tained 2.6% lipid, endosperm 6.2%, and the average for scutellum plus embryonic axis was 19.7%. This is in good agreement with our data from James Hulless; hulls 4.4%, endosperm 7.1%, and embryo 21.2%. The fatty acid analyses of Dal and Froker were run on free lipids (mainly neutral lipids) and bound lipids (mainly glycolipids and phospholipids). The neutral lipids in the endosperm, embryonic axis, and scutellum of Dal oats had a fatty acid distribution similar to that of James Hulless (Table III) and reported previously for Chief oats (Price and Parsons, 1975). Oats have lower amounts of the polyunsaturated fatty acids, linoleic and linolenic, than barley (Table III) and (Price and Parsons, 1975) and are higher in saturated palmitic acid. If the amount of unsaturated acid present can be directly related to oil quality, barley is superior to oats in this aspect.

The results of this study suggest that genetic improvement in the lipids of barley need only be quantitative as it relates to oats. The hull and bran–endosperm of barley contain less lipid than comparable fractions of oats. The barley hull is much thinner and lighter than the oat hull, so the opportunity for a substantial increase in percentage distribution in this fraction is severely limited. The barley kernel is heavier and has a larger bran–endosperm than that of oats. It is to this fraction that attention will be directed to achieving an increase in the lipid content of barley. Nuclear magnetic resonance spectroscopic (NMR) analyses (Price, unpublished data) of over 17 000 entries in the U.S. Department of Agricultural World Collection of Barley indicate that genetic increase in lipid content to 5% may be possible. If so, the caloric content and nutritional value of barley can be increased and its competitive stance with other feed grains improved.

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On Chemical Problems of the Mechanism of Action of the Chlorothiobenzamides

Müfit Bahadir,* Siegfried Nitz, Harun Parlar, and Friedhelm Korte

Both the direct and the photoinduced oxidation reactions of chlorine-substituted thiobenzamides (**1b–3e**) have been investigated. Asymmetric chlorophenylthiadiazoles (**4b–4e**) were identified as the principal transformation products. Oxidation of 2,6-dichlorothiobenzamide (**1e**) with nitrous acid unexpectedly afforded the corresponding 2,6-dichlorophenyl mustard oil (**3e**), which has a strongly enhanced herbicidal action.

Chlorine-substituted benzoic acid derivatives are among the most important systemic-action leaf and root herbicides (Linser, 1956). 2,6-Dichlorothiobenzamide (**1e**) especially has achieved great importance because of its excellent biological properties. The mechanism of action of these compounds is attributed primarily to the rapid formation of the corresponding nitriles (Wegler, 1977), which are known to display a strong herbicidal activity. This also explains the stronger action of 2,6-dichlorothiobenzamide (**1e**) relative to 2,6-dichlorobenzamide. The present work attempts to characterize the possible biologically active transformation products with the aid of the direct and the photoinduced oxidation reactions of the thiobenzamides (**1a–1e**). Determination of the herbicidal activity of these compounds should provide information about whether perhaps products other than nitriles (**2a–2e**) are responsible for the activity of the thiobenzamides (**1a–1e**) in the environment.

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MATERIALS AND METHODS

Chemicals. Thiobenzamide (**1a**), *p*-chlorobenzamide, and *o*-chlorobenzonitrile (**2b**) were purchased from E. Merck, Germany, while 2,6-dichlorobenzonitrile (**2e**) was obtained from Ega-Chemie. *o*-Chlorothiobenzamide (**1b**), *m*-chlorothiobenzamide (**1c**), and 2,6-dichlorothiobenzamide (**1e**) were prepared from the corresponding nitriles (**2b** and **2e**) by reaction with hydrogen sulfide in pyridine, while *p*-chlorothiobenzamide was prepared from *p*-chlorobenzamide with phosphorus pentasulfide in toluene.

Preparation of the Thiadiazoles (4a–4d). Twenty milliliters of a 10% solution of hydrogen peroxide was added drop by drop to a stirring suspension of 4 g of the relevant thiobenzamide (**1a–1d**) in 100 mL of 5% HCl at room temperature. After an hour the yellow precipitate

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Table I. Pnysical Data of the Compounds 3e and 4a-4d

compd	mp, °C	t_R, min	R_f[a]
3e	44	10.0[b]	0.47
4a	86	5.4	0.53
4b	86	17.6	0.49
4c	122	18.0	0.52
4d	163	19.2	0.56

[a] Hexane-ethyl acetate (2:1). [b] Column temperature, 120 °C; 3% OV-101.

was filtered off, washed with water, and dissolved in dichloromethane. The simultaneously formed sulfur was separated by filtration. The filtrate was dried over $MgSO_4$, concentrated on a rotatory evaporator, and recrystallized from aqueous methanol (yield, 40–60%).

Preparation of 2,6-Dichlorophenyl Isothiocyanate (3e). Ten milliliters of a 5% solution of aqueous sodium nitrile was added dropwise to an ice-cooled suspension of 1 g of 2,6-dichlorothiobenzamide in 30 mL of concentrated hydrochloric acid. The reaction mixture was slowly warmed up to room temperature and after 2 h diluted with water to 100 mL and extracted twice with chloroform. Isolation of the reaction products was carried out by column chromatography (silica gel 0.06–0.20 mm, Merck, Darmstadt) with hexane. The yield of isothiocyanate was 30%. Less than 2% thiadiazole (**4e**) was identified as byproduct. Table I shows the physical data of compounds **3e** and **4a–4d**.

Chromatography. Routine investigations were carried out with a Carlo Erba Fractovap Model 2101 AC gas chromatograph (column length 2 m, diameter 4 mm; 3% SE-30 on Chromosorb W-AW-DMCS, 80–100 mesh; injector temperature, 250 °C; detector temperature, 250 °C; carrier gas, nitrogen, 30 mL/min). For thin-layer chromatography, silica gel 60 TLC plates with a layer thickness of 0.25 mm (Merck, Darmstadt) were used with hexane–ethyl acetate (2:1) as developing solvent system.

Spectroscopy. Mass spectra of gas chromatographically pure substances were obtained by the direct inlet technique and of mixtures by combined GC–MS with an LKB 9000 S instrument (column length, 2 m, diameter, 4 mm; 3% OV-1 on Chromosorb W-AW-DMCS) at 70 eV.

The IR spectra were recorded as KBr pellets on a Perkin-Elmer Model 577 instrument. ^1H and ^{13}C NMR spectra were recorded on R 24 Perkin Elmer and Varian CFT 20 spectrometer, respectively. $CDCl_3$ was used as solvent and $(CH_3)_4Si$ as internal reference.

Irradiation Experiments. Solutions (2×10^{-2} M) of the corresponding thioamides or thiadiazoles in tetrahydrofuran were irradiated with an HPK 125 or Philips high-pressure mercury lamp.

For the irradiations in the adsorbed phase silica gel (0.3–0.06 mm; Merck, Darmstadt) or flower-growing soil containing much humus was used. Application of the substances was performed by mixing a solution of 100 mg of the corresponding thiobenzamide in acetonitrile with the solid materials and removing the solvent under reduced pressure.

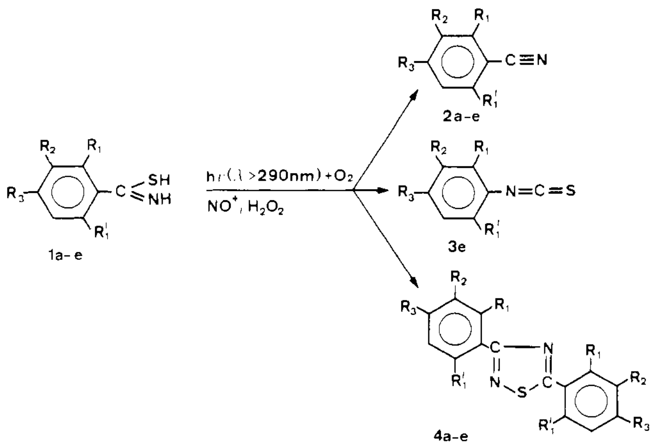
RESULTS

We were able to show from our laboratory investigations that the thiobenzamides (**1a–1e**) react to 1,2,4-phenylthiadiazoles (**4a–4e**) when UV light ($\lambda > 290$ nm) acts on them in the presence of oxygen. These compounds are also formed by direct oxidation with hydrogen peroxide and with nitrous acid, benzonitriles (**2a–2e**) being formed as well under these reaction conditions. The most important product of this series, 2,6-dichlorothiobenzamide (**1e**), is an exception here because with nitrous acid it surprisingly reacts to form the isothiocyanate (**3e**) (Figure 1). UV irradiation of the thiobenzamides (**1a–1e**) in tetrahydrofuran and in the soil under a nitrogen atmosphere points to the essential role of oxygen on these photoreaction, since no thiadiazole (**4a–4e**) are formed under these conditions. The behavior to UV light of the compounds **1a–1e** on sufaces seems to be of interest. When adsorbed on silica gel the thiobenzamides (**1a–1e**) show no change even after several hours of irradiation with UV light. In contrast, as already mentioned, the photooxidation afforded the thiadiazoles (**4a–4e**) in identifiable amounts if the compounds are irradiated with UV light when adsorbed on soil.

The structure determination of compounds **2a–2e** and **3e** was carried out with the aid of reference substances. Both the physical properties (mp, t_R, R_f) and the spectroscopic data (IR, MS, ^1H and ^{13}C NMR) agreed with those of the authentic samples. In contrast, the satisfactory structure determination of the compounds (**4a–4e**) is problematic. In principle, four theoretically possible thiadiazoles can be anticipated (Figure 2). The symmetrical representatives of this substance class, 1,2,5-thiadiazole and 1,3,4,-thiadiazole, can be excluded reliably because the ring C atoms (C_3, C_4 and C_2, C_5) should give identical ^{13}C NMR peaks in both cases. In fact, different signals are found in the spectra of the relevant compounds

Table II. Spectroscopic Data of the Compounds 3e and 4a-4d

compd	MS data, m/e (rel intensity, %)	IR data (KBr), cm^{-1}	^1H NMR data chemical shifts, δ		^{13}C NMR data chemical shifts, δ
3e	203 (100)	3060, 2340, 2100	3 H (7.25, m)		127.35, 128.25, 132.33
	171 (11)	2030, 1540, 1420			
4a	238 (28)	3030, 2330, 1495	2 H (8.38, m)	(Ph)	127.56, 128.48, 128.68
					129.27, 130.32, 130.92
	135 (100)	1465, 1430, 1400	2 H (8.03, m)		131.84, 133.11
	103 (31)	1320	6 H (7.50, m)	(C_3, C_5)	173.94, 188.17
4b	306 (22)	3050, 2330, 1580	1 H (8.63, m)	(Ph)	126.75, 127.47, 129.69
					130.42, 130.73, 130.93
					132.09, 132.31, 133.44
	169 (100)	1480, 1450, 1420	1 H (8.02, m)		133.84
	137 (22)	1395, 1300	6 H (7.45, m)	(C_3, C_5)	169.85, 183.04
			1 H (8.37, s)	(Ph)	125.62, 126.45, 127.33
4c	306 (40)	3050, 2330, 1590	1 H (8.23, m)		128.54, 129.92, 130.49
			1 H (8.06, s)		131.91, 132.11, 134.31
	169 (100)	1570, 1490, 1460	1 H (7.90, m)		134.85, 135.53
	137 (40)	1430, 1390, 1300	4 H (7.47, m)	(C_3, C_5)	172.50, 185.37
4d	306 (34)	3040, 2330, 1585	2 H (8.26, d)	(Ph)	128.70, 129.00, 129.17
			2 H (7.92, d)		129.72, 131.32, 136.69
	169 (100)	1460, 1410, 1390	2 H (7.44, d)		138.27
	137 (31)	1300	2 H (7.42, d)	(C_3, C_5)	172.89, 187.05



Compound	R_1	R_1'	R_2	R_3
1a-4a	H	H	H	H
1b-4b	Cl	H	H	H
1c-4c	H	H	Cl	H
1d-4d	H	H	H	Cl
1e-4e	Cl	Cl	H	H

Figure 1. Photoinduced and direct oxidation reactions of thiobenzamides **1a**–**1e**.

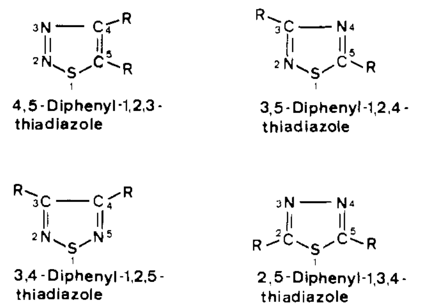


Figure 2. Structure alternatives of present thiadiazoles.

(Table II). On the other hand, 1,2,3- and 1,2,4-thiadiazoles cannot be easily distinguished by means of their spectroscopic data. A decision in favor of 1,2,4-thiadiazole may be made on the basis of the work by Mack (1967), who prepared compound **4a** by reacting benzonitrile (**2a**) with sulfur in the presence of tri-*n*-octylamine. The same compound (**4a**) was also prepared by Howe and Franz (1974) by thermology of 1,3,4-oxathiazol-2-one. Kresze et al. (1965) have also described an elegant method for preparing 1,2,4-thiadiazoles, involving the reaction of some thioamides with *N*-sulfinyl-*p*-toluenesulfonamide. The physical and spectroscopic data obtained in the above work agree satisfactorily with those of **4a** and confirm the structures of the compounds **4a**–**4e** proposed here. In the literature the following possible routes of phenylthiadiazole formation from the corresponding thiobenzamides have been proposed (Figure 3). One formulation is the oxidation of the thioamides to thioamide *s*-oxides or similar intermediates as the first step, followed by cyclization with unreacted thioamide (pathway A) (Kitamura and Suzuki, 1937). Alternatively, a 1,3-dipolar cycloaddition is postulated in which the intermediately formed [RC≡N→S) react with the nitriles of the 1,2,4-thiadiazoles (pathway B) (Howe and Franz, 1974). The results of our product analysis show that during oxidation of the thiobenzamides (**1a**–**1e**) both nitriles and sulfur are formed. Whether these findings are adequate for confirming the latter route (pathway B) cannot be stated with certainty.

The herbicidal activity determination show clearly that compound **3e**, unlike the thiadiazoles (**4a**–**4d**), has an excellent herbicidal action (Table III). It follows that the

Table III. Herbicidal Activity of the Compounds 3e and 4a–4d[a]

		phytotoxicity rating (0–9)																								
		postemergence (plants)																seeds								
		soil drench, 10/kg/ha								foliar spray								preemergency								
compd	dose, kg/ha	Mz	R	BG	O	L	M	SB	S	Mz	R	BG	O	L	M	SB	S	Mz	R	BG	O	L	M	SB	S	LD_{50}, mg/kg, A.O.M.
3e	5	8	9	8	9	8	8	8	8	4	3	2	3	0	0	6	3	5	5	9	9	9	9	9	9	
	1																									
4a	5	0	2	3	2	0	2	3	3	0	0	2	3	3	5	3	5	0	0	0	0	0	0	0	0	~250
	1										0	0	2	1	4	3	2	0	0	0	0	0	0	0	0	
4b	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	>250
	1									0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
4c	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	>250
	1									0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
4d	5	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	>250
	1									0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

[a] Mz, maize; R, rice; SB, sugar beet; BG, barnyard grass; O, oats; L, linseed; M, mustard; S, soya bean.

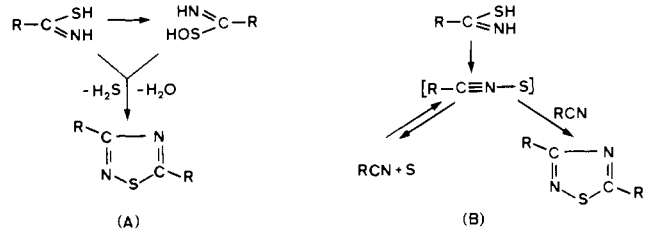


Figure 3. Possible modes of formation of the 3,5-diphenyl-1,2,4-thiadiazoles.

hitherto generally held opinion of the action of 2,6-dichlorothiobenzamide (**1e**) being due entirely to the formation of 2,6-dichlorobenzonitrile (**2e**) is only of limited validity.

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Studies on the Effect of Heat on the Dissociation, Denaturation, and Aggregation of Sesame α-Globulin

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The protein α-globulin, the major fraction of sesame seed (*Sesamum indicum* L.) proteins, coagulates on heating. Dissociation, denaturation, and aggregation of the protein upon heating have been studied by gel filtration, polyacrylamide gel electrophoresis, sedimentation velocity, pK_{app} of tyrosyl groups, and fluorescence measurements. The addition of β-mercaptoethanol does not reduce the extent of heat coagulation. The reassociation of the heat denatured subunits through hydrophobic interaction results in the formation of insoluble precipitate.

Sesame seed (*Sesamum indicum* L.) is a source of nutritionally important proteins due to their relatively high methionine content. The protein upon heating results in precipitation which restricts its use in certain food formulations, e.g., milk extender or beverage formulation. Recently, we have reported the association–dissociation and denaturation behavior of the major constituent (65–70%) α-globulin of sesame protein in different solutions (Prakash and Nandi, 1976, 1977a,b,c, 1978; Lakshmi and Nandi, 1977, 1978). In the present paper we report a study of the sequence and mechanism of heat aggregation of the protein.

MATERIALS AND METHODS

The protein α-globulin was isolated from sesame seeds (*Sesamum indicum* L., white variety) following the procedure developed in this laboratory (Prakash and Nandi, 1978). The total protein extract in 1 M NaCl obtained from defatted sesame flour was diluted 1:5.5 times with distilled water when α-globulin with some other protein fraction precipitated. The redissolution of the precipitate in 1 M NaCl, followed by dilution as above, yielded a protein which was found to be homogeneous (~95%) by gel electrophoresis, sedimentation analysis, and DEAE-cellulose chromatography (Prakash and Nandi, 1978). Phosphate buffer prepared from reagent grade chemicals and Tris (hydroxymethylaminomethane) obtained from Sigma were used in most of the experiments. Sepharose 6B-100 (Sigma) and urea (Sarabhai M. Chemicals) were used. $NaDodSO_4$ (Hindustan Levers) was crystallized twice from ethanol.

Heat coagulation experiments were carried out with protein solution in Tris–HCl buffer of pH 8.6 at 98 °C for 20 min. The absorbance of the supernatant was measured at 280 nm. The percentage of protein precipitated was determined by calculating the amount of protein present in the supernatant compared to the initial concentration of the protein solution. Protein concentration was calculated using $E_{1cm}^{1\%} = 10.8$.

A Sepharose 6B-100 column 46 × 2.5 cm (bed volume, $V_t \sim 200$ mL), was used for gel filtration experiments. The gel was equilibrated thrice the bed volume of the column with Tris–HCl buffer, 0.01 M, pH 8.6. The flow rate after loading the protein solution was adjusted to 18–20 mL/h and the protein concentration in the fractions was determined by measuring the absorbance at 280 nm.

Polyacrylamide gel electrophoresis (PAGE) was carried out in a Metrex gel electrophoresis unit using 0.02 M phosphate buffer at pH 7.5. A 10% gel in tubes having 7.5 × 0.5 cm dimensions was used. Protein samples (10 μg/μL) containing ~5% sucrose and 0.05% bromophenol blue (indicator dye) were used, and electrophoresis was carried out at a constant current of 3 mA/tube for 1 h and 40 min. The gels were stained for 45 min in 0.5% amido black in 7.5% (v/v) acetic acid, and destaining was carried out in 7.5% acetic acid solution.

Sedimentation velocity values were measured in a Spinco Model E analytical ultracentrifuge equipped with phase plate schlieren optics. A standard 12-mm duraluminum cell centerpiece was used. Plates were read on a Gaertner microcomparator and $s_{20,w}$ values calculated

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