S-2571-oxon isomers should be determined for confirmation of the stereochemical course of the oxidation reaction, as reported for fonofos.⁷⁾

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要 約

S-2571 (O-エチル O-2-ニトロ-5-メチルフェニル N-イソプロピル ホスホロアミド チオエート) のラセミ体は、ウサギ肝 mixed function oxidase (mfo) により代謝され右旋性のオキシ体および5位のメチル基が水酸化された代謝物を与えた。また、回収された S-2571 も右旋性を示した。(+)S-2571 も同様に代謝され、得られた主代謝物は右旋性であった。また、(-)S-2571 からの代謝物は左旋性であった。したがって、S-2571 両光学異性体は mfo により立体特異的に代謝されると考えられる。他方、S-2571 両光学異性体の紫外線によるオキシ体への酸化も立体特異的であった。m-chloro-peroxybenzoic acid による酸化と比較することにより、mfo および紫外線による P=S から P=O への酸化はリン原子における立体配置を保持したまま進行していると考えられる。

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