concept that glycine is a direct metabolite of DPX-3217 rather than a naturally occurring compound in which the radiolabel has been metabolically incorporated.

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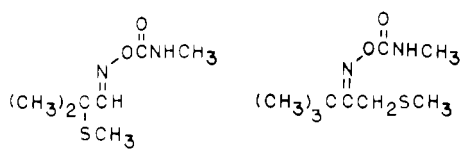
Stereochemical Effects in Carbamate Insecticides: Effect of *E,Z* Configuration on the Insecticide Activity of 1,1-Bis(methylthio)-3,3-dimethyl-2-butanone *O*-[(Alkylamino)carbonyl]oximes

H. Glenn Corkins,* Edmond R. Osgood, Louis Storace, Lawrence E. Limpel, and P. David Simcox

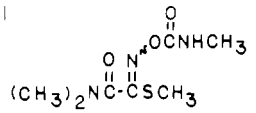
A series of 1,1-bis(methylthio)-3,3-dimethyl-2-butanone *O*-[(alkylamino)carbonyl]oximes and the *S*-oxides of 1,1-bis(methylthio)-3,3-dimethyl-2-butanone *O*-[(methylamino)carbonyl]oxime were synthesized. The *E* and *Z* isomers of the oximes were separated and their absolute stereochemistry established by use of the Beckmann rearrangement. The carbamates were found to be effective insecticides having activity against the Mexican bean beetle (*Epilachna varivestis*), two-spotted spider mite (*Tetranychus urticae*), and Black Bean aphid (*Aphis fabae*). The *Z* isomers were consistently more active than the *E* isomers. In vitro studies indicated the compounds to be cholinesterase inhibitors.

Since the early 1950s many structural variations of oxime carbamates have been made and reported as effective insecticides. Although the *E–Z* isomerism (also called syn–anti) that results from the stereochemical integrity of the oxime functionality has been recognized as an important structural feature of this class of compounds as well as carbamoylated thiohydroximidates, reported comparisons of the insecticidal activity of such isomers are few. For example, the aldoxime, aldicarb (1), was proposed as the single *E* isomer and its insecticidal activity reported



E aldicarb
1

Z thiofanox
2



oxamyl
3

(Payne et al., 1966). The insecticidal data for the *Z* isomer was not given. An undefined single configurational isomer of the ketoxime thiofanox (2) was more recently reported to have superior insecticidal activity over a 1:1 mixture of isomers (Magee and Limpel, 1977). Although the two isomers of oxamyl (3) have been prepared, their absolute configurations and their biological activities were not reported (Buchanan, 1971).

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The *E* and *Z* isomers of isophorone *O*-[(methylamino)-carbonyl]oxime have been prepared and differences in the insecticidal activity reported to be insignificant (Figure 1) (Metcalf and Fukuto, 1967). A similar result was reported for the isomeric pair of *exo*-3-chloro-*endo*-6-cyano-2-norbornanone *O*-[(methylamino)carbonyl]oximes (Figure 2) (Payne et al., 1967). The insecticidal activity and configurational assignments of the related thiohydroximidate carbamate, methomyl (4), have been carefully examined. The configuration of the *E* and *Z* isomers of methomyl (4) were determined via Beckmann rearrangement (Davies et al., 1968) and later confirmed by X-ray crystallography (Waile and Sim, 1971).

The insecticidal activity of the *Z* isomer was found to be significantly and consistently (over several species of insects) greater than that of the *E* isomer (Figure 3). A 40-fold increase of the *Z* over the *E* isomer on the Vetch aphid was the greatest difference noted. Consistent with the potent activity of these compounds, the *Z* isomer was 100 times more effective in inhibiting flyhead cholinesterase.

The *Z* isomer of the corresponding oxygen analogue of methomyl, methyl *N*-[(methylamino)carbonyl]acetimidate, was reported to have greater activity than the *E* isomer (Felton, 1968). This is consistent with the report by Donninger et al. (1968) on the activity of a series of oxygen analogs.

From the limited number of reported comparisons of insecticidal data, it appears that the isomeric nature of the oxime is important for activity in the thio and oxy hydroximidate type compounds and has limited effect on carbamates of ald- and ketoximes. This is unusual in that both types of compounds are reported to be cholinesterase inhibitors and presumably have a similar, if not identical, mode of action. One might expect the isomerism to be important since acetylcholinesterases are known to be highly stereospecific in the hydrolysis of the optical isomers

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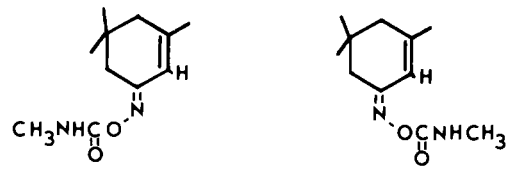
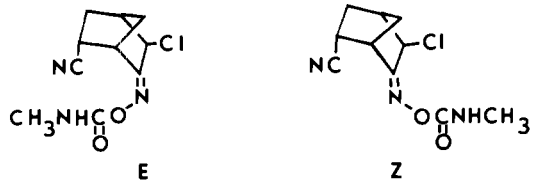


E (Anti) Z (Syn)

	LD_{50} μg/g		LC_{50} ppm	I_{50}
	HF	HF (synergized)	M	HF ChE
Z	>500	14	1.85	1×10^{-6}
E	>500	12	1.15	9×10^{-7}

HF = adult housefly; M = mosquito larvae

Figure 1. Insecticidal activity of the *E* and *Z* isomers of isophorone *O*-[(methylamino)carbonyl]oxime (Metcalf and Fukuto, 1967).

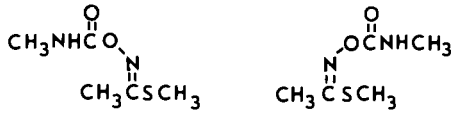


E (syn) Z (anti)

	LC_{50}, ppm			I_{50}
	BB[1]	M[1]	A[1]	HF ChE
E	100	14	54	2×10^{-6}
Z	120	20	28	1×10^{-6}

1. BB = Mexican bean beetle; M = Two spotted spider mite; A = bean aphid.

Figure 2. Insecticidal activity of the *E* and *Z* isomers of *exo*-3-chloro-*endo*-6-cyano-2-norbornanone *O*-[(methylamino)-carbonyl]oxime (Payne et al., 1967).



E Z

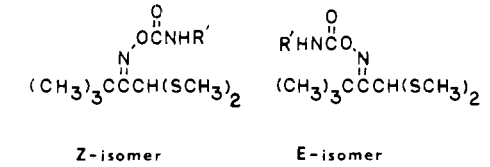
	$100\left[LD_{50}(\text{parathion})/LD_{50}(\text{Cmpd})\right]^4$				I_{50}
	HF	DB	VA	M	HF ChE
Z	10	2	80	20	2.6×10^{-7}
E	1	< 1	2	< 4	2.9×10^{-5}

HF = adult housefly; DB = Diamond-back moth larvae; Vetch aphid; M = red spider mite

Figure 3. Insecticidal activity of the *E* and *Z* isomers of methomyl (4) (Felton, 1968).

of acetyl β-methylcholine (Augustinson and Isacsher, 1957) and since a cis type of structure has been assigned to acetylcholine (Canepa et al., 1966).

The present work describes the synthesis of the *Z* and *E* isomers of a new series of previously unknown bis-



Z-isomer E-isomer

(methylthio) oxime carbamates and several related sulfur oxides. The absolute configurations of the corresponding oximes were determined by Beckmann rearrangement chemistry that has not been previously reported. Where possible, comparisons of the effectiveness of the *E* and *Z* isomers as insecticides are made.

EXPERIMENTAL SECTION

Chemical Methods. Nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Perkin-Elmer R24B spectrometer with Me_4Si as an internal standard. Natural abundance ^{13}C NMR spectra were obtained at 22.53 MHz with complete proton decoupling in $CDCl_3$ for field/frequency locking, and chemical shifts were referenced to Me_4Si. Infrared spectra (IR) were recorded on a Perkin-Elmer Model 137 infrared spectrometer. All boiling points and melting points are reported uncorrected. A Varian CH 7 mass spectrometer interfaced with a Varian 1740 gas chromatograph was used in the GC–MS phase of this study. The column used was an 8-ft stainless steel column packed with SP 2100 + 0.1% Carbowax on 100/120 Supelcoport. All mass spectra were recorded at 70 eV. The spectral data (^{1}H NMR, ^{13}C NMR, IR, and MS) are reported in the supplemental material for this paper.

Oximation of Bis(methylthio)pinacolone (bis-MTP). Recrystallized bis(methylthio)pinacolone (mp 48–50 °C, 385 g, 2 mol), hydroxylamine hydrochloride (139 g, 2 mol), and pyridine (158 g, 2 mol) were dissolved in 2 L of absolute ethanol and the solution was refluxed under argon for 12 days. An additional equivalent of hydroxylamine hydrochloride and pyridine was added every 3 days. The course of the reaction was followed by ^{1}H NMR. Additional reaction time beyond 12 days did not significantly increase the yield of the oxime. The ethanol was then removed by rotary evaporation and the residue partitioned between ether and water. The ether layer was washed with water, dried ($CaCl_2$), and rotary evaporated to yield 414 g of an oil containing (by ^{1}H NMR) 42% (α)-3,3-dimethyl-1,1-bis(methylthio)-2-butanone oxime (**5**), 12–15% (β)-3,3-dimethyl-1,1-bis(methylthio)-2-butanone oxime (**5**), and 24–26% bis(methylthio)pinacolone. Since the absolute configurations of the oximes were unknown, the oximes were designated the α and β isomers according to the chemical shifts of their methine protons. The α isomer absorbs at 4.27 ppm while the β isomer absorbs at 4.53 ppm. The three components were separated by preparative liquid chromatography [ISA Jobin Yvon Chromatospac Prep 100 [1.5 kg of EM silica gel 60 (60–200 particle size), UV detection and stop-flow gradient elution using hexane, CH_2Cl_2, acetone at a rate 42 mL/min]] of an 80.0-g sample of the crude reaction mixture. A total of 18 g of β-oxime was isolated. The 18-g sample of β-oxime was recrystallized from heptane to yield 16.8 g, mp 123–124 °C. A total of 35 g of the α isomer was obtained by chromatography. Recrystallization from heptane resulted in 32.6 g, mp 124–127 °C.

Reaction of the α Isomer of 3,3-Dimethyl-1,1-bis-(methylthio)-2-butanone Oxime (5) with Phosphorus Pentachloride. A solution of phosphorus pentachloride (2.15 g, 0.01 mol) in 200 mL of ether was added dropwise over 0.5 h to an ethereal solution of the α isomer (2.07 g, 0.01 mol, in 30 mL) with stirring at −25 °C under argon. The solution was stirred 0.5 h at −25 °C and then 1 h at 25 °C. An aliquot was subjected to GC–MS and ^{1}H NMR analysis. The major products were found to be *N*-*tert*-butyl-2,2-bis(methylthio)acetamide (**11**) and bis(methylthio)acetonitrile (**10**). The reaction solution was poured into H_2O. The ether layer was separated, washed with H_2O, dried ($MgSO_4$), and concentrated by rotary evapo-

ration to give 1.36 g of a semisolid. The ^{1}H NMR spectrum of this sample indicated a mixture of amide, nitrile, and unreacted starting material (50%, 35%, and 15%, respectively). The semisolid was slurried in pentane and the undissolved solids were collected by vacuum filtration. Recrystallization from hexane gave 0.100 g of **11** as a white crystalline solid, mp 91–95 °C. The spectral properties were identical with that obtained from material synthesized independently. Bis(methylthio)acetonitrile (**10**) had the following spectral properties: ^{1}H NMR (CCl_4) δ 2.30 (6 H, s, SCH_3), 4.48 (1 H, s, CH), MS, *m/e* (relative %) M^+ 133 (45), 118 (5), 94 (3.5), 86 (100), 70 (18), 59 (16), 45 (58).

***N*-*tert*-Butyl-2,2-dichloroacetamide (12).** Compound **12** was prepared in 57% yield as a white solid, mp 155–160 °C, by using the method of Speziale (1956).

Preparation of *N*-*tert*-Butyl-2,2-bis(methylthio)-acetamide (11). To a dimethylformamide solution of methanethiol (5.5 g, 0.115 mol, in 25 mL) stirring at 0 °C was added 1.95 g (0.0106 mol) of *N*-*tert*-butyl-2,2-dichloroacetamide (**12**). Powdered anhydrous potassium carbonate (10 g, 0.07 mol) was added and the mixture stirred 16 h at 25 °C. The mixture was poured into water and the product extracted with ether. The ether layer was washed successively with water and saturated NaCl solution. After drying ($MgSO_4$) and concentrating this layer, a total of 1.6 g (76%) of white solid was obtained. Recrystallization from hexane gave 0.35 g of the acetamide as a white crystalline solid, mp 92–95 °C.

Reaction of the β Isomer of 3,3-Dimethyl-1,1-bis-(methylthio)-2-butanone Oxime (5) with Phosphorus Pentachloride. A solution of phosphorus pentachloride (0.52 g, 0.0024 mol) in 50 mL of ether was added dropwise over 0.5 h to an ethereal solution of the β isomer (0.5 g, 0.0024 mol, in 15 mL) with stirring at −25 °C under argon. The solution was stirred 0.5 h at −25 °C and 1 h at room temperature. A GC-MS and ^{1}H NMR analysis of a sample of the solution showed pivalonitrile [MS *m/e* (relative %) M^+ −1, 82 (4), 68 (49), 52 (11), 42 (100), 41 (87), 27 (20), 15 (11), ^{1}H NMR (Et_2O); δ 1.38 (s, *tert*-butyl)] and bis-(methylthio)chloromethane as the major products. The spectra were identical with those of authentic samples. The reaction solution was poured into water. The ether layer was separated, washed with water, dried ($MgSO_4$), and concentrated at reduced pressure to give 0.2 g (80% yield) of tris(methylthio)methane (**14**).

Preparation of Bis(methylthio)chloromethane (17). Compound **17** was prepared according to the procedure of Bohme and Roehr (1961) and isolated as a mixture (by ^{1}H NMR) of 63% bis(methylthio)chloromethane, 29% tris(methylthio)methane, and 8% dichloromethyl methyl sulfide. Because of the thermal instability of **17**, this mixture was used "as is". Bis(methylthio)chloromethane had the following spectral properties: ^{1}H NMR (CCl_4) δ 2.28 (6 H, s, SCH_3), 5.91 (1 H, s, CH). A sample of bis-(methylthio)chloromethane was stirred 16 h with a small amount of water. Carbon tetrachloride was added and the layers were separated. The CCl_4 layer was dried ($MgSO_4$). ^{1}H NMR of this solution showed a complete conversion to tris(methylthio)methane (**14**).

Preparation of the Carbamates of 3,3-Dimethyl-1,1-bis(methylthio)-2-butanone Oximes. *Method A.* To a stirring methylene chloride solution of either the pure *E* or *Z* isomers of 3,3-dimethyl-1,1-bis(methylthio)-2-butanone oxime, e.g., 0.018 mol in 35 mL, was added a 5% excess of the isocyanate. The solution was stirred at room temperature 6 h and then concentrated by rotary evaporation to give a solid. The *N*-phenyl and *N*-methyl carbamate derivatives of the *Z* and *E* isomers were severally prepared and were recrystallized from hexane. The following *E* and *Z* isomers of the carbamates were prepared: 3,3-dimethyl-1,1-bis(methylthio)-2-butanone *O*-[(methylamino)carbonyl]oxime and 3,3-dimethyl-1,1-bis(methylthio)-2-butanone *O*-[(phenylamino)carbonyl]oxime.

Method B. To a chilled solution of phosgene in anhydrous ether was added 1 equiv of *N,N*-dimethylaniline followed by the dropwise addition of an equivalent amount of either the (*Z*)- or (*E*)-3,3-dimethyl-1,1-bis(methylthio)-2-butanone oxime in diethyl ether. The mixture was stirred at 0 °C for 2 h and allowed to come to room temperature. The white precipitate that was formed was removed by vacuum filtration. The filtrate was cooled (ice bath) while 3 equiv of the appropriate amine was added. After being stirred for 1 h, the reaction mixture was poured into water. The ether layer was separated, dried ($MgSO_4$), and concentrated to give the desired carbamate. The following *N*-allyl and the unsubstituted derivatives of the *Z* and *E* isomers were severally prepared: 3,3-dimethyl-1,1-bis(methylthio)-2-butanone *O*-[aminocarbonyl]oxime and 3,3-dimethyl-1,1-bis(methylthio)-2-butanone *O*-[(2-propenylamino)carbonyl]oxime.

Preparation of (*Z*)-3,3-Dimethyl-1-(methylsulfinyl)-1-(methylthio)-2-butanone *O*-[(Methylamino)carbonyl]oxime (28). (*Z*)-3,3-Dimethyl-1,1-bis-(methylthio)-2-butanone *O*-[(methylamino)carbonyl]oxime (**20**) (19.83 g, 0.075 mol) was dissolved in 800 mL of methanol and the solution cooled to 0 °C. A solution of sodium periodate (16.70 g, 0.078 mol) in 250 mL of water was added dropwise over a period of 30 min. The mixture was stirred at 0 °C for 14 h and at room temperature for 24 h. The sodium iodate was removed by filtration and the filtrate rotary evaporated to give an oil which was dissolved in 200 mL of methylene chloride. This solution was dried ($MgSO_4$) and filtered. The filtrate was rotary evaporated to yield 21.2 g of white solid. This material contained (by ^{1}H NMR) about 90% product as a diastereomeric mixture (ratio 2:1). The material was dried 14 h in an Abderhalden drying apparatus at 56 °C under vacuum. The residue was recrystallized twice from acetone–hexane to yield 3.0 g of one diastereomer as a white crystalline solid, mp 128–130 °C dec.

Preparation of (*Z*)-3,3-Dimethyl-1,1-bis(methylsulfinyl)-2-butanone *O*-[(Methylamino)carbonyl]-oxime (31). Using 2 equiv of sodium periodate in the previously described procedure resulted in a quantitative conversion to **31**. The ^{1}H NMR spectrum showed the solid product as three diastereomers (ratio 2:2:1). Recrystallization from acetone–hexane gave one diastereomer as a white crystalline solid, mp 164–166 °C.

Preparation of (*Z*)-3,3-Dimethyl-1-(methylsulfonyl)-1-(methylthio)-2-butanone *O*-[(Methylamino)carbonyl]oxime (30), (*Z*)-3,3-Dimethyl-1,1-bis-(methylsulfonyl)-2-butanone *O*-[(Methylamino)-carbonyl]oxime (34), (*E*)-3,3-Dimethyl-1-(methylsulfinyl)-1-(methylthio)-2-butanone *O*-[(Methylamino)carbonyl]oxime (29), and (*E*)-3,3-Dimethyl-1-(methylsulfonyl)-1-(methylthio)-2-butanone *O*-[(Methylamino)carbonyl]oxime (29a). For the preparation of these compounds see Corkins et al. (1980).

Preparation of (*Z*)-3,3-Dimethyl-1-(methylsulfinyl)-1-(methylsulfonyl)-2-butanone *O*-[(Methylamino)carbonyl]oxime (33). (*Z*)-3,3-Dimethyl-1-(methylsulfonyl)-1-(methylthio)-2-butanone *O*-[(methylamino)carbonyl] oxime (**30**) (13.56 g, 0.0439 mol) was dissolved in 25 mL of methylene chloride and the solution cooled to 0 °C. A slurry of *m*-chloroperbenzoic acid

(Aldrich, 85%, 9.95 g, 0.049 mol) in 100 mL of methylene chloride was added in portions over 15 min. The mixture was stirred 6 h at 0 °C and then 14 h at room temperature. The mixture was cooled to −78 °C and the *m*-chlorobenzoic acid removed by filtration. The filtrate was rotary evaporated to yield 12.2 g of white solid. The ^{1}H NMR spectrum showed this solid to be about 85% product as two diastereomers (ratio 5:1). The solid was dried under vacuum 14 h at 25 °C and 4 h at 50 °C. Recrystallization from acetone–hexane gave 4.45 g of **33** as a single diastereomeric, white crystalline solid, mp 170–172 °C.

Biological Methods. The compounds were evaluated for insecticidal and acaricidal activity against five species: Mexican bean beetle larvae (*Epilachna varivestis*), Southern armyworm larvae (*Spodoptera eridania*), adult housefly (*Musca domestica*), two-spotted spider mite (*Tetranychus urticae*), and black bean aphid (*Aphis fabae*). Contact activity was determined against all five species while systemic activity was determined against the mite and aphid.

For purposes of comparing the observed insecticidal activity, the dosage–mortality data for each compound was transformed to an LC_{50} value by a modified computer program that calculated the regression line relating PROBITS and log dose (Busuine, 1971). The LC_{50} value is recorded in parts per million for the bean beetle (BB), mite (M), and aphid (A) tests and is recorded in kg/Ha for the mite systemic and aphid systemic tests.

For determinations of the in vitro acetylcholinesterase inhibition, the test chemicals were dissolved in dimethyl sulfoxide. Electric eel acetylcholinesterase was dissolved in 0.1 M tris(hydroxymethyl)aminomethane-HCl–0.66% NaCl, pH 7.4, buffer. The test chemical solutions were diluted 100-fold in buffered enzyme and incubated at 26 °C for 10 min. The residual enzyme activity was assayed according to Ellman et al. (1961). The residual enzyme activity was compared to that of the dimethyl sulfoxide control and the percent inhibition was plotted vs. the concentration of inhibitor present in the incubation mixture. The I_{50} values were calculated by using computer-assisted PROBIT analysis.

Stock formulations containing 1024 ppm of each test chemical were prepared by using 102.4 mg of the test chemical (or 0.05 mL of a liquid), 4.0 mL of acetone containing 0.25% (v/v) Triton X-155, and 96.0 mL of deionized water and are used in both soil drench and spray treatments. The stock formulations were diluted to obtain the appropriate lower concentration maintaining the concentration level of all adjuvants.

Bean Aphid Spray and Systemic Test. The bean aphid was cultured on nasturtium plants (var. Tall Single); no attempt was made to select insects of a given age in these tests. Single nasturtium test plants growing in soil contained by 2.25-in. fiber pots were infested with populations of 100–200 aphids. In the spray application, 50 mL of stock or diluted formulation was uniformly sprayed onto the plants. In the systemic application, 11.2 mL of stock or diluted formulation is added to the soil containing the plant. Throughout the test, the plant test units were maintained under fluorescent lights and watered through the bottom. The percent mortality was determined 3 days after treatment.

Red Spider Mite Spray and Systemic Test. The stock culture of mites was maintained on Scarlet runner bean foliage. Approximately 18–24 h before testing, mites were transferred to the primary leaves of two Lima bean plants (var. Sieva) grown in 2.25-in. pots. The spray and systemic application methods described were used to apply the test formulations to the infested plants and soil. Mortality was determined by examination of two treated leaves 3 days after treatment.

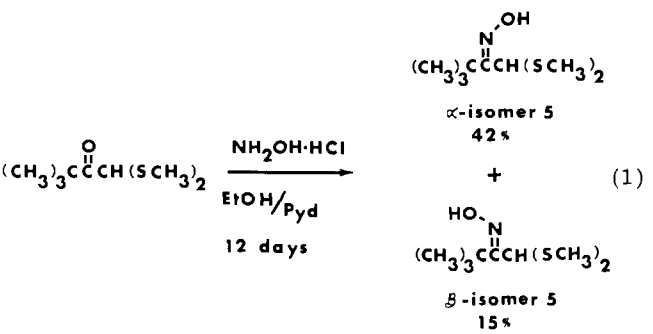
Housefly Spray Test. Ten adult flies were placed in a cylindrical screened cage 1.5 × 4 in. fabricated from 20-mesh stainless steel and are sprayed with 50 mL of the test solution. The flies are supplied food and drink from a dextrose solution. The percent mortality was determined 3 days after treatment.

Southern Armyworm Spray Test. Paired fully expanded primary leaves excised from Scarlet runner bean plants were maintained in plastic tubes containing water and sprayed with the test formulation. After the spray deposit on the leaves was dry, the paired leaves were separated. One leaf is placed onto a 1.5% water agar and infested with 10 newly hatched larvae. The covered test unit is stored at 22 °C for 4 days and then percent mortality was determined.

Mexican Bean Beetle Leaf Spray Test. This test is the same as that described for the armyworm test except that 1-day-old larvae of the bean beetle are used. The percent mortality is determined after 4 days.

RESULTS AND DISCUSSION

Preparation of the *E* and *Z* Isomers of 3,3-Dimethyl-1,1-bis(methylthio)-2-butanone Oxime (5). The reaction of bis(methylthio)pinacolone with hydroxylamine hydrochloride in refluxing ethanol–pyridine required 12 days for production of a 57% yield of a mixture of the *E* and *Z* isomers of bis(methylthio)pinacolone oxime (**5**) (15% and 42%, respectively). The success of this reaction was surprising because of a report by Pearson and Keaton (1963) on the special conditions required for oximation of hindered ketones. The isomers were separated by preparative medium-pressure chromatography and arbitrarily designated as α and β isomers. The spectral data (IR, ^{1}H NMR, MS, and ^{13}C NMR) were completely consistent with the oxime structures. Although the NMR methods clearly distinguished between the two isomers, assignment of the absolute configuration could not be made by ^{1}H NMR.

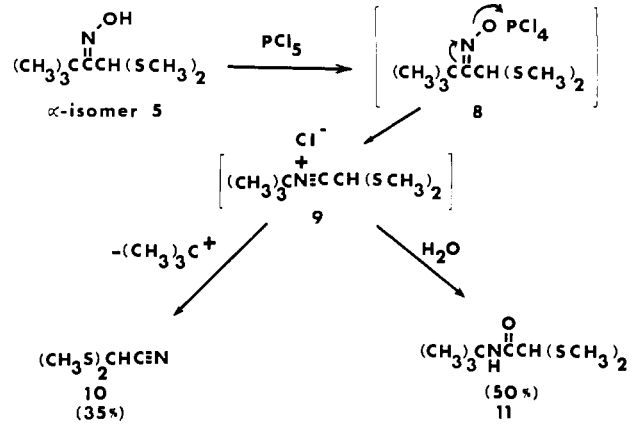


(1)

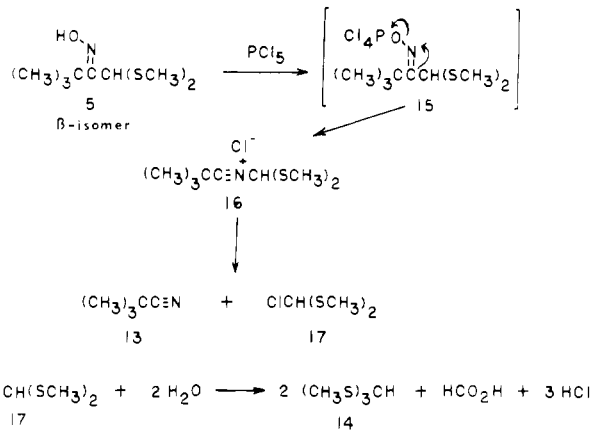
Since the oximes were produced under conditions that would have given a thermodynamic mixture of isomers, the 3:1 ratio of isomers indicated about a 0.6 kcal/mol difference in free energy between the isomers. With such a small difference in energy between the isomers, care had to be taken to prevent possible isomerization in future transformations of the compounds. At room temperature over a 24–48-h period, it has been noted that pure (*E*)-3,3-dimethyl-1-(methylthio)-2-butanone oxime slowly isomerizes to a 95% *Z*–5% *E* mixture.

Establishing the Absolute Configuration of the *E* and *Z* Isomers of Bis(methylthio)pinacolone Oxime (5). The absolute configurations of the oximes were determined by the Beckmann rearrangement under conditions where no isomerization occurred (Scheme I). An

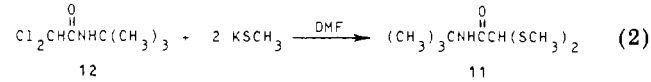
Scheme I



Scheme II



ethereal solution of the α isomer of 3,3-dimethyl-1,1-bis(methylthio)-2-butane oxime (**5**) cooled to −25 °C was treated with a slightly less than stoichiometric amount of PCl_5 in ether. After workup a mixture of *N-tert*-butyl-2,2-bis(methylthio)acetamide (**11**) (50%), bis(methylthio)acetonitrile (**10**) (35%), and starting oxime (15%) was obtained. Since the recovered starting material consisted of only the α isomer, isomerization did not appear to be a problem. The products can be rationalized by rearrangement of the *tert*-butyl group onto the nitrogen atom of the phosphorylated oxime (**8**) to give the iminium ion **9** which can either fragment to give the nitrile or react with water to give the acetamide. From the products of the reaction it can be concluded that the α isomer has the *Z* configuration. The structure of the bis(methylthio)acetonitrile (**10**) was established by ^1H NMR and GC–MS. The *N-tert*-butyl-2,2-bis(methylthio)acetamide (**11**) was isolated from the reaction and its spectral properties were compared to those of an authentic sample synthesized by reacting *N-tert*-butyl-2,2-dichloroacetamide (**12**) with a



(2)

DMF solution of potassium thiomethylate (eq 2). An ethereal solution of the β isomer of 3,3-dimethyl-1,1-bis(methylthio)pinacolone oxime (**5**) cooled to −25 °C was treated with PCl_5 in ether. After workup an 80% yield of tris(methylthio)methane (**14**) was obtained. The spectral properties of **14** were identical with those of a sample synthesized independently. An examination of the ether solution by ^1H NMR, GLPC, and GC–MS obtained during the Beckmann rearrangement but prior to the water workup indicated the presence of pivalonitrile (**13**) and bis(methylthio)chloromethane (**17**).

The products can be rationalized by the rearrangement of **15** to give the iminium structure **16** which fragments to pivalonitrile (**13**) and bis(methylthio)chloromethane (**17**; Scheme II). The reaction of **17** with the water that is added during the workup produces the observed tris(methylthio)methane (**14**). This intermediate reaction was demonstrated independently. The synthesis of **17** by the method of Bohme and co-workers (1961) and reaction of **17** with water indeed gave tris(methylthio)methane (**14**). Thus, it can be concluded from the isolated products of the Beckmann rearrangement that the β isomer has the *E* configuration.

Preparation of the (*E*)- and (*Z*)-Oxime Carbamates. Having prepared the *E* and *Z* isomers of 3,3-dimethyl-1,1-bis(methylthio)-2-butanone oxime and having established their absolute configuration, it was of interest to prepare the corresponding carbamates for insecticidal evaluation. The isomerically pure oximes were reacted with either the isocyanate to give the carbamates directly or with phosgene followed by a substituted amine (Scheme III). It is important to note that *E,Z* isomerization did not occur under either set of reaction conditions. The (*E*)- and (*Z*)-carbamates having a methyl, allyl, or phenyl nitrogen substituent were prepared along with the unsubstituted derivative.

Preparation of the Sulfur Oxides of 3,3-Dimethyl-1,1-bis(methylthio)-2-butanone *O*-[(Methylamino)carbonyl]oxime (20). Because of the increased

Scheme III

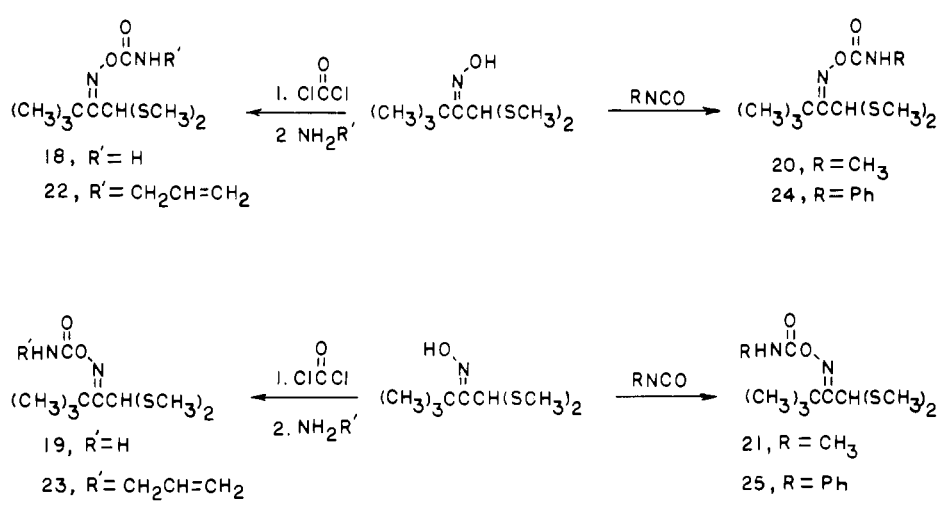
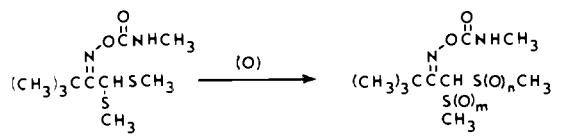


Table I. Synthesis of the Sulfur Oxides of 20

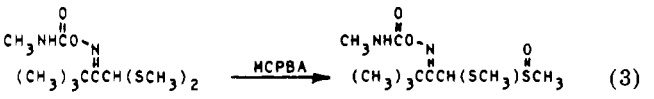


Oxidizing Agent (eqv)	Solvent	n/m	Possible Diast.	% Yield	Diast. ratio
$NaIO_4$ (1)	$MeOH/H_2O$	0/1	2	90	2:1
$NaIO_4$ (2)	$MeOH/H_2O$	1/1	3	100	2:2:1
$KMnO_4$ (2)[1]	Acetone/H_2O	0/2	0	90	–
MCPBA (1)[2]	CH_2Cl_2	1/2	2	76	5:1
MCPBA (4)	CH_2Cl_2	2/2	0	100	–

1. According to the method of Chatterway and Kellet (1930).
2. MCPBA = m-Chloroperbenzoic acid; starting material for this reaction was the mono-sulfone.

activity observed upon oxidation of the sulfur atom in thiofanox, it was of interest to prepare the *S*-oxides of the most active compound, i.e., the *N*-methyl derivative. Unfortunately, the preparation of the *S*-oxides was complicated by having two oxidizable sulfur atoms. Excluding the possible optical and diastereomeric isomers, five different sulfur oxides were possible. The syntheses of the *S*-oxides of the *Z* isomer are summarized in Table I. In all of the oxidations very acceptable yields were achieved. In the cases where diastereomeric mixtures were obtained, recrystallization resulted in the isolation of one pure diastereomer.

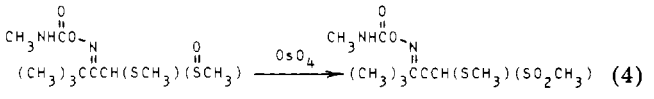
Under the identical oxidative conditions used in oxidizing the (*Z*)-carbamates (Table I), the oxidation of the (*E*)-carbamate (**21**) was unsuccessful. The synthesis of the sulfur oxides of **21** was limited to two compounds. The (*E*)-3,3-dimethyl-1-(methylsulfinyl)-1-methylthio-2-butanone *O*-[(methylamino)carbonyl]oxime (**29**) was produced in essentially quantitative yield by *m*-chloroperbenzoic acid oxidation of **21** (eq 3). Further reaction of the (*E*)-



(3)

21 **29**

monosulfoxide **29** with osmium tetroxide gave (*E*)-monosulfone **29a** in essentially quantitative yield (eq 4). Un-



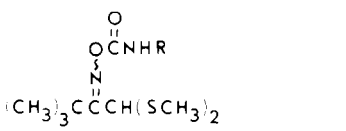
(4)

29 **29a**

fortunately, the monosulfone was unstable and decomposed upon recrystallization (Corkins et al., 1980). Attempts at isolation of **29a** by chromatography failed because of decomposition on the column. The monosulfoxide was the only stable oxide isolated.

Insecticidal Activity of the *E* and *Z* Isomers of 3,3-Dimethyl-1,1-bis(methylthio)-2-butanone *O*-[(Substituted-amino)carbonyl]oximes and the Sulfur Oxides of 3,3-Dimethyl-1,1-bis(methylthio)-2-butanone *O*-[(Methylamino)carbonyl]oxime. The spectrum of insecticidal activity of the various N-substituted carbamates when tested over five insect species was limited to the bean beetle, mite, and aphid. Table II summarizes the biological data. At the 128-ppm test concentration, the compounds were inactive on the armyworm and adult housefly. In general, the *Z* isomer, which has the *O*-carbamoyl and the bis(methylthio)methyl moieties on the same side, was significantly more effective than the *E* isomer. The difference between the *E* and *Z* isomers was greater than the difference between standard *E* and *Z*

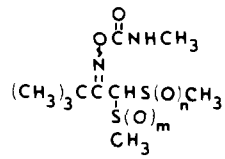
Table II. Insecticidal Activity of the *Z* and *E* Isomers of 3,3-Dimethyl-1,1-bis(methylthio)-2-butanone *O*-[(Substituted-amino)carbonyl]oximes



			LC_{50}, ppm			LC_{50}, Kg/Ha		I_{50}^2
No	R	Configuration	BB[1]	M[1]	A[1]	MS[1]	AS[1]	$\times 10^7$ M
18	H	Z	71	48	11	>4	1.5	6.3
19	H	E	615	1066	619	>4	>4	750
20	CH_3	Z	30	37	4	>4	0.9	15
21	CH_3	E	170	227	30	>4	2.2	110
22	$CH_2CH{=}CH_2$	Z	31	117	10	>4	2.6	960
23	$CH_2CH{=}CH_2$	E	187	>1024	243	>4	>4	>1000
24	Ph	Z	>1024	>1024	>1024	>4	>4	320
25	Ph	E	>1024	>1024	>1024	>4	>4	1800
26	Thiofanox	Z	26	9	1.4	0.51	0.09	9.5
27	Thiofanox	E	56	30	3.4	3.2	1.0	960

1. BB = bean beetle; M = two spotted spider mite; A = bean aphid; MS = mite systemic; and AS = aphid systemic. 2. Electric eel acetylcholinesterase.

Table III. Insecticidal Activity of the Sulfur Oxides of 3,3-Dimethyl-1,1-bis(methylthio)-2-butanone O-[(Methylamino)carbonyl]oxime



No	n/m	Diastereomer	Configuration	LC_{50}, ppm BB[1]	M[1]	A[1]	LC_{50}, Kg/Ha MS[1]	AS[1]	I_{50}^{2} x 10^7 M
20	0/0	–	Z	30	37	4	>4	0.9	15
28	1/0	a	Z	44	32	3.8	>4	1	580
29	1/0	a	E	350	>1024	117	>4	>4	>1000
30	2/0	–	Z	4	8.6	5.2	1.6	3.1	74
31	1/1	a	Z	8.5	124	18	>4	2.0	>1000
32	1/1	b & c	Z	11	40	14	>4	2.5	2500
33	2/1	a	Z	2.4	48	12	>4	>4	>1000
34	2/2	–	Z	>128	>128	85	>4	2.9	>1000
26	Thiofanox		Z	26	9	1.4	.51	.09	9.5

1. BB = Mexican bean beetle; M = two spotted spider mite; A = black bean aphid; MS = mite systemic; AS = aphid systemic. 2. Electric eel acetylcholinesterase.

thiofanox. The largest difference between the isomeric pairs was noted for compounds **18** and **19** on aphids while the smallest difference, 6-fold, was observed over several species and several isomeric carbamate pairs. The most active compound, (*Z*)-3,3-dimethyl-1,1-bis(methylthio)-2-butanone *O*-[(methylamino)carbonyl]oxime (**20**), was slightly less active than the standard (*Z*)-thiofanox (**26**), and it had the same spectrum of activity.

The insecticidal activity of the sulfur oxides of the *E* and *Z* isomers **20** and **21** are summarized in Table III. A comparison of the *E* and *Z* isomers of the monosulfoxides **28** and **29** continues to show a significant difference in their insecticidal activity. On the aphid the activity difference is really a case of having one isomer active while the other is not. It should be noted that each sulfoxide has two diastereomeric forms. Only one diastereomer (arbitrarily designated **a**) for each isomer was tested. The absolute configurations of the asymmetric centers in these diastereomers is not known. However, in the case of the disulfoxide where three possible diastereomers of the *Z* isomer are possible, the insecticidal activity of diastereomer **a** was not significantly different from the activity of a mixture of diastereomers **b** and **c** (ratio 3:1, respectively). Thus, the observed difference in biological activity appears to result from configurational differences in the oxime portion of the molecule rather than diastereomeric differences resulting from S-oxidation. Further work is necessary, however, to clarify this point.

In general, the insecticidal activity increases with oxidation of the sulfur atoms. The maximum activity appears to occur in the monosulfone of the *Z* isomer, i.e., compound **30**. Compound **30** was as potent as the standard (*Z*)-thiofanox on the bean beetle (BB), mite (M), and aphid (A) but it had systemic activity inferior to that of thiofanox. Complete oxidation of the sulfur atoms caused significant loss in activity as shown by the activity of **34**.

The results of the cholinesterase inhibition are also presented in Tables II and III. Of all the carbamates tested, **18** was the best acetylcholine esterase inhibitor having an I_{50} equal to 6.3×10^{-7} M. The corresponding *E* isomer, **19**, was considerably less active, as was the case for all *Z*/*E* isomeric pairs examined. (*Z*)-Thiofanox (**26**) was moderately more active than the corresponding bis(methylthio) derivative **20**.

The decreasing order of activities for the *Z* isomers of *N*-R substituents was H > CH_3 > Ph > allyl. Although the NH_2 carbamate moiety displayed the greatest in vitro inhibition, compounds of this class are relatively inactive in in vivo insecticidal bioassays (Magee and Limpel, 1977). Rapid reactivation of the carbamylated enzyme through hydrolysis was apparent in the present in vitro assays and probably accounts for the lack of insecticidal activity.

The oxidation state of the bis(methylthio) group strongly affects in vitro inhibition. The order of decreasing activity was –S– > $-SO_2-$ > –SO– for the *Z* isomer. Oxidation of the remaining sulfur atom further decreases the activity. A comparison of the diastereomeric pairs **31** and **32** was inconclusive due to the poor inhibitory properties of the disulfoxide substitution.

In summary, a new series of bis(methylthio)-3,3-dimethyl-2-butanone oxime carbamates is reported. Examination of the insecticidal data of the *E* and *Z* isomers of these carbamates indicated that the *Z* isomer is significantly and consistently more active than the *E*. This is the first report of large differences between the *E*/*Z* isomeric pairs of ketoxime carbamates.

Supplementary Material Available: Analytical and spectral data (^{1}H NMR, ^{13}C NMR, IR, and MS) (6 pages). Ordering information is given on any current masthead page.

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Insecticide Inhibition of Growth and Patulin Production in *Penicillium expansum*, *Penicillium urticae*, *Aspergillus clavatus*, *Aspergillus terreus*, and *Byssochlamys nivea*

Frances A. Draughon*[1] and John C. Ayres

Since patulin is produced by a wide variety of fungi under natural conditions, experiments were undertaken to determine (1) if the inhibition of naled, Sevin, pyrethrum, and methoxychlor was species dependent and (2) if the breakdown products of naled were active inhibitors of patulin production. At 100 ppm, naled completely inhibited patulin production and growth by the fungi *Penicillium expansum*, *Penicillium urticae* NRRL 1952, *Penicillium urticae* NRRL 994, *Byssochlamys nivea*, *Aspergillus clavatus*, and *Aspergillus terreus*. Sevin (100 ppm) inhibited patulin production by 16.6, 80.3, 100, 81.7, 31.5, and 89.5%, respectively, by these fungi. Pyrethrum (100 ppm) inhibited production of patulin in these fungi by 33.3, 1.2, 59.4, 34.9, 59.2, and 90.9%, respectively. Methoxychlor did not significantly inhibit patulin production. Six breakdown products of naled were important contributors to the effectiveness of naled as an inhibitor of patulin production.

The mycotoxin patulin [4-hydroxy-4*H*-furo[3,2-*c*]-pyran-2(6*H*)-one] is a carcinogenic heterocyclic lactone produced by species of *Aspergillus*, *Penicillium*, and *Byssochlamys*. Originally isolated as an antibiotic (Karow and Foster, 1944), patulin is toxic to experimental animals and carcinogenic to rats (Dickens and Jones, 1961). Patulin is stable during processing in products such as cooked cornmeal mush (Lieu and Bullerman, 1977) and grape and apple juices (Scott and Somers, 1968). Patulin is not stable in high protein products (Ciegler et al., 1972) although Ciegler et al. (1976) reported that teratogenicity of patulin adducts may be retained. Patulin represents a potential health hazard to humans because of its widespread occurrence as a contaminant of agricultural products, especially apples and apple products (Stoloff, 1975).

The presence of patulin and other toxic and carcinogenic mycotoxins in foods and feedstuffs has led to extensive research concerning chemicals which inhibit fungal growth and/or mycotoxin production. Draughon and Ayres (1978) reported that several commonly used insecticides inhibit production of the mycotoxin citrinin. The insecticide dichlorvos completely inhibits aflatoxin production at a concentration of 10 ppm (Rao and Harein, 1973; Hsieh, 1973), and zearalenone production and patulin production are completely inhibited by 100 ppm of the insecticide naled (Berisford and Ayres, 1976; Draughon and Ayres, 1979). In addition, the effectiveness of various acids and salts such as the benzoates, malonates, propionates, and acetates as antifungal agents is well documented (Uriah and Chipley, 1976; Stewart et al., 1977).

Table I. Fungal Strains Tested for Inhibition of Patulin Production

organism	strain	media[b]	source
P. (urticae)[a] *patulum*	NRRL 994	PDB	University of Georgia, Athens, GA
P. (urticae) patulum	NRRL 1952	PDB	A. Ciegler, NRRL, Peoria, IL
P. expansum	NRRL 2304	PDB	A. Ciegler
A. clavatus	NRRL 1980	YES	A. Ciegler
A. terreus	NRRL 255	YES	A. Ciegler
B. nivea	NRRL 2615	Czapek-Dox	University of Georgia, Experiment, GA

[a] The culture *P. patulum* will be referred to as *P. urticae* since the designation *P. patulum* is no longer considered a valid species by taxonomists. [b] See the text for formula modifications.

The organophosphate family of insecticides has been studied as potential inhibitors of mycotoxin production due to the effectiveness of certain of its members and because they have a very short half-life in the environment. Subsequently, they do not accumulate or persist in foodstuffs for any lengthy period of time and the problems associated with toxicity are avoided. However, the organophosphate insecticide naled demonstrated complete inhibition of patulin production in cultures treated for 30 days at an initial concentration of 100 ppm in stationary culture media (Draughon et al., 1980). After 7 days, less than 1 ppm of naled was present in the culture media. Since the prolonged inhibition of patulin production following the breakdown of naled in the culture was unexplained, studies were undertaken to determine if the breakdown products of naled are the active inhibitors of

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