Synthesis, Separation, and Fungicidal Activity of the Rotationally Hindered Isomers (Atropisomers) of *N*-(Methoxyacetyl)-*N*-[2-methyl-6-(methylthio)phenyl]-D,L-alanine Methyl Ester

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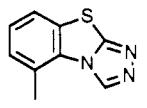
The synthesis of *N*-(methoxyacetyl)-*N*-[(2-methylthio)phenyl]-D,L-alanine methyl ester was successfully carried out in two steps from 2-methyl-6-(methylthio)aniline. It exists as two, separable, rotationally hindered isomers (atropisomers) that were isolated, characterized, and shown to have differential fungicidal activity against *Phytophthora infestans* and *Plasmopara viticola*.

During the work leading to the identification of the rat urine metabolites of tricyclazole (1) (Jourdan et al., 1986), the active component in the rice blast fungicide Beam (Froyd et al., 1976), a number of potential metabolites were synthesized to confirm the structural assignments.

It was discovered that the major metabolite, **2**, a glucuronide, isolated as the acylated methyl ether, existed as a mixture of rotational isomers (rotamers) due to the hindered rotation about the triazole–phenyl bond. A synthesized sample of the acylated methyl ester of the glucuronide also existed as a mixture of rotamers. Further, these rotamers could be separated by liquid chromatography and characterized by their different NMR spectra (Jourdan et al., 1986). This led us to examine the role of the *S*-methyl group in the rotational hindrance of other 2,6-disubstituted aniline derivatives and to determine what effect, if any, this hindered rotation had on biological activity.



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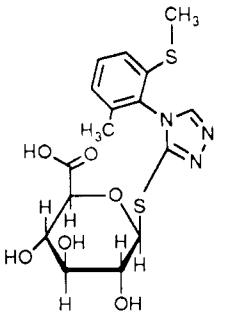
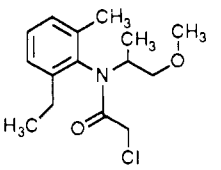


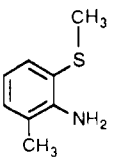
2

Because of our interest in fungicides, we sought to determine whether this kind of chiral axis isomerism could lead to differential fungicidal activity in vivo against important plant pathogens. Since the rotationally hindered tricyclazole metabolites were inactive biologically, as were their precursors, there was no way to determine with these compounds the contribution of each rotamer to fungicidal activity. There are numerous examples (Kawano et al., 1981; Oki et al., 1983a,b) of compounds exhibiting hindered rotational isomerism (atropisomerism), but none involving *S*-alkyl groups. Little is known about the effect of atropisomerism on biological activity. Some work was reported by Moser on the herbicidally active phenylacetamide metolachlor (**3**) (Moser et al., 1982), which concluded that different rotational isomers of the same acetanilide possessed different herbicidal activity. Moser's synthetic approach only gave only mixture of two torsion diastereomers of **3** that could not be separated by physical means, and separation was only achieved by synthetic techniques involving derivatization and dederivatization. Although metalachlor possesses only weak fungicidal activity, the authors also were able to detect differential fungicidal activity with the different rotamers in an in vitro test against *Pythium ultimum*.



3 (metolachlor)

From the tricyclazole metabolite synthetic approaches, we had available a ready source of 2-methyl-6-(methylthio)aniline (**4**) (Jourdan et al., 1986), so we chose potential target fungicides that could possibly utilize that intermediate. Metalaxyl (**5**), a phenylalanine known to have superior fungicidal activity against late blight, *Phytophthora infestans*, and grape downy mildew, *Plasmopara viticola*, seemed a likely target. By replacing one of the *o*-methyl groups in metalaxyl with an *S*-methyl group to provide **6**, we hoped to determine whether the resulting compound existed as two rotamers, whether these rotamers could be separated by simple physical methods, and, if so, whether they possessed fungicidal activity different from metalaxyl and from each other.



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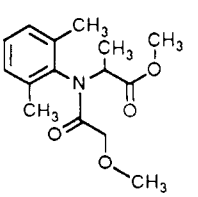
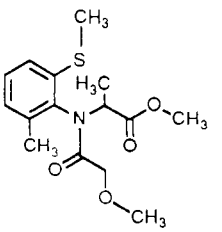


5 (metalaxyl)



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Fungicidal phenylalanines like metalaxyl are generally synthesized from substituted anilines in two steps (Hubele, 1975); i.e., the alkylation of the aniline with a

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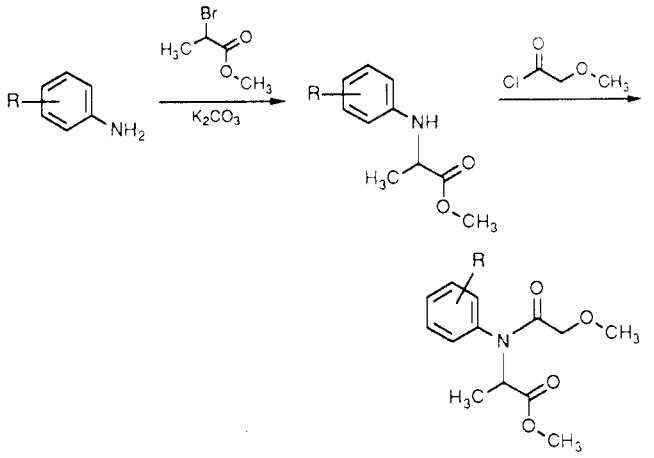
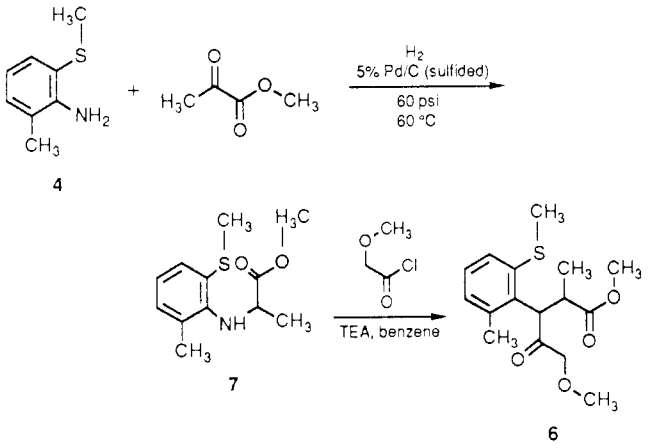
Scheme I



Scheme II



methyl α-bromopropionate followed by acylation with an α-methoxyacetyl chloride as in Scheme I.

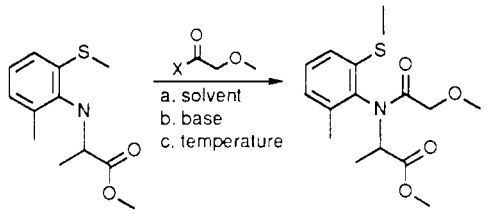
However, when Scheme I was carried out with 2-methyl-6-(methylthio)aniline, the first step resulted in a low-yielding reaction with multiple side products. The very characteristics of the phenylalanine that would hinder rotation, i.e. the bulky *S*-methyl group, made the alkylation with methyl α-bromo propionate difficult. An alternate route was sought and, after many trials, phenylalanine **7** was formed in good yield by the reductive alkylation of the aniline with methyl pyruvate, using a "sulfided" 5% palladium on carbon catalyst. Compound **7** was successfully acylated with α-methoxyacetyl chloride to form **6** in high yield (Scheme II).

MATERIALS AND METHODS

Column chromatography was carried out with use of low-pressure Michel–Miller chromatography columns dry-packed with Woelm silica gel and pressured by an FMI pump. NMR data were obtained on a Varian T-60 and a 360-MHz Bruker. Mass spectral data were obtained on a Hewlett-Packard 5985 GC/MS. Melting points were determined on a Mel-Temp and are uncorrected.

2-Methyl-6-(methylthio)aniline (4) (Fridman, 1950). A 70-g portion (0.43 mol) of 2-amino-4-methylbenzothiazole was added slowly to 200 g of KOH in 200 mL of water. The reaction mixture was heated to 135 °C slowly, allowing the water to evaporate. The temperature was then increased to 165–170 °C and held there for 2 h. The reaction was then cooled to room temperature and dissolved in 600 mL of water. The water solution was filtered through a Celite bed to remove unreacted benzothiazole, the filtrate was collected, and the water was removed in vacuo. To the residue were added 500 mL ethanol and 60 g (0.43 mol) of methyl iodide, the slurry was stirred at room temperature for 16 h, and the ethanol was removed in vacuo. The residue was then dissolved in water, neutralized to pH 7.0 with concentrated HCl, and extracted with methylene chloride. The methylene chloride solution was separated and concentrated in vacuo, yielding 51.0 g of crude **4**. This was chromatographed over a silica gel column to yield 47.0 g of **4** (72% yield) as an oil: NMR (deuteriochloroform) δ 2.11 (s, 3 H), 2.23 (s, 3 H), 4.18 (br s, 2 H), 6.43–7.26 (m, 3 H); MS, *m/e* 153.

Table I



X	solvent	base[a]	temp, °C	ratio of rotamers **6a** to **6b**
Cl	benzene	TEA	80	60:40
Cl	benzene	TEA	50	66:33
Cl	toluene	TEA	23	66:33
Cl	pyridine		23	60:40
Cl	DMF	NaH	0	trace of product
Cl	hexane	TEA	23	80:20
Cl	hexane	TEA	0	90:10
OCH_3	(neat)		reflux	no desired product
imidazole	THF		23	no desired product
imidazole	THF		reflux	trace of product

[a] TEA = triethylamine.

***N*-[2-Methyl-6-(methylthio)phenyl]-D,L-alanine Methyl Ester (7).** A mixture of 19.4 g (0.127 mol) of **4** and 14.5 g (0.142 mol) of methyl pyruvate in 360 mL of methanol was hydrogenated for 16 h at 60 psi and 60 °C with use of 10 g of 5% palladium on carbon (sulfided) catalyst. The reaction was cooled, the catalyst was removed by filtration, and the filtrate was concentrated in vacuo until crystallization commenced. The crystals were collected and recrystallized from methanol, yielding 19.2 g (63% yield) of a white crystal: mp 58 °C; NMR (deuteriochloroform) δ 1.33 (d, 3 H), 2.26 (s, 3 H), 2.33 (s, 3 H), 3.61 (s, 3 H), 4.21 (q, 1 H), 4.5 (br s, 1 H), 6.63–7.2 (m, 3 H); MS, *m/e* 239. The NMR spectrum was consistent with that of the desired product.

***N*-(Methoxyacetyl)-*N*-[2-methyl-6-(methylthio)phenyl]-D,L-alanine Methyl Ester (6).** A 34-g (0.143-mol) sample of phenylalanine **7** was dissolved in 175 mL of benzene, and 19.5 g (0.19 mol) triethylamine was added. This was followed by the addition of 21 g (0.19 mol) of freshly distilled methoxyacetyl chloride in 50 mL of benzene. The reaction was stirred at room temperature for 16 h, then 200 mL of water was added, the benzene layer was removed, washed once with 100 mL of water and once with 100 mL of a saturated sodium bicarbonate solution, isolated, and dried over sodium sulfate, and the benzene solution was reduced in vacuo to a mobile oil. The IR, NMR, mass spectrum, and elemental analysis of the oil indicated that it was compound **6**, but a TLC of the oil (eluted with ether on Whatman LK5 silica gel plates) suggested a mixture of two products and the NMR indicated that there were two chiral methyl peaks (0.97 vs 1.20 ppm) with an approximate ratio of 2:1. We arbitrarily assigned the isomer with the highest field NMR chiral methyl absorption (0.97 ppm) as rotamer **6a** and the other (1.20 ppm) as rotamer **6b**.

Variation of Acylation Conditions. As indicated above, the acylation of **7** with α-methoxyacetyl chloride gave the desired compound, **6**, as a 2:1 mixture of rotamers, as determined by the NMR resonance of the chiral methyl group. By varying the acylation conditions, we were able to alter the ratio of rotamers, but rotamer **6a** always predominated. Different acylation agents failed to give more than a trace of either rotamer (see Table I).

Separation and Characterization of Rotamer 6a. Under the best reaction conditions, with hexane and TEA at 0 °C,

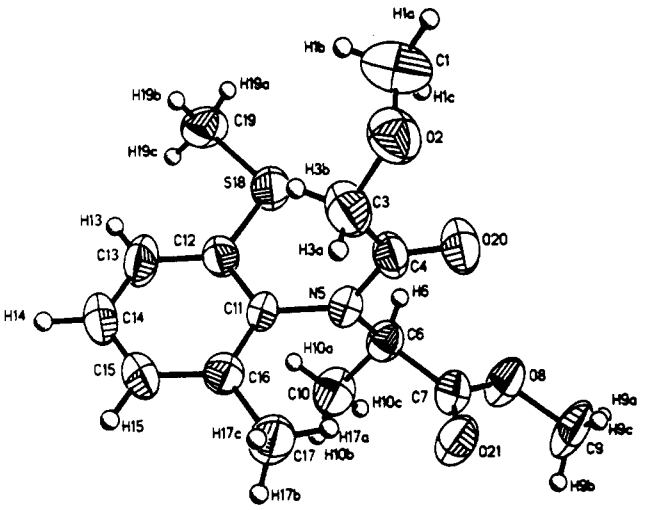


Figure 1. ORTEP plot of **6a**.

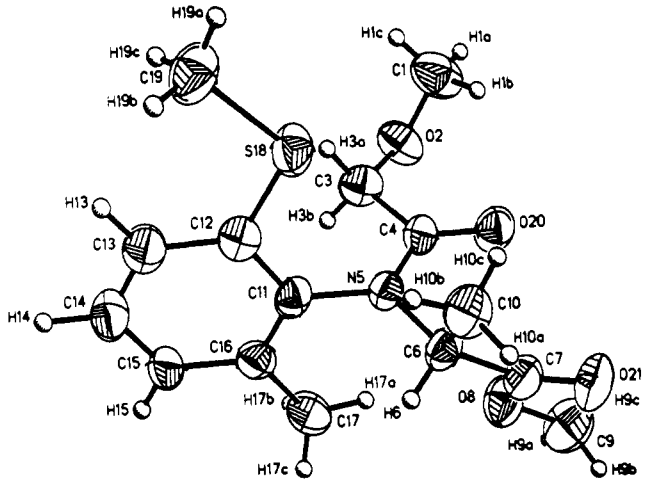


Figure 2. ORTEP plot of **6b**.

rotamer **6a** predominated 9:1 over rotamer **6b** and fractional crystallization from large volumes of hexane gave pure rotamer **6a**. It melted at 84–85 °C and had a chiral methyl nuclear magnetic resonance signal of δ 0.97 (d, 3 H). The complete NMR spectrum ($CDCl_3$) is as follows: δ 0.97 (d, 3 H), 2.41 (s, 3 H), 2.43 (s, 3 H), 3.3 (s, 3 H), 3.56 (s, 2 H), 3.75 (s, 3 H), 4.7 (m, 1 H), 7.15 (m, 3 H).

Rotamer **6a** was crystallized from ether, and an X-ray crystal structure determination was carried out. Compound **6a** crystallized in the space group *Cc*, with 4 molecules in a unit cell having dimensions a = 11.273 (2) Å, b = 16.177 (4) Å, c = 8.964 (2) Å, β = 91.892 (2)°, and a calculated density of 1.26 g cm^{-3}. A total of 1134 unique reflections with 2θ less than 116.0° were measured on an automated four-circle diffractometer using monochromatic copper radiation. The structure was solved with use of the direct methods routine SOLV of the SHELXTL program library (Sheldrick, 1983) and was refined by the least-squares method with anisotropic temperature factors for all atoms except hydrogen. All hydrogen atoms were included with isotropic temperature factors at calculated positions. The final R factor was 0.046 for 2501 observed reflections. Figure 1 shows an ORTEP plot of the molecule, and Tables IV–VII (supplementary material) give the atomic coordinates, bond lengths, bond angles, and anisotropic temperature factors.

Separation and Characterization of Rotamer 6b. The mother liquor contained a mixture of the two rotamers that could be separated by silica gel column chromatography (medium pressure) with ethyl ehter as the eluent. Rotamer **6b** was subsequently crystallized from hexane to yield a white crystalline solid, mp 73–74 °C, which had a chiral methyl ^{1}H NMR resonance at δ 1.20 (d, 3 H). The complete NMR spectrum ($CDCl_3$) is as follows: δ 1.20 (d, 3 H), 2.16 (s, 3 H), 2.40 (s, 3 H), 3.3 (s, 3 H), 3.64 (d, 2 H), 3.74 (m, 1 H), 7.10 (m, 3 H).

Rotamer **6b** was crystallized from ether, and an X-ray crystal structure determination was carried out. Compound **6b** crystallized in the space group $P\bar{1}$, with 2 molecules in a unit cell having dimensions a = 9.239 (2) Å, b = 11.144 (3) Å, c = 8.695 (2) Å, α = 108.18 (2)°, β = 103.26 (2)°, γ = 73.50 (2)°, and a calculated density of 1.28 g cm^{-3}. A total of 2125 unique reflections with 2θ less than 116.0° were measured on an automated four-circle diffractometer using monochromatic copper radiation. The structure was solved with use of the direct methods routine SOLV of the SHELXTL program library (Sheldrick, 1983) and was refined by the least-squares method with anisotropic temperature factors for all atoms except hydrogen. All hydrogen atoms were included with isotropic temperature factors at calculated positions. The final R factor was 0.050 for 2202 observed reflections. Figure 2 shows an ORTEP plot of the molecule, and Tables VIII–XI (supplementary material) give the atomic coordinates, bond lengths, bond angles, and anisotropic temperature factors.

When either pure rotamer was slowly heated in deuterated DMSO and the NMR resonance in the δ 0.95–1.3 range monitored, no change was observed below 130 °C. At 140–180 °C,

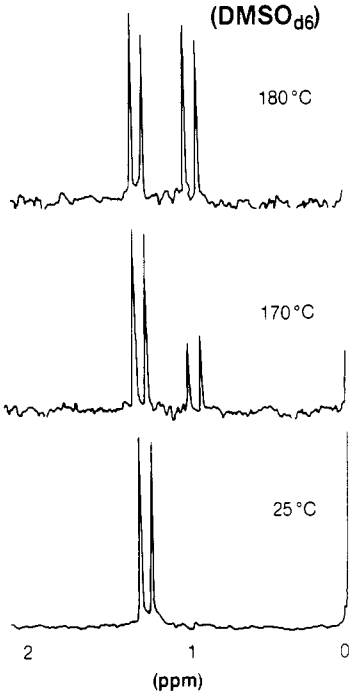


Figure 3. Thermal conversion of rotamer **6b** to a mixture of rotamers **6a** and **6b**.

regardless of which pure rotamer we began with, a mixture of rotamers resulted in a final rotamer ratio **6a** to **6b** of 1:1. The NMR results of heating a pure sample of rotamer **6b** in DMSO at varying temperatures is shown in Figure 3. This confirmed that we were dealing with rotational isomers of the same compound.

SCREENING METHODS

In all the in vivo screening studies, young plants (8–15 days) were used. The formulated compounds were sprayed on all foliar surfaces to runoff, the treatments were allowed to dry, and the plants were innoculated with the appropriate pathogens within 2–4 h. After 7–9 days the effectiveness of the compounds in controlling disease on each test plant was compared to that of an untreated control and rated as percent disease control. A reference-treated check was included in every trial.

The fungicidal activity in vitro was evaluated by the agar growth technique at four different concentrations. A fair number of controls were used. The number of replications was 3 in each case. The technique in brief involves the mixing of the compounds with agar medium and allowing the planted fungus to grow on such treated food

$$\% \text{ inhbn} = \frac{C - T}{C} \times 100$$

Table II. Comparison of Rotamer Activity in Plant Disease Control

rate applied, ppm	downy mildew (squash)		late blight (tomato)	
	6a	6b	6a	6b
400	100	100	100	95
100	100	100	95	70
25	100	100	95	60
10	100	80		
5	100	80		
2.5	100	40		
1.25	100	20		
0.625	100	20		
0.3125	100	0		
0.1562	90	0		
0.098	80	0		

Table III. Percent Colony Growth Inhibition

rotamer	rate, ppm	% control			
		Pythium ultimum		*Phytophthora capsici*	
		wild	resistant	wild	resistant
6a	0.1	53	0	6	0
	1	96	0	52	0
	10	100	0	80	0
	100	100	0	89	18
6b	1	36	8	0	0
	10	71	8	29	0
	100	84	10	67	4
metalaxyl	100	100	0	100	8

where C = diameter of fungus colony (mm) in the control plate and T = diameter of fungus colony (mm) in the treated plate. Inhibition in fungus growth was determined as the difference in diameter of fungus colony between control plates and those treated with either the rotamers or the reference (metalaxyl).

TESTING RESULTS

A. Greenhouse Comparison of Fungicidal Activity of Rotamer 6a with Rotamer 6b. Both pure rotamers as well as mixtures of rotamers were screened in vivo for the control of a variety of plant pathogens in the greenhouse and were found to have a disease spectrum similar to that of metalaxyl. They were shown to have significant fungicidal activity against both late blight of tomato and downy mildew of squash. The greenhouse studies demonstrate that rotamer **6a** is significantly more active than rotamer **6b** (see Table II).

B. Activity against Organisms Insensitive to Metalaxyl. Rotamers **6a** and **6b** were also tested in vitro, compared to metalaxyl, against both "wild" and metalaxyl-insensitive strains of *P. ultimum* and *P. capsici* to determine whether either rotamer controlled the resistant organism. These organisms were grown on "clarified" V-8 juice agar into which was incorporated both **6a** and **6b** at four concentrations varying from 0.1 to 100 ppm. Metalaxyl was also tested at 100 ppm under similar test conditions. Rotamer **6a** was more active than **6b** against the wild organism but only about half as active as metalaxyl. Both rotamers were equally ineffective against the insensitive strain (see Table III).

CONCLUSION

We have shown that replacing an *o*-methyl group in metalaxyl with an *S*-methyl results in a compound that exists as an unequal mixture of rotamers that can be readily separated and purified by column chromatography. These rotamers exhibit the same disease spectrum but to different degrees, with the major rotamer, **6a**, significantly more active than the minor rotamer, **6b**. Rotamer **6a** also proved to be a very active foliar fungicide in vivo against squash downy mildew and late blight of tomato. Work is under way to better understand the steric requirements necessary for activity against *Phycomycetes*.

ACKNOWLEDGMENT

We gratefully acknowledge the technical assistance of Paul Unger of LRL for his help in the interpretation of the NMR data.

Supplementary Material Available: Tables IV–XI, which list the data used in the X-ray crystal structure determination (8 pages). Ordering information is given on any current masthead page.

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Registry No. 4, 100305-95-1; DL-**6**, 124482-53-7; L-**6**, 124482-55-9; D-**6**, 124482-56-0; **7**, 124482-54-8; 2-amino-4-methylbenzothiazole, 1477-42-5; methyl pyruvate, 600-22-6.