O-Aryl O-ethyl N-sec-butylphosphoramidothioate類の合成と抗菌活性

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Original Article

Synthesis and Antifungal Activity of *O*-Aryl *O*-ethyl *N*-*sec*-butylphosphoramidothioates

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Thirty-nine *O*-aryl *O*-ethyl *N*-*sec*-butylphosphoramidothioates were synthesized and tested for their antifungal activity against *Pseudopernospora cubensis*. Structure-activity relationship of various aryl substituents were examined. A 5-methoxy group on the 2-nitrophenyl moiety possessed an optimum van der Waals volume. A 4,5-methylenedioxy substituent on the 2-nitrophenyl group was comparable to the most effective 5-methoxy group. A strong electron-withdrawing group on the 2-position, for instance a nitro group, was an important molecular requirement. Compounds with only a 3,4-methylenedioxyphenyl or a 3,5-dimethoxyphenyl group were also effective.

INTRODUCTION

In our previous paper,[1] we reported on the synthesis and antifungal activity of *O*-ethyl *O*-(5-methoxy-2-nitrophenyl) *N*-*sec*-butylphosphoramidothioate **6** (Fig. 1) and the related compounds focusing on structural requirements for the *O*-alkyl and *N*-alkyl moieties.

To evaluate the effect of the phenyl moiety in *O*-ethyl *N*-*sec*-butylphosphoramidothioates, we introduced thirty-eight substituent groups on the benzene ring, and examined the antifungal activity against *Pseudopernospora cubensis*. The present paper describes the synthesis and a structure-activity relationship study discussing the test results in terms of electronic and steric factors, and positions of various benzene ring substituents.

MATERIALS AND METHODS

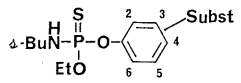
Melting points are uncorrected. IR spectra were measured on a Hitachi 270-30 spectrophotometer. ^{1}H NMR spectra were measured at 60 MHz with a Hitachi R24B instrument using tetramethylsilane as an internal standard.

1. *Synthesis of Phenols*

Most of the nitrophenols were synthesized according to the known procedures[2] and references. Unless otherwise noted, phenols were commercially available. New phenols were prepared as follows:

1.1 *4,5-Ethylenedioxy-2-nitrophenol*

A solution of 3,4-ethylenedioxyacetophenone (6.00 g) and *m*-chloroperbenzoic acid (9.00 g) in dichloromethane (200 ml) was mechanically stirred at room temperature for 3 hr. The solution was washed with aq. sodium hydrogen carbonate and 5% sodium hyposulfite solution in sequence and repeatedly until no peroxide was detected. The solution was dried ($MgSO_4$), filtered, and evaporated to give 3,4-ethylenedioxyphenyl acetate (5.93 g, 91%) as a solid. To a mechanically stirred solution of acetate (5.93 g) in acetic acid (15 ml) was added dropwise a mixture of nitric acid ($d=1.5$, 2.0 ml) and acetic acid (3.0 ml) at 0°C in 30 min. Stirring was continued for 1 hr after the addition at ambient temperature. The reaction mixture was poured into ice water, and the solid was collected by suction. Collected 4,5-ethylenedioxy-2-nitrophenyl acetate (mp 90–92°C) was hydrolyzed with sodium hydroxide solution to give 4,5-ethylenedioxy-2-nitrophenol (4.50 g, 74.8%). mp 173–174°C. Found: C, 48.79; H, 3.55; N, 7.04, Calcd. for



6 : 2-NO_2,5-OMe

34 : 2-NO_2,4,5-(OCH_2O)

37 : 3,5-$(OMe)_2$

Fig. 1 Chemical structures of antifungal phosphoramidothioates.

$C_8H_7NO_5$: C, 48.73; H, 3.55; N, 7.10%.

1.2 5-Dimethylamino-2-nitrophenol

A mixture of 50% dimethylamine solution (50 ml) and 5-fluoro-2-nitrophenol (3.0 g) in ethanol (50 ml) was refluxed for 0.5 hr. Ethanol was removed *in vacuo*, and the residue was extracted with chloroform. The combined layer was washed with brine and dried over magnesium sulfate. Removal of the solvent gave 5-dimethylamino-2-nitrophenol (2.24 g, 64%) as a solid. mp 141–143°C. ^{1}H NMR (60 MHz) $\delta_{TMS}^{CDCl_3}$ ppm: 3.10 (6H, s, N-$\underline{CH_3}$), 6.05–6.40 (2H, m, $H_{4,6}$), 7.91 (1H, d, J=5Hz, H_3), 11.40 (1H, s, O$\underline{H}$). Found: C, 52.61; H, 5.58; N, 15.36, Calcd. for $C_8H_{10}N_2O_3$: C, 52.7; H, 5.5; N, 15.4%.

2. Synthesis of Phosphorus Compounds

O-Ethyl *O*-aryl *N-sec*-butylphosphoramidothioates were synthesized by reacting *O*-ethyl *N-sec*-butylphosphoramidothiochloridate with the phenols in the presence of a base, as shown in the following example.

O-Ethyl O-(4, 5-ethylenedioxy-2-nitrophenyl) N-sec-butylphosphoramidothioate, ***35***

To a stirred solution of 4,5-ethylenedioxy-2-nitrophenol (1.97 g) in acetonitrile (100 ml) were added anhydrous potassium carbonate (1.40 g) and cuprous chloride (10 mg), and kept 40–50°C for 0.5 hr. To the acetonitrile solution was added *O*-ethyl *N-sec*-butylphosphoramidothiochloridate (2.2 g) in 40 min, and the mixture was refluxed for 3 hr. Filtered solution was evaporated to give an oil, which was dissolved in toluene and washed with 5% hydrochloric acid solution, 5% sodium hydroxide solution and water. Dried solution was evaporated. The residue was purified by column chromatography to give **35** as a solid (2.33 g, 62%). mp 59–61°C. Found: C, 44.88; H, 5.66; N, 7.31; P, 8.43, Calcd. for $C_{14}H_{21}N_2PSO_4$: C, 44.68; H, 5.62; N, 7.44; P, 8.23%.

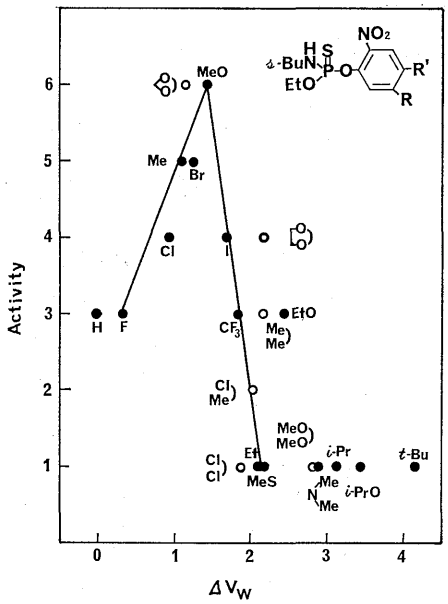


Fig. 2 Plot of antifungal activity values vs. relative group van der Waals volume of substituents: (●)=ΔV_w(5-R), (○)=ΔV_w(4-R′)+ΔV_w(5-R).

The line is to guide the reader's eyes.

3. Antifungal Activity against P. cubensis on Cucumber

Antifungal activity was based on the protective value which was determined in the same way as we reported.[1] In Fig. 2, the antifungal activity was expressed as a score from 6 through 1 according to the following criteria; 6 (protective value more than 90% at 12.5 ppm), 5 (protective value more than 90% at 50 ppm), 4 (protective value more than 90% at 200 ppm), 3 (protective value more than 90% at 500 ppm), 2 (protective value more than 50% and less than 90% at 500 ppm), 1 (protective value less than 50% at 500 ppm).

4. Physicochemical Parameter

The relative group van der Waals volume, ΔV_w was used as the steric parameter, and the

term was defined by the following equation.

$\Delta V_w(R) = V_w(R) - V_w(H)$. $V_w(R)$ and $V_w(H)$ are van der Waals volume values for the substituent R and H calculated according to Bondi.[3,4] The values are scaled by 0.1 for convenient comparison with the activity values.

RESULTS AND DISCUSSION

The physical properties, yields and antifungal activity of the synthesized compounds

Table 1 Antifungal activity and physical properties of *O*-aryl *O*-ethyl *N*-*sec*-butyl (or *i*-propyl)phosphoramidothioates.

Compound No.	Benzene ring substituent 2	3	4	5	n_D (°C)	Yield (%)	Protective value (%) 500 ppm	200 ppm	50 ppm	12.5 ppm	ΔV_w[a] (5-R)
1	NO_2	H	H	H	1.5231 (22.5)	60	100	81	19	0	0.00
2	NO_2	H	H	Me[5,6]	1.5340 (20)	70	100	100	94	19	1.12
3	NO_2	H	H	Et[2]	1.5325 (18)	66	0				2.14
4	NO_2	H	H	*i*-Pr[2]	1.5280 (18)	62	0				3.16
5	NO_2	H	H	*t*-Bu[2]	1.5260 (18)	66	0				4.18
6	NO_2	H	H	OMe[7]	1.5353 (26)	49	100	100	100	100	1.44
7	NO_2	H	H	OEt[8]	mp 65–67	35	100	50	19	0	2.46
8	NO_2	H	H	*i*-OPr[2]	1.5362 (23.5)	72	31	b)	b)	b)	3.48
9	NO_2	H	H	F[9]	1.5259 (19.5)	61	100	0	0	0	0.33
10	NO_2	H	H	Cl[10]	1.5438 (23.5)	69	100	100	75	44	0.95
11	NO_2	H	H	Br[11]	1.5562 (19.5)	69	100	100	100	31	1.26
12	NO_2	H	H	I[12]	1.5630 (20.5)	67	100	98	69	13	1.71
13	NO_2	H	H	CF_3[2]	1.4960 (20.5)	60	94	63	0	0	1.88
14	NO_2	H	H	$N(Me)_2$	mp 67–73	63	13	b)	b)	b)	2.92
15	NO_2	H	H	SMe[13]	1.5740 (25.0)	61	30	b)	b)	b)	2.20
16	H	NO_2	H	H	1.5351 (20)	56	0				
17[c]	H	H	NO_2	H	1.5505 (19.0)	72	0				
18	CN	H	H	H	1.5314 (27.0)	67	0				
19[c]	H	H	CN	H	1.5400 (19.0)	62	0				
20	COOMe	H	H	H	1.5240 (24.5)	4	0				
21	COMe	H	H	H	1.5299 (23.0)	3	0				
22[c]	Cl	H	H	H	1.5370 (19.0)	51	0				
23	H	H	H	OMe	1.5263 (23)	41	17				
24	CN	H	H	OMe[14]	1.5403 (19.5)	68	88	69	38	0	
25	CHO	H	H	OMe	1.5747 (23.5)	21	44	b)	b)	b)	
26	Cl	H	H	OMe	1.5294 (20.0)	39	67	b)	b)	b)	
27	Cl	H	H	Cl	1.5393 (26.0)	35	0				
28	OMe	H	H	NO_2	1.5422 (24)	88	0				
29	OMe	H	H	CN[14]	mp 70–72.5	34	0				
30	NO_2	H	OMe	OMe[2]	1.5503 (27.5)	23	0				2.88
31	NO_2	H	Me	Me[15]	1.5350 (19.0)	52	100	81	0	0	2.24
32	NO_2	H	Cl	Me[16]	1.5411 (21.0)	74	63	b)	b)	b)	2.07
33	NO_2	H	Cl	Cl[2]	1.5489 (20)	50	31	b)	b)	b)	1.90
34	NO_2	H	$O-CH_2-O$[17]		1.5500 (21.5)	52	100	100	100	97	1.16
35	NO_2	H	$O-(CH_2)_2-O$		mp 59–61	62	100	100	50	30	2.19
36	NO_2	H	NO_2	OMe[7]	1.5531 (25.5)	37	0				
37	H	OMe	H	OMe	1.5262 (23.5)	18	100	100	97	37	
38	H	H	$O-CH_2-O$		1.5327 (21.5)	44	100	100	0	0	
39	H	H	OMe	OMe	1.5313 (22.0)	5	0				

a) The values for **30–35** are the sum of ΔV_w(4-R′) and ΔV_w(5-R).

b) not determined.

c) An *i*-propylamino group in place of a *sec*-butylamino group.

are listed in Table 1.

Various substituents (compounds **1–15**) were introduced to the 5-position of the 2-nitrophenyl moiety. Although these substituents included both a strongly electron-withdrawing trifluoromethyl group and an electron-donating alkyl or an alkoxy group, the electronic characters of the substituents were not correlated well with the activity values in our preliminary consideration. Next, we turned our attention to steric factors and chose van der Waals volume. The ΔV_w value of each substituent (5-R) is plotted against the antifungal activity in Fig. 2. The activity increased with an increase of ΔV_w value from hydrogen to the methoxy substituent having the highest activity. Then the activity declined till bulkier substituents (compounds **3–5**) were no longer effective. From the bending line, we see that the 5-substituents of a 2-nitrophenyl moiety require an optimum van der Waals volume.

One more substitution on the 4-position of the 2-nitro-5-substituted phenyl groups mostly resulted in reduction in activity (compounds **30–33**). Noticeably, a compound (**30**) with a 4,5-dimethoxy-2-nitrophenyl group was not active at all. On the other hand, its cyclic analog, a 4,5-ethylenedioxy-2-nitrophenyl group provided high antifungal activity for a compound (**35**) at 200 ppm, and a compound (**34**) having a 4,5-methylenedioxy-2-nitrophenyl group was as effective as compound **6**. When the sum of ΔV_w(4-R′) and ΔV_w(5-R) was plotted with open circles in Fig. 2, they lined near the bending line for ΔV_w(5-R). The correlation of efficacy and steric bulkiness seems reasonable as a whole. We infer from the correlation that the effective 5-R (and 4-R′) substituents bind to a hydrophobic region of a target site. A highly polar 3- or 4-nitro substituent, therefore, may not be preferable, whereas a 2-nitro isomer is highly active at 500 ppm. For a more precise discussion it would be necessary to take account of the conformation of the ethoxy group and extension of 4,5-substituents around the benzene ring.

Substitution of electron-withdrawing groups for the 2-nitro group of the 5-methoxy-2-nitrophenyl moiety (*i.e.*, compounds **24–26**) mostly reduced the activity in comparison with the most electron-withdrawing nitro compound (**6**). These compounds, however, still showed moderate and stronger activity than compound **23** having only a 3-methoxy group at 500 ppm. These 2-substituents (compounds **18** and **22**) were not effective enough in the absence of a 5-methoxy group. Hence, electron-withdrawing 2-substituents seem to be another molecular requirement of this class of compounds.

Focusing solely on the methoxy substituent of the 5-methoxy-2-nitrophenyl group, we introduced a 3,5-dimethoxyphenyl group and a 3,4-methylenedioxyphenyl group that were devoid of a 2-nitro group. Both compounds (**37** and **38**) were highly effective at 50 ppm and 200 ppm, respectively. Probably, the two substituent groups satisfied unique steric requirements on the interaction with a target site.

Compound **34** clearly showed that two independent substituent effects attributable to each substituent (the 2-NO_2 and 4,5-methylenedioxy groups) exist.

Our conclusion: *O*-aryl *O*-ethyl *N*-*sec*-butylphosphoramidothioates having those phenyl groups such as 5-methoxy-2-nitrophenyl, 4,5-methylenedioxy-2-nitrophenyl, 4,5-ethylenedioxy-2-nitrophenyl, 2-nitrophenyl, 3,4-methylenedioxyphenyl and 3,5-dimethoxyphenyl groups showed good antifungal activity against *P. cubensis*. Both steric and electronic molecular requirements were found for the aryl substituents; an optimum van der Waals volume was found for the 5-substituents of the 2-nitrophenyl group: the presence of an electron-withdrawing 2-substituent on the benzene ring, especially a 2-nitro group, was another important molecular requirement.

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要　　約

O-Aryl *O*-ethyl *N*-*sec*-butylphosphoramidothioate 類の合成と抗菌活性

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リード化合物の 5-メトキシ-2-ニトロフェニル基を変換し，*Pseudopernospora cubensis* に対する抗菌活性と置換基との関係を検討した．5-メトキシ基だけを変換した結果，ある限られたファンデルワールス体積（エトキシ基）以下の 5 位置換基（4,5-二置換体はその和）が 500 ppm 以下の濃度で活性を示した．そして，至適値があり，それはメトキシ基，あるいはメチレンジオキシ基のものであった．5-メトキシ基をそのままにし 2-ニトロ基を変換したなかでは，シアノ基のみが活性を示した．電子求引性の一置換基の位置異性体群のなかでは 2-ニトロ基のみが活性を示した．また，3,4-メチレンジオキシ基または 3,5-ジメトキシ基のみを有する化合物も良好な活性を示した．ニトロ基のような電子求引性の 2 位の置換基と，適度な大きさを有する 5 位，または，4, 5 位の 2 置換基が活性発現に必要であることがわかった．