Table II. TMSOTf-Promoted Annulation of 4-Oxoheptanal with 4

entry	product	R_1	R_2	% isoltd yield (5 + 6)[a]	diastereoselectivity (5:6)[b]
1	**5a + 6a**	Me	Me	73	>40:1
2	**5b + 6b**	Me	*t*-Bu	80	1.3:1
3	**5c + 6c**	Me	OEt	76	1:>35
4	**5d + 6d**	*i*-Pr	OEt	73	1:25
5	**5e + 6e**	$-CH_2CH_2O-$		75	1:1.3

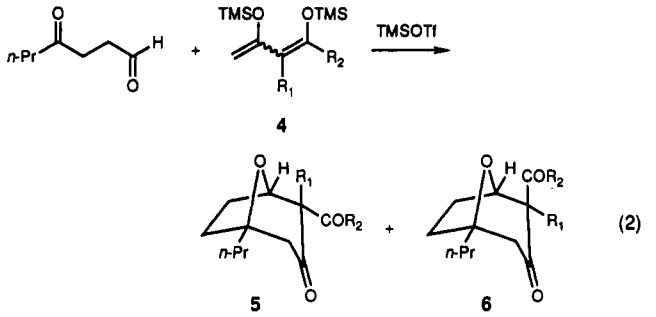
[a] Refers to yields of purified products. All of these compounds have been fully characterized spectroscopically (^1H NMR, ^{13}C NMR, IR), and elemental composition has been established by combustion analysis and/or exact mass. [b] Diastereoselectivities and regioselectivities were determined by fused silica capillary GLC analysis.

results suggested that the origin of unusual chemoselectivity was much more complex in nature than we had originally anticipated.

Stereoselectivity in the process was next examined by utilizing chiral, racemic 1,4-keto aldehydes in conjunction with **2** and TMSOTf.[11] The observed stereoselectivities of the annulation products (5.4:1 to >160:1, Table I, entries 9–14) were much higher than that obtained in the intermolecular reaction of **2** with a simple acyclic ketone like 3-methyl-2-pentanone (1.8:1, 56% yield). Another interesting feature of the annulation process is that the relative 1,3-asymmetric induction (Table I, entries 12–14) appears to be as good as, if not better than, 1,2-asymmetric induction (Table I, entries 9–11).[12]

On the basis of the remarkable trends in chemoselectivity and stereoselecivity delineated previously, a plausible mechanism invoking a unique neighboring group participation is outlined in Scheme I. Activation of the *less hindered* carbonyl by TMSOTf[13] followed by intramolecular participation by the remaining, *more hindered* carbonyl forms an electrophilic oxonium ion. Nucleophilic attack of the terminal carbon of **2** at the electron-deficient, more hindered carbon center followed by cyclization explains the remarkable regiochemistries observed for unsymmetrical 1,4-diketones. The unusual sense and relative magnitudes of the diastereoselectivities obtained in this reaction is also rationalized by initial formation of a cyclic intermediate.[14]

Stereoselectivity engendered in the [3 + 4] annulation reaction of α-substituted β-dicarbonyl dianionic synthons **4** with 4-oxoheptanal in the presence of TMSOTf was also studied (eq 2). One unusual feature of these reactions is



(2)

that the product derived from the reaction of 3-substituted bis(trimethylsilyl) enol ethers of β-keto esters (entries 3 and 4, Table II) has the opposite relative stereochemistry as that obtained by reaction of 3-substituted bis(trimethylsilyl) enol ethers of β-diketones (entry 1, Table II).[15] While the origin of this phenomenon is as yet unknown, the ability to access both stereoisomeric product manifolds with functionalized quaternary stereogenic centers is impressive.[16]

In summary, TMSOTf promotes the [3 + 4] annulation of bis(trimethylsilyl) enol ethers with a variety of 1,4-dielectrophiles. Unusual chemoselectivities and diastereoselectivities are observed in these transformations. A mechanism involving neighboring group participation has been invoked to account for these remarkable results. Further mechanistic and synthetic studies are underway to elucidate the scope and nature of these reactions.

Acknowledgment. We thank the National Science Foundation for their generous support of this research. We are also grateful to Curt Haltiwanger for obtaining the X-ray crystal structure data.

Supplementary Material Available: Complete experimental details and spectral data for all of the annulations described herein. Details of the X-ray crystallographic structural determination and NMR assignments pertaining to the determination of product regio- and diastereoselectivities (26 pages). Ordering information is given on any current masthead page.

(11) Diastereoselectivities were determined by first derivatizing the resulting bicyclic keto ester (**3**) to the enol acetate, thereby eliminating the methoxycarbonyl stereocenter.

(12) Stereochemistry at C-7 was determined from ^1H NMR coupling constants. The bridgehead proton (H-1) couples only to the exo substituent at C-7. H-1 in the major diastereomer (R^3 exo) appeared as a singlet (**3l–n**), but in the minor diastereomer (R^3 endo) it appeared as a doublet (J = 5.9 Hz, **3l**). An X-ray crystal structure of **3j** allowed stereochemical determination of that compound.

(13) (a) Maruoka, K.; Araki, Y.; Yamamoto, H. *Tetrahedron Lett.* **1988**, *29*, 3101. (b) Maruoka, K.; Araki, Y.; Yamamoto, H. *J. Am. Chem. Soc.* **1988**, *110*, 2650. (c) Maruoka, K.; Nagahara, S.; Yamamoto, H. *J. Am. Chem. Soc.* **1990**, *112*, 6115. (d) Maruoka, K.; Nagahara, S.; Yamamoto, H. *Tetrahedron Lett.* **1990**, *31*, 5475.

(14) A similar trend of 1,2- and 1,3-relative asymmetric induction was observed for nucleophilic addition of allylsilanes to substituted 2-hydroxytetrahydrofurans. Schmitt, A.; Reissig, H. *Synlett* **1990**, *1*, 40.

(15) An example of the method utilized for stereochemical determination is given. The bicyclic compound derived from annulation of the bis(trimethylsilyl) enol ether of 2,4-pentanedione with 4-oxoheptanal was prepared. Alkylation (NaH, MeI, THF, 0 °C to rt) provided the 2-*exo*-methyl-substituted bicyclic compound, which had physical and spectral characteristics identical with **5a**.

(16) Isolated products resubjected to the reaction conditions shown no sign of epimerization. Consequently, the reaction appears to be kinetically controlled.

Metabolic Transformation of the Phytoalexin Brassinin by the "Blackleg" Fungus

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Summary: The metabolism of a phytoalexin from *Brassica* species, brassinin (**1**), by the phytopathogenic fungus *Phoma lingam* was investigated and the unusual structure of the first metabolic intermediate was assigned as methyl (3-indolylmethyl)dithiocarbamate *S*-oxide (**2**) based on spectroscopic data and synthesis of a methyl derivative.

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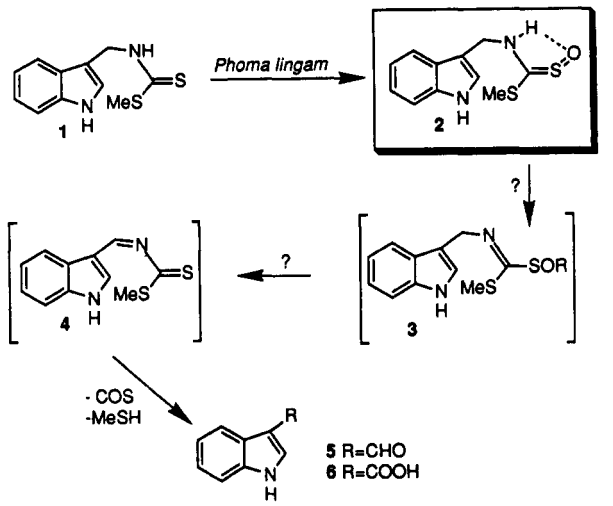
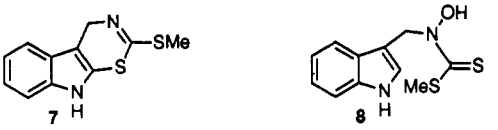
Dithiocarbamates have long been known as a very important class of bioactive products.[1] Interestingly, a novel class of phytoalexins,[2] containing a dithiocarbamate group attached to a 3-methylindolyl moiety, has been recently reported.[3-5] These phytoalexins are produced by plants from *Brassica* species (e.g. rapeseed, canola, broccoli, cabbage, turnip, etc.) in response to stress. In general, phytoalexins are produced in response to pathogen infection as a defense mechanism. The causative agent of "blackleg disease" of cruciferous plants (comprising *Brassica* species) is the fungal pathogen *Phoma lingam* (Tode ex Fr.) Desm. [asexual stage of *Leptosphaeria maculans* (Desm.) Cas. et de Not.], a serious agricultural problem worldwide. This pathogen produces a complex array of metabolites, some of which are phytotoxic and appear to be related to virulence.[6] Plants from diverse *Brassica* species show resistance to blackleg disease; however, there is no general understanding of this process or of the chemistry involved in this interaction. Regarding this last aspect, we have been studying the effect of *Brassica* phytoalexins on *P. lingam*. Herein we report novel aspects of the chemistry involved in the metabolism of a *Brassica* phytoalexin, brassinin (1), by a virulent isolate of *P. lingam*. The unusual structure of the dithiocarbamate *S*-oxide metabolic intermediate 2 and the chemistry involved in the structure elucidation of this compound are particularly noteworthy.

In preliminary experiments, the phytoalexins brassinin (1) and cyclobrassinin (7) were synthesized[4] and tested on various isolates of *P. lingam*.[7] Brassinin (1) was found to be twice as active as cyclobrassinin (7), and, therefore, it was chosen to initiate this study. *P. lingam* (highly virulent isolate Leroy, 10^8 spores/mL) was grown at 26 ± 2 °C in shake culture on minimal medium supplemented with thiamine.[8] After 48 h, brassinin (1) solutions in Me_2SO were fed to fungal cultures and uninoculated media (final concentration 5×10^{-4} M). Cultures were incubated and samples were withdrawn at 1–12-h intervals (up to 7 days), extracted first with Et_2O, and then with ethyl acetate. Extracts were analyzed by TLC and by HPLC to determine the optimum incubation time for isolation of the putative intermediates. Subsequently, chromatographic purification of ethereal extracts obtained from larger scale cultures afforded the so-called "metabolite A" (2, 4–5-h incubation), indole-3-carboxaldehyde (5, 12–18-h incubation), and indole-3-carboxylic acid (6, 24–30-h incubation) (Scheme I). The structures of compounds 5 and 6 were readily determined by comparison of their spectroscopic data with those of authentic samples.[9] The structure of metabolite A was assigned as methyl (3-indolylmethyl)dithiocarbamate *S*-oxide (2) on the basis of the following discussion. Brassinin (1) feeding experiments to various fungal cultures showed that metabolite A was

Scheme I



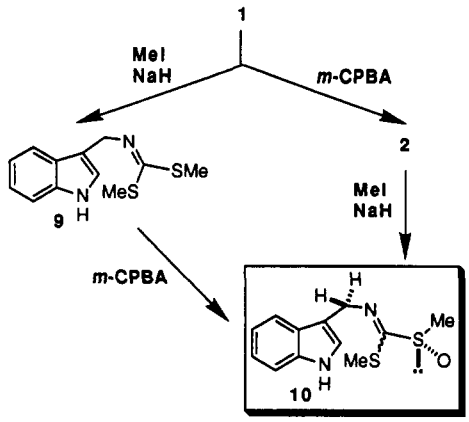
likely the first metabolic intermediate and was more rapidly metabolized than either brassinin (1) or aldehyde 5 or acid 6. The isolation of metabolite A in a multimilligram scale was not viable because of this fast transformation. However, sufficient sample was isolated and purified to obtain all of the spectroscopic data necessary for its structure elucidation.[10] The HRMS (plasmaspray)[11] of metabolite A indicated a molecular formula of $C_{11}H_{13}N_2OS_2$ ([M + 1]$^+$, 253.0469, found 253.0487) which represented addition of one oxygen atom to brassinin (1). The ^{1}H NMR spectrum of metabolite A showed only 11 hydrogens and indicated clearly that the structural differences between 1 and its oxidized metabolite resided in the side chain.[12] The resonances attributable to the five indolic hydrogens of metabolite A (2) were similar to the corresponding ones in 1. In contrast with 1 (CH_2 at δ 5.04, d, J = 4.5 Hz), the methylene hydrogens of metabolite A (2) appeared as a sharp single (δ 4.75) and the Me(S) hydrogens resonated at higher field (δ 2.45 for 2 vs δ 2.62 for 1). In the ^{13}C NMR spectrum, the resonances of metabolite A were comparable with those of 1 (within 1 ppm) except for the signals attributable to thiocarbonyl (δ 198.2 in 1 vs δ 193.7 in 2) and thiomethyl (δ 18.2 in 1 vs δ 12.7 in 2) groups.[13] The spectroscopic data of metabolite A appeared to be compatible with alternative structures 2 or 8. A third possible structure, having the oxygen on the



(Me)S group, was not compatible with both ^{13}C NMR and

(1) Thorn, G. D.; Ludwig, R. A. *The Dithiocarbamates and Related Compounds*; Elsevier Publishing Company: New York, 1962; pp 169–276.
(2) For recent reviews, see for example, (a) Brooks, C. J. W.; Watson, D. G. *Nat. Prod. Rep.* **1985**, 427–459. (b) *Phytoalexins*; Bailey, J. A., Mansfield, J. W., Eds.; John Wiley and Sons: New York, 1982. (c) VanEtten, H. D.; Mathews, D. E.; Mathews, P. S. *Annu. Rev. Phytopathol.* **1989**, *27*, 143–164.
(3) Takasugi, M.; Katsui, N.; Shirata, A. *J. Chem. Soc. Chem. Comm.* **1986**, 1077–1078.
(4) Takasugi, M.; Monde, K.; Katsui, N.; Shirata, A. *Bull. Chem. Soc. Jpn.* **1988**, *61*, 285–289.
(5) Monde, K.; Sasaki, K.; Shirata, A.; Takasugi, M. *Phytochemistry* **1990**, *29*, 1499–1500.
(6) Pedras, M. S. C.; Séguin-Swartz, G.; Abrams, S. R. *Phytochemistry* **1990**, *29*, 777–782.
(7) The active concentrations were determined by comparing the radial mycelial growth of *P. lingam* incubated on agar plates containing minimal media[8] and the phytoalexins 1 or 2 and control plates containing media.
(8) Tinline, R. D.; Stauffer, J. F.; Dickson, J. G. *Can. J. Bot.* **1960**, *38*, 275–282.
(9) Authentic samples were obtained from Aldrich Chemical Company, Inc., Madison, WI.
(10) Over several experiments, from a total amount of 400 mg of brassinin in 1.5 L of cultures, a total of 10 mg of metabolite A were obtained.
(11) This HRMS technique was used because EIMS gave a very weak molecular ion. EIMS m/z (relative intensity): 252 [M]$^+$ (0.3), 234 [M − 18]$^+$ (9), 161 [M − 91]$^+$ (46), 130 [C_9H_8N]$^+$ (100). CIMS (NH_3) m/z (relative intensity): 270 [M + 18]$^+$ (6), 253 [M + 1]$^+$ (78), 235 [M − 17]$^+$ (70), 205 [M − CH_3S]$^+$ (7), 162 [M − 90]$^+$ (30), 130 [C_9H_8N]$^+$ (100).
(12) ^{1}H NMR ($CDCl_3$, 360 MHz): δ 9.30 (br s, indole NH), 7.54 (d, J = 7.8 Hz, H-4), 7.35 (d, J = 8.1 Hz, H-7), 7.18 (t, J = 8.1 Hz, H-6), 7.11 (t, J = 7.5 Hz, H-5), 6.87 (br s, H-2), 4.75 (s, CH_2), 2.45 (s, CH_3). For complete NMR data for 1 see refs 3 and 4.
(13) ^{13}C NMR ($CDCl_3$, 90.5 MHz): δ 193.67 (s), 136.47 (s), 126.12 (s), 123.95 (d), 122.50 (d), 120.07 (d), 118.16 (d), 111.72 (d), 109.36 (s), 41.17 (t), 12.74 (q).

Scheme II



IR data. Firstly, the ^{13}C NMR resonance of the MeS group was located at higher field (δ 12.74) in metabolite A (**2**) than it was in brassinin (**1**) (δ 18.2). This result is contrary to expected shift increments increase caused by a higher oxidation state of sulfur.[14] Moreover, the α effect of a (C)(Me)S=O group should bring the α carbon (δ 198.2 in **1**) downfield,[15] whereas an upfield shift (δ 193.67 in **2**) was observed. Additionally, the IR spectrum did not show the expected strong absorption of a (Me)S=O group (1060–1050 cm^{-1}).[16,17] Structure **8** was initially favored because it would also account for the observed lack of coupling ($^2J_{HH}$) between the CH_2 and NH groups of metabolite A. Initial attempts to form a derivative of **2** by acylation or methylation were unsuccessful. Eventually, oxidation of brassinin (**1**) with *m*-CPBA gave a product which was identical in every respect with metabolite A. Having larger quantities of metabolite A in hand was crucial for unequivocal assignment of its structure. It was reasoned that metabolite A should form a derivative, whether it had structure **2** or **8**. Surprisingly, on attempted derivatization (under a variety of conditions, e.g. CH_3I/Et_2O, CH_3I, Ac_2O/pyridine, AcCl/pyridine, etc.), or even on standing at room temperature (in $CHCl_3$, CH_2Cl_2, or MeOH), metabolite A (**2**) decomposed to brassinin (**1**) and other undetermined products. These puzzling results explained the difficulties encountered previously while working on a "submilligram" scale. Ultimately, a methylated derivative (**10**) was obtained by treatment with CH_3I and NaH at 0 °C. Although a different product might be expected, **10** is the only structure accounting for the spectroscopic properties of the methylated metabolite A. Namely, the hydrogens of the CH_2 group present in **10** are diastereotopic due to the presence of the stereogenic (C)(CH_3)S=O group and appeared as an AB quartet in the ^{1}NMR spectrum.[18] To corroborate that assignment, brassinin (**1**) was methylated (CH_3I/NaH) and oxidized (*m*-CPBA, 0 °C) to yield **10**. These results are summarized in Scheme II. The oxidation of methylbrassinin (**9**) was a key experiment in the structure elucidation of metabolite A. Prior to this report, there had been only three other accounts on compounds containing a dithiocarbamate *S*-oxide group.[19–21] However, it should be noted that structure **2** represents a distinct type. The N-substituents, H and $ArCH_2$, will likely confer different reactivity to the group in question.[22] It is worthwhile to comment on derivative **10** and its formation, apparently unprecedented.[23] This reaction yielded virtually one product by TLC and HPLC. Nevertheless, both ^{1}H and ^{13}C NMR spectra showed doubled resonances for each atom (carbons and hydrogens), indicating the presence of two compounds (ca. 2:1).[18] Moreover, on cooling (0 °C), the proton resonances of **10** sharpened and certain signals resolved completely, whereas, on heating (60 °C), both the two AB quartets and methyl groups coalesced. These results might be caused by *E*/*Z* isomerization brought about by rotation of the C=N bond. Perhaps significantly, the oxidation of **9** furnished a ratio of isomers identical with that obtained by methylation of **2**. These results warrant further studies. On the other hand, metabolite A (**2**) could exhibit *E*/*Z* isomerism due to the C=S bond.[21] Considering the sharpness of its NMR resonances, it appeared that only one isomer was present and an *E* stereochemistry would allow intramolecular hydrogen bonding, as shown in Scheme I.

A possible pathway for brassinin degradation is depicted in Scheme I which shows two hypothetical intermediates, **3** and **4**. This pathway could explain the conversion of **2** to aldehyde **5**, with concomitant loss of COS and MeSH.[24] Preliminary experiments indicate that metabolite A (**2**) is metabolized two to three times faster than brassinin (**1**). It has been reported that fungal metabolism (detoxification) of potato, chickpea, and bean phytoalexins is related with pathogenicity.[2c] Perhaps this is also true for the metabolism of phytoalexins from *Brassica* species by the blackleg fungus. The carboxylic acid **6** is at least 10 times less toxic to *P. lingam* than phytoalexin **1**. We are currently investigating these aspects with the goal of devising more effective strategies for controlling blackleg disease.

Acknowledgment. We would like to thank Prof. D. E. Ward, University of Saskatchewan, for helpful discussions and Mr. B. Chatson, Mr. L. Hogge and Mr. D. Olson, Plant Biotechnology Institute, Saskatoon, for NMR and MS spectral determinations. NRCC no. 32460.

(14) Consider, for example, the ^{13}C NMR resonances of Me_2S (δ 19.1) and Me_2S=O (δ 40.0).[15]

(15) Breitmaier, E.; Voelter, W. *Carbon-13 NMR Spectroscopy*; VCH Publishers: New York, 1987; pp 233–234.

(16) The strongest absorption in the FTIR spectrum appeared at 895 cm^{-1} (C=S=O), although at that time it could not be conclusively assigned. FTIR: ν_{max} 3565, 1514 (s), 1426, 1335, 1238, 895 (s), 876 cm^{-1}. UV (MeOH): λ_{max} 230 (log ϵ 1.07), 280 (log ϵ 1.04), 290 (sh), 320 (log ϵ 0.80) nm.

(17) (a) Durst, T. In *Comprehensive Organic Chemistry*; Neville Jones, D., Ed.; Pergamon: Oxford, England; 1979; Vol. 3, pp 121–156. (b) Duus, F. In *Comprehensive Organic Chemistry*; Neville Jones, D., Ed.; Pergamon: Oxford, England; 1979; Vol. 3, pp 373–487.

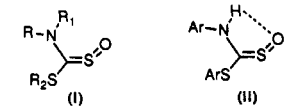
(18) ^{1}H NMR ($CDCl_3$, 15 °C, 360 MHz): δ 8.33 and 8.28 (2 br s, indole NH), 7.65 and 7.62 (2 d, J = 7.9 Hz), 7.37 and 7.36 (d, J = 7.9 Hz), 7.21–7.08 (m), 5.10 and 4.90 (AB q, J = 14.4 Hz, CH_2), 5.00 and 4.93 (AB q, J = 14.8 Hz, CH_2), 2.78 and 2.72 (2 s, (O)SCH_3 and SCH_3), 2.67 and 2.36 (2 s, (O)SCH_3 and SCH_3). EIMS *m*/*z* (relative intensity): 202 [M – CH_4SO]$^+$ (1), 155 [M – (CH_4SO + CH_3S)]$^+$ (5), 130 [C_9H_8N]$^+$ (100). CIMS (NH_3) *m*/*z* (relative intensity): 284 [M + 18]$^+$ (1), 267 [M + 1]$^+$ (5), 219 [M – CH_3S]$^+$ (4), 147 [M – 119]$^+$ (53), 130 [C_9H_8N]$^+$ (100).

(19) Walter, W.; Wohler, K. *Justus Liebigs Ann. Chem.* **1971**, *752*, 115–135.

(20) Segall, Y.; Casida, J. E. *J. Agric. Food Chem.* **1983**, *31*, 242–246.

(21) Watanabe, Y.; Ishimura, Y. *J. Org. Chem.* **1988**, *53*, 2119–2120.

(22) Compounds previously reported are of structure type i or ii.[19–21] Structures like ii appeared to be stable and were assigned *E* configuration,[19] whereas structures like i were reported to undergo spontaneous decomposition by deoxygenation,[20] except in one case.[21]



(23) A similar reaction can be found for sulfenate ions. See: Hogg, D. R. In *Comprehensive Organic Chemistry*; Neville Jones, D., Ed.; Pergamon: Oxford, England; 1979; Vol. 3, pp 261–310.

(24) GC-EIMS analysis of head-space volatiles of fungal cultures containing brassinin (**1**) indicated the presence of COS and MeSH during early stages of incubation (ca. 1–6 h). These volatiles were not detected in control cultures. A pathway involving (3-indolylmethyl)amine as an intermediate does not seem likely; (3-indolylmethyl)amine was not detected in cultures containing brassinin at any stage of incubation. In addition, this amine appears to be more toxic to the fungus than brassinin (**1**).