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Unprecedented Vilsmeier Formylation: Expedient Syntheses of the Cruciferous Phytoalexins Sinalexin and Brassilexin and Discovery of a New Heteroaromatic Ring System

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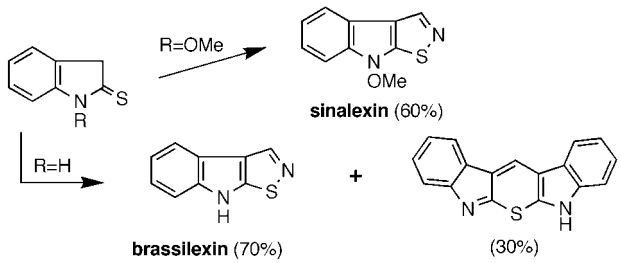
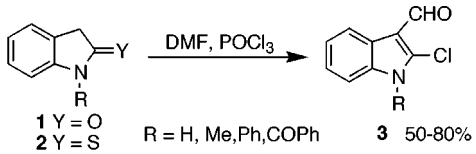
ABSTRACT



A very concise first synthesis of sinalexin was achieved by regioselective formylation of 1-methoxyindoline-2-thione under Vilsmeier conditions followed by unprecedented ammonia workup. Similar formylation of indoline-2-thione yielded brassilexin and a novel pentacyclic heteroaromatic compound resulting from condensation of the Vilsmeier adduct of indoline-2-thione. Both sinalexin and brassilexin displayed strong antifungal activity against several pathogens of crucifers.

The wide scope of the Vilsmeier−Haack formylation renders it an extremely useful reaction in organic synthesis. Since it was first reported in 1925, its application to a variety of both aromatic and heteroaromatic substrates is well-documented.[1] Nonetheless, this formylation reaction is only applicable to substrates more reactive than benzene. For example, the formylation of indole under Vilsmeier conditions occurred regioselectively in high yield, whereas the formylation product of benzo[*b*]thiophene was obtained in very poor yield.[1] Although the reactivity of substrates such as indolin-2-one (**1**) is expected to be lower than that of indole, a number of indolin-2-ones (**1**, R = H, Me, Ph, COPh) have been formylated to yield the corresponding 2-chloro-3-formyl indoles (Scheme 1).[1]

Scheme 1



On the other hand, indoline-2-thiones (**2**) do not appear to have been formylated under Vilsmeier conditions, whereas 2-thioethers such as 2-thiomethylindole were formylated in good yield.[2] Significant interest in the formylation of indolin-

(1) (a) Jones, G.; Stanforth, S. P. In *Organic Reactions*; Paquette, L. A., Ed.; Wiley: New York, 1997; Vol. 49, pp 1−330. (b) Downie, I. M.; Earle, M. J.; Heaney, H.; Shuhaibar, K. F. *Tetrahedron* **1993**, *49*, 4015.
(2) Pedras, M. S. C.; Khan, A. K. *J. Agric. Food Chem.* **1996**, *44*, 3403.

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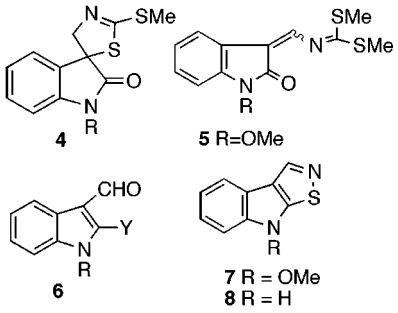
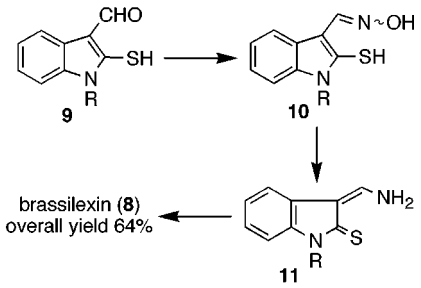
2-ones (**1**) and indoline-2-thiones (**2**)[3] derives from their application to the synthesis of a number of tryptophan-derived alkaloids, in particular, some cruciferous phytoalexins such as spirobrassinin (**4**), wasalexins (**5**), brassicanals (**6**, R = H, Y = SMe, S(O)OMe), sinalexin (**7**), and brassilexin (**8**).[4] Phytoalexins are secondary metabolites biosynthesized de novo by plants in response to diverse forms of stress, including pathogen attack.[5,6] Sinalexin (**7**)[7] appears to have a notable role in defense mechanisms of white mustard (*Sinapis alba*);[8] however, partly because of the extremely small amounts isolable from plants, the biological activity and ecological significance of sinalexin (**7**) remains to be established. Toward this end, it was of great interest to develop a chemical synthesis of sinalexin (**7**).



Here we report the first synthesis of sinalexin (**7**) through an unprecedented application of the Vilsmeier formylation to *N*-methoxyindoline-2-thione (**2**, R = OMe). Most interestingly, application of these conditions to the synthesis of brassilexin (**8**) led to the isolation of a new product containing a heterocyclic aromatic ring system. In addition, the antifungal activity of both sinalexin and brassilexin against four economically important plant pathogens was determined and is reported.

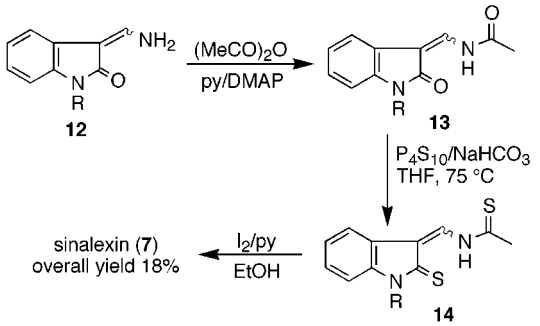
Initially, we sought an apparently direct route to sinalexin (**7**) based on the convenient biomimetic synthesis of the structurally related phytoalexin brassilexin (**8**), as shown in Scheme 2.[9] During this synthesis it was established that the

Scheme 2



required intermediate **9** (R = H), readily obtained from reaction of indoline-2-thione (**2**, R = H) with NaH and ethyl formate, could not be obtained from *N*-methoxyindoline-2-thione (**2**, R = OMe) under similar reaction conditions. Instead of the expected formylation product, extensive decomposition of the starting material (**2**, R = OMe) was observed. Subsequently, to avoid decomposition, sulfur was introduced at a later stage in the synthesis, as shown in Scheme 3. Thus thioamides **14** (R = OMe) were prepared

Scheme 3



from **13** (obtained quantitatively by acetylation of enamine **12**)[10] but in poor yield as a result of extensive decomposition.[11] Eventually, oxidation[12] and solvolysis of **14** yielded sinalexin in low overall yield (Scheme 3).

The rather lengthy route required to obtain sinalexin (**7**, Scheme 3) coupled with low overall yield (18%) directed us to investigate the direct Vilsmeier formylation of 1-methoxyindoline-2-thione (**2**, R = OMe) and/or its thiol tautomer.[13] Although this key intermediate had not been described previously, the electron-withdrawing effect of the MeO substituent at N-1 suggested that its reactivity toward electrophilic substitution would be significantly lower than that of indoline-2-thione (**2**, R = H). Furthermore, considering that indoline-2-thione (**2**, R = H, pk_{HA} = 10.0) is significantly more acidic than indolin-2-one (**2**, R = H, pk_{HA}

(3) Indoline-2-thiones are also useful in the preparation of certain tyrosine kinase inhibitors, i.e., the corresponding 3-substituted 2,2′-dithiobis(1*H*-indoles); see for example: Palmer, B. D.; Rewcastle, G. W.; Thompson, A. M.; Boyd, M.; Showalter, H. D. H.; Sercel, A. D.; Fry, D. W.; Kraker, A. J.; Denny, W. A. *J. Med. Chem.* **1995**, *38*, 58.

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(5) . (a) Bailey, J. A.; Mansfield, J. W., Eds. *Phytoalexins*; Blackie & Son: Glasgow, U.K., 1982; p 334. (b) Brooks, C. J. W.; Watson, D. G. *Nat. Prod. Rep.* **1985**, 427.

(6) For a recent review see ref 4.

(7) Pedras, M. S. C.; Smith, K. C. *Phytochemistry* **1997**, *46*, 833.

(8) Pedras, M. S. C.; Zaharia, I. L.; Gai, Y., Zhou, Y.; Ward, D. E. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 747.

(9) (a) Pedras, M. S. C.; Okanga, F. I. *Chem. Commun.* **1998**, 1565. (b) Pedras, M. S. C.; Okanga, F. I. *J. Agric. Food Chem.* **1999**, *47*, 1196.

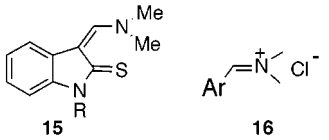
(10) Pedras, M. S. C.; Sorensen, J. L.; Okanga, F. I.; Zaharia, I. L. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3015.

(11) Satisfactory spectroscopic data were obtained for all synthetic intermediates.

(12) (a) Joule, J. A.; Mills, K.; Smith, G. F. *Heterocyclic Chemistry*; Chapman & Hall: London, U.K., 1995; p 404. (b) Goerdeler, J.; Krone, U. *Chem. Ber.* **1969**, *102*, 2273.

(13) Spectroscopic data of 1-methoxyindoline-2-thione (**2**, R = OMe). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37 (dd, J = 7.5, 7.5 Hz, 1H), 7.30 (d, J = 7.5 Hz, 1H), 7.20 (dd, J = 7.5, 7.5 Hz, 1H), 7.11 (d, J = 8 Hz, 1H), 4.16 (s, 3H), 4.04 (s, 2H). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 191.9 (s), 142.8 (s), 128.4 (d), 126.1 (s), 125.1 (d), 124.8 (d), 108.5 (d), 62.3 (q), 47.2 (t). HREIMS *m*/*z* measured 179.0407 (179.0405 calcd for C_9H_9NOS). EIMS *m*/*z* (% relative abundance) 179 (M^+, 100), 149 (69), 148 (60), 121 (32), 117 (23), 104 (13). FTIR ν_{max} 2937, 1620, 1464, 1391, 1364, 1285, 1192, 1138, 1067, 949, 749 cm^{-1}.

= 18.5; R = Me, pk_{HA} = 18.5) or 1-acetylindolin-2-one (**2**, R = Ac, pk_{HA} = 13.7),[14] it was suspected that formylation of 1-methoxyindoline-2-thione (**2**, R = OMe) might occur at both C-3 and S with potential ring opening and decomposition. Preliminary attempts to convert 1-methoxyindoline-2-thione (**2**, R = OMe) under mild Vilsmeier conditions ($POCl_3$/DMF, rt, 1 h, followed by NaOH work up at rt) yielded enamine thione **15** (R = OMe) in 15−25% yield along with multiple undetermined products. When the work up was carried out with boiling NaOH, **15** (R = OMe) (in similar yield) and traces of the desired aldehyde **9** (R = OMe) were obtained.

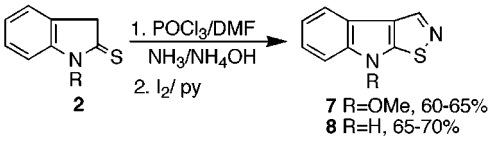


15 **16**

Subsequently, we reasoned that the product of a Vilsmeier formylation with $POCl_3$/DMF is an iminium salt, e.g. **16**, that could be converted into groups other than aldehydes. For example, treatment of the Vilsmeier iminium salt **16** (Ar = pyrrole) with hydroxylamine[1] yielded oximes, whereas treatment of **16** (Ar = indolizine) with hydrogen sulfide[1] lead to thioaldehydes.[1] Thus we sought a new workup procedure that might lead directly to the desired intermediate 3-(amino)methylene-1-methoxyindoline-2-thione (**11**, R = OMe). Thione **2** (R = OMe) dissolved in DMF was first treated with $POCl_3$ (1 h at 40 °C), and then the reaction mixture was cooled to 0 °C, basified with aqueous NH_3 (pH > 11), and extracted and concentrated in the usual way. The resulting reaction mixture was dissolved in pyridine and oxidized (I_2)[12] to yield, after FCC fractionation, sinalexin (**7**) identical in all respects to the natural product (Scheme 4, 60−65% overall yield).

Hence the regioselective formylation of 1-methoxyindoline-2-thione (**2**, R = OMe) under Vilsmeier conditions followed by ammonia workup is the key to a very concise synthesis of the phytoalexin sinalexin (**7**). This unprecedented workup procedure[1] is extremely useful in the preparation of primary imines/enamines, since these products cannot be obtained directly from reaction of aldehydes with ammonia.[15] In this procedure the imine **11** (R = OMe) is formed by reaction of enamine **15** (R = OMe) with ammonia, thus avoiding formation of an unstable aldehyde and the need for two additional steps to prepare sinalexin (**7**) (oximation and oxime reduction shown in Scheme 2).

Scheme 4



To extend the application of this method to additional and useful substrates, the formylation of indoline-2-thione (**2**, R = H) was carried out under reaction conditions similar to those described for sinalexin (**7**). The expected enamine/imine intermediate **11** (R = H) was isolated in good yield (65−70%) along with a significant amount of an undetermined orange product (30−35%). Oxidation of 3-(amino)-methylene-2-thione (**11**, R = H) with iodine gave brassilexin (**8**, Scheme 4) quantitatively. The structure of the orange product was established from its spectroscopic data to be the novel heterocycle **17**.[16] Compound **17** represents a new heteroaromatic ring system of 22 π-electrons with two equivalent tautomeric structures **17a** and **17b**. Reaction of **17** with MeI yielded quantitatively the expected methyl derivative **18**, whose spectroscopic data corroborated the structure of **17**. The formation of product **17** is rationalized in Scheme 5 (structures inside brackets are possible reactive intermediates); **17** may be formed by condensation of the Vilsmeier adduct **15** (R = H) and subsequent elimination of hydrogen sulfide or equivalent. Subsequently, we established that the crude reaction mixture containing both thione **11** (R = H) and **17** could be directly oxidized to brassilexin (**8**, R = H) without affecting its overall yield.

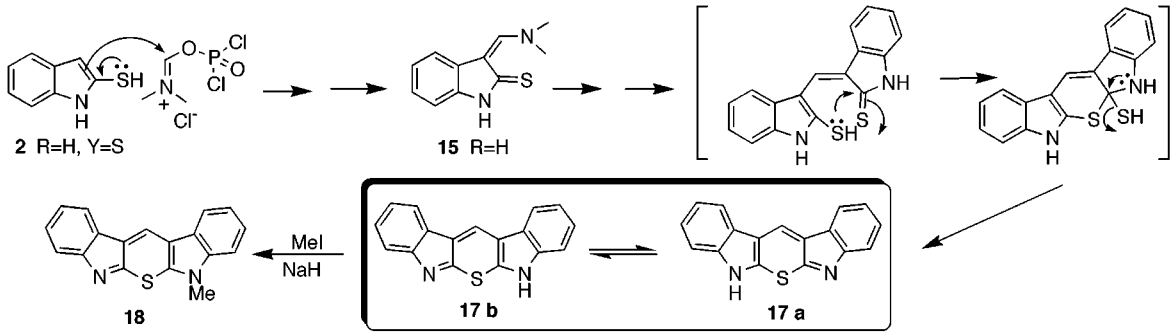
In conclusion, the ammonia workup procedure applied to the Vilsmeier formylation provides an extremely simple and unique two-step synthesis (with only one chromatographic separation) of two important cruciferous phytoalexins.

Table 1. Antifungal Activity[a] of Sinalexin (**7**) and Brassilexin (**8**) against Pathogens of Crucifers: *Alternaria brassicae* (isolate AB 11, 7-day incubation), *Phoma lingam* (perfect stage = *Leptosphaeria maculans*) (isolate BJ 125, 4-day incubation), *Rhizoctonia solani* (isolate group AG 2-1, 2-day Incubation), and *Sclerotinia sclerotiorum* (clone 33, 2-day incubation)

compound	concentration (in growth medium)	*A. brassicae* B 11	*R. solani* AG 2-1	*S. sclerotiorum* clone 33	*P. lingam* BJ 125
sinalexin (**7**)	5×10^{-4} M	100%	100%	100%	60 ± 11%
	1×10^{-4} M	31 ± 8%	100%	100%	25 ± 9%
	2×10^{-5} M	n. i.[b]	33 ± 4%	37 ± 4%	n. i.
brassilexin (**8**)	5×10^{-4} M	100%	100%	100%	100%
	1×10^{-4} M	42 ± 10%	100%	100%	81 ± 4%
	2×10^{-5} M	n. i.	66 ± 7%	86 ± 4%	n. i.

[a] Percent of inhibition = 100 − [(growth on medium containing compound/growth on control medium) × 100)] ± standard deviation; incubation time (fungal growth on control medium) depends on each species. [b] n.i. = no inhibition (growth on control medium and on medium containing compound is similar).

Scheme 5



Although several synthesis of brassilexin (**8**) have been published,[6] the present synthesis represents a very significant improvement in both overall yield and simplicity. Furthermore, our facile synthesis of intermediates **11** (R = OMe or H), potential biosynthetic precursors of **7** and **8**, respectively,[9] will allow an efficient one-carbon isotopic labeling and should facilitate future biosynthetic and other metabolic studies of sinalexin (**7**) and brassilexin (**8**).[4]

The antifungal activities of sinalexin and brassilexin against four of the major pathogens of crucifers, i.e., *Alternaria brassicae*, *Phoma lingam* (perfect stage = *Leptosphaeria maculans*), *Rhizoctonia solani*, and *Sclerotinia sclerotiorum* were determined as previously described.[17] As shown in the Table, brassilexin (**8**, R = H) caused complete inhibition of fungal mycelium growth in all four species at a concentration of 5×10^{-4} M for the duration of the experiment (up to 3 weeks), whereas sinalexin (**7**, R = OMe) showed less growth inhibition (60%) to *P. lingam* at this concentration. In addition, both sinalexin and brassilexin inhibited the growth of *S. sclerotiorum* and *R. solani* at 1×10^{-4} M. These results confirm a possible role for sinalexin in the resistance of white mustard to *A. brassicae*.[8] The availability of synthetic sinalexin and brassilexin should allow further investigation on their mechanism of action against economically important cruciferous pathogens.

Acknowledgment. We thank the Natural Sciences and Engineering Research Council of Canada (research grant to M.S.C.P.) and the University of Saskatchewan (graduate fellowship to I.L.Z.) for financial support. Isolates of *A. brassicae*, *P. lingam*, *S. sclerotiorum*, and *R. solani* were obtained from G. Séguin-Swartz, P. R. Verma, and D. Makenzie, Agriculture and Agri-Food Canada, Saskatoon Research Centre, Saskatoon SK.

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(15) The reaction of ammonia with aldehydes leads to spontaneous polymerization of the resulting imines. (a) March, J. *Advanced Organic Chemistry*, 5th ed.; J. Wiley & Sons: New York, 2001; p 1186. (b) Hull, W. E.; Sykes, B. D.; Babior, B. M. *J. Org. Chem.* **1973**, *38*, 2931.

(16) Spectroscopic data for compound **17**: ^{1}H NMR (500 MHz, $(CD_3)_2$-SO) δ 13.14 (br s, 1H), 9.48 (s, 1H), 8.24 (d, J = 7.5 Hz, 2H), 7.66 (d, J = 8 Hz, 2H), 7.43 (dd, J = 7.5, 7.5 Hz, 2H), 7.35 (dd, J = 7.5, 7.5 Hz, 2H). ^{13}C NMR (125.8 MHz, $(CD_3)_2$SO) δ128.1 (d), 125.3 (d), 125.2 (d), 121.2 (2×, d), 119.2 (2×, d), 114.9 (2×, br d). HREIMS m/z measured 274.0564 (274.0566 calcd for $C_{17}H_{10}N_2S$). EIMS m/z (% relative abundance) 274 (M^+, 100), 137 (11). FTIR ν_{max} 3400 (br), 3060, 2923, 2692, 1598, 1449, 1387, 1364, 1308, 1247, 1186, 1132, 744 cm^{-1}. Spectroscopic data for compound **18**: ^{1}H NMR (500 MHz, CD_2Cl_2) δ 8.83 (s, 1H), 8.09 (d, J = 7.5 Hz, 1H), 8.05 (d, J = 7.5 Hz, 1H), 7.74 (d, J = 8 Hz, 1H), 7.51 (d, J = 7.5 Hz, 1H), 7.48 (m, 2H), 7.42 (dd, J = 8, 8 Hz, 1H), 7.33 (dd, J = 7.5, 7.5 Hz, 1H), 3.85 (s, 3H). ^{13}C NMR (125.8 MHz, CD_2Cl_2) δ 156.7 (s), 154.2 (s), 141.1 (s), 139.8 (s), 127.2 (d), 126.8 (s), 126.6 (d), 125.2 (s), 124.8 (d), 123.2 (s), 122.4 (d), 121.9 (d), 120.1 (d), 118.8 (2×, d), 111.2 (s), 110.0 (d), 31.4 (q). HREIMS m/z measured 288.0716 (288.0721 calcd for $C_{18}H_{12}N_2S$). EIMS m/z (% relative abundance) 288 (M^+, 100), 273 (14), 144 (11). FTIR ν_{max} 2926, 1593, 1392, 1383, 1307, 1255, 1126, 1170, 744 cm^{-1}.

(17) Bioassays against *Phoma lingam* and *Rhizoctonia solani* were conducted as described in ref 9b but utilizing 4-well plates and liquid instead solid medium (400 μL medium); bioassays against *Alternaria brassicae* and *Sclerotinia sclerotiorum* were conducted similarly to assays with *R. solani*. Different incubation times were used for each pathogen: *A. brassicae*, 7-day incubation; *P. lingam*, 4-day incubation; *R. solani*, 2-day incubation; and *S. sclerotiorum*, 2-day incubation (if complete inhibition was observed the assay plates were kept for 3 weeks).