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Synthesis of Camalexin and Related Phytoalexins

William A. Ayer*, Peter A. Craw, Yu-ting Ma, and Shichang Miao

Department of Chemistry, University of Alberta,
Edmonton, Alberta, Canada T6G 2G2

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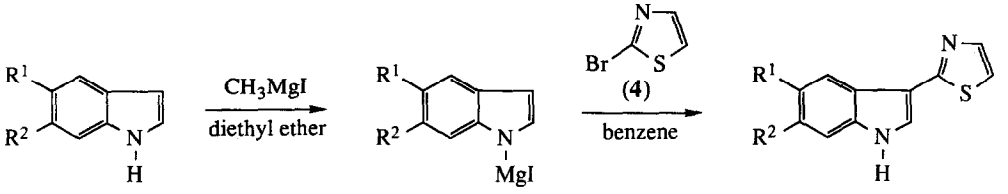
Abstract: *A number of 3-(2'-thiazoyl)indoles, including two recently reported phytoalexins, have been prepared from the Grignard reaction of the corresponding substituted indole and 2-bromothiazole. The antifungal activity of these 3-(2'-thiazoyl)indoles and 3-(2'-oxazolyl)indole have been examined.*

Recently we reported the isolation of two new thiazoyl substituted phytoalexins, camalexin (**1**) and 6-methoxycamalexin (**2**), produced in the leaves of *Camelina sativa* (Cruciferae) in response to infection by the fungus *Alternaria brassicae* [1]. In order to permit further testing of the antifungal activity of these phytoalexins we have developed efficient short syntheses of camalexin (**1**), 6-methoxycamalexin (**2**), and related 3-(2-thiazoyl)indoles.

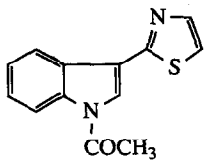
Indolylmagnesium halides are known to react with alkylating reagents to give C3-alkylated indoles[2]. We have found that reaction of two equivalents of indolylmagnesium iodide (prepared *in situ* from indole (**3**) and methylmagnesium iodide) with 2-bromothiazole (**4**)[3] in refluxing benzene[4] affords camalexin (**1**) in reproducible yields of 68-76% based on 2-bromothiazole (**4**). The reaction in one case also produced small amounts of 1-acetylcamalexin (**5**) during workup when the reaction mixture was poured into ethyl acetate prior to aqueous washing. The 1-acetylcamalexin (**5**) presumably arises from equilibration between camalexin (**1**) and



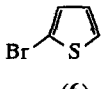
(**3**) R^1, R^2 = H
(**12**) R^1 = H, R^2 = OCH_3
(**15**) R^1 = OCH_3, R^2 = H

(**1**) R^1, R^2 = H
(**2**) R^1 = H, R^2 = OCH_3
(**16**) R^1 = OCH_3, R^2 = H

the excess indolylmagnesium iodide, and acetylation of the Grignard reagent of camalexin (**1**) with ethyl acetate prior to quenching with aqueous solutions. 1-Acetylcamalexin (**5**) was not detected when the reaction mixture was quenched with water prior to extraction with ethyl acetate. When the reaction was carried out using equimolar amounts of indolylmagnesium iodide and 2-bromothiazole (**4**), camalexin (**1**) is produced in only 44% yield. Presumably a portion of the indolylmagnesium iodide is consumed by equilibration in the reaction mixture to form the Grignard reagent of camalexin (**1**). Attempted reaction between indolylmagnesium iodide and 2-bromothiophene (**6**) failed to give any 3-(2-thiophenyl)indole (**7**).



(5)



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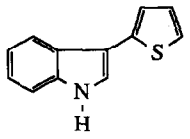
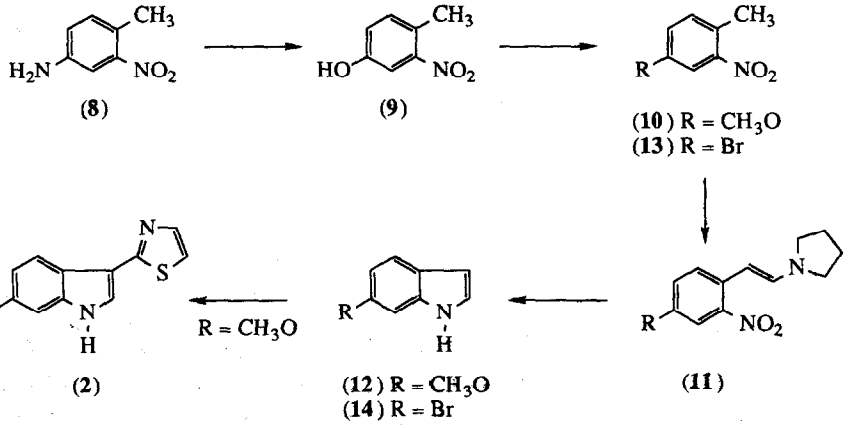


(7)

The synthesis of 6-methoxycamalexin (**2**) is outlined in Scheme 1. 4-Methyl-3-nitrophenol (**9**) was prepared in 75% overall yield from 4-methyl-3-nitroaniline (**8**) following the procedure of Ungnade and Orwell[5] for preparation of 3-bromo-4-hydroxytoluene from 3-bromo-4-aminotoluene. Methylation of the 4-methyl-3-nitrophenol (**9**) gave 4-methyl-3-nitroanisole (**10**) which was converted into 6-methoxyindole (**12**) using a modification of the procedures reported by Rinehart *et al* [6], Batcho and Leimgruber[7], and Feldman and Rapoport[8]. Thus, treatment of 4-methyl-3-nitroanisole (**10**) with dimethylformamide dimethyl acetal and pyrrolidine gave a red solution of the intermediate β-pyrrolidinostyrene (**11**)[7,8]. Direct reduction of the reaction mixture containing the β-pyrrolidinostyrene (**11**) with titanous chloride in a 4M ammonium acetate buffer gave



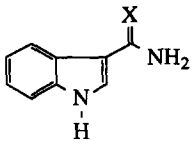
Scheme 1

6-methoxyindole (**12**) in 73-78% overall yield. This represents an improvement both in terms of yield and/or experimental simplicity over the previously reported procedures in which the intermediate β-pyrrolidinostyrene is isolated[6-8] and then reduced with either Raney nickel[7] or hydrogen over a palladium catalyst[8]. Application of the present procedure gives a higher yield (76%) of 6-bromoindole (**14**) from 4-bromo-2-nitrotoluene (**13**) than the 63% yield reported by Rinehart *et al* [6].

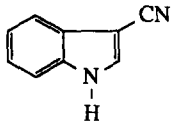
Alkylation of 6-methoxyindolyl magnesium iodide with 2 equivalents of 2-bromothiazole (**4**), according to the procedure used to prepare camalexin (**1**), gave a 77% yield of 6-methoxycamalexin (**2**). The m.p., uv and ^{1}H nmr spectra of the synthetic 6-methoxycamalexin (**2**) were in excellent agreement with those of naturally occurring (**2**)[1]. Alkylation of the commercially available 5-methoxyindole (**15**) with 2-equivalents of 2-bromothiazole (**4**) gave 5-methoxycamalexin (**16**) in 98% yield based on recovered 5-methoxyindole (**15**).

Camalexin (**1**) has also been prepared, albeit in lower yield, from indole *via* indole-3-carboxamide (**17**)[10]. Attempts to prepare the thioamide **18** by treating **17** with Lawesson's reagent[11] in refluxing benzene lead to the

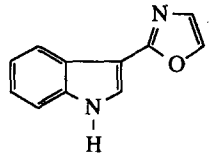
formation of 3-cyanoindole (**19**) as the only isolable product. However, when **17** was heated with phosphorus pentasulfide in benzene for 3 hours, then a solution of chloroacetaldehyde diethyl acetal in ethanol was added and heating was continued for 15 hours, camalexin (**1**) was obtained in 35% yield. The oxazole analog **20** of camalexin was prepared in 50% yield by reaction of **17** with chloroacetaldehyde diethyl acetal in ethanol.



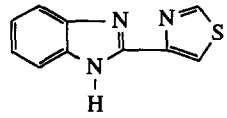
(**17**) X = O
(**18**) X = S



(**19**)



(**20**)



(**21**)

The antifungal activities of camalexin (**1**), 1-acetylcamalexin (**5**), 6-methoxycamalexin (**2**), 5-methoxycamalexin (**16**), the oxazole **20**, and the systemic fungicide thiabendazole (**21**) were examined using bioautography[12, 13] with *Cladosporium sp.* as the test organism (Table 1). Thiabendazole (**21**) and camalexin (**1**) displayed antifungal activity at an application level of 1 μg (zones of inhibition with diameters of 25 mm and 6 mm, respectively), but significant antifungal activity for 6-methoxycamalexin, 5-methoxycamalexin (**16**), and the oxazole **20** was only observed at application levels of 10μg or greater. Interestingly, 5-methoxycamalexin (**16**) was significantly more active than 6-methoxycamalexin (**2**) at an application level of 100μg, and this together with the fact that 1-acetylcamalexin (**5**) showed only weak activity at 100μg suggests that the basicity of the indole nitrogen in these compounds may be important for antifungal activity[14].

Table 1. Antifungal activity of camalexin (**1**) and related compounds.

	Quantity / Antifungal Activity*		
	1μg	10μg	100μg
Camalexin (**1**)	+	+++	+++
6-Methoxycamalexin (**2**)	δ	++	++
5-Methoxycamalexin (**16**)	δ	++	+++
1-Acetylcamalexin (**5**)	-	-	+
3-(2'-Oxazolyl)indole (**20**)	-	++	+++
Thiabendazole (**21**)	++	+++	+++

* Diameter of zone of inhibition: +, 0-10 mm; ++, 10-20 mm; +++, 20-40 mm; -, no inhibition; δ, trace of inhibiton.

EXPERIMENTAL

General procedure for alkylation of indole (3) and substituted indoles with 2-bromothiazole(4). *Preparation of Camalexin (1).*

Methylmagnesium iodide-diethyl ether complex (prepared by treating magnesium turning (1.05 g) in ether with excess methyl iodide (3.7 ml) and then removing the ether and excess methyl iodide) was dissolved in benzene (50 ml) and stirred under argon while a solution of indole (**3**) (5.00 g, 42.7 mmol, dried under vacuum) in benzene (13 ml) was added by means of a cannula (gas evolution). The solution of indolyl- magnesium iodide was stirred for 10 minutes then 2-bromothiazole (**4**) (1.93 ml, 21.4 mmol) was added and the solution

was refluxed under argon for 67 hours during which time the reaction mixture became dark red-brown. The reaction mixture was then cooled and poured into ethyl acetate. The ethyl acetate extract was washed with saturated ammonium chloride, water, and saturated sodium chloride. Evaporation gave an oil which was chromatographed on silica gel. Elution with hexanes-acetone (4:1) gave successively unreacted 2-bromothiazole (**4**), unreacted indole (**3**), 2-acetylcamalexin (**5**) (0.27g, 6%), and camalexin (**1**) [3.26 g, 76% based on 2-bromothiazole (**4**)] which crystallised on standing as as an off-white solid. Crystallisation from acetone-hexanes gave pale brown needles of camalexin (**1**), m.p. 147-148°C (lit.[1] m.p. 134-137°C, from benzene): uv (CH_3OH) λ_{max} (ε): 215 (19000), 278 (8000), 318 nm (13900); Anal. Calcd for $C_{11}H_8N_2S$: C, 66.0; H, 4.0; N, 14.0; S, 16.0. Found: C, 65.8; H, 4.0; N, 13.5; S, 16.0; correct ^1H nmr spectrum[1].

1-Acetylcamalexin (**5**) crystallised from acetone-hexanes as long needles, m.p. 117.5-118°C; uv (CH_3OH) λ_{max} (ε): 219 (14500), 241 (13300), 258 (14700), 314 nm (15900); ^1H nmr ($CDCl_3$): δ 8.42 (1H, br d, J = 7.5 Hz), 8.17 (1H, br d, J = 7.2 Hz), 7.91 (1H, s), 7.85 (1H, d, J = 3.3 Hz), 7.36 (1H, m), 7.28 (1H, d, J = 3.4 Hz), 2.60 (3H, s); ^{13}C nmr ($CDCl_3$, APT): δ 23.8 (CH_3), 116.5 (CH), 116.9 (C), 117.5 (CH), 120.6 (CH), 124.1 (CH), 124.3 (CH), 125.9 (CH), 126.9 (C), 135.9 (C), 142.9 (CH), 160.6 (C), 168.3 (C); hreims (probe 130°C): m/z calc'd for $C_{13}H_{10}N_2OS$ (M^+): 242.0515; found: 242.0515 (39), 201 (14), 200 (M^+-CH_2CO, 100), 142 (12). Anal. Calcd for $C_{13}H_{10}N_2OS$: C, 64.4; H, 4.2; N, 11.6; S, 13.2. Found: C, 64.5; H, 4.0; N, 11.5; S, 13.4.

*4-Methyl-3-nitrophenol (**9**).*

4-Methyl-3-nitroaniline (**8**) (3.0 g, 19.7 mmol) was added to concentrated sulfuric acid (22 ml) in water (60 ml) and the mixture was heated to 95°C. The solution was then cooled to 15°C, ice was added and the temperature maintained below 5°C while a solution of sodium nitrite (1.61 g, 23.3 mmol) in water (10 ml) was added. The solution was stirred and urea (0.15 g) and 10 g of ice were added. This solution was added to a boiling stirred solution of sodium sulfate (30 g), concentrated sulfuric acid (20 ml) and water (20 ml) in a flask fitted with a condenser for downward distillation. The rate of addition was adjusted to match the rate of collection of the yellow distillate. The distillate collected over about 2 hours was extracted with diethyl ether. The ether solution was extracted twice with 10% aqueous sodium hydroxide. This solution was acidified with concentrated hydrochloric acid and extracted with diethyl ether to give 4-methyl-3-nitrophenol (**9**) as a pale yellow oil which crystallised on standing (0.76 g, 75%), m.p. 71-72°C (lit.[8] m.p. 75-76°C); ^1H nmr ($CDCl_3$): δ7.47 (1H, d, J = 2.7 Hz), 7.20 (1H, d, J = 8.4 Hz), 7.02 (1H, dd, J = 8.4, 2.7 Hz), 2.51 (3H, s), 5.52 (1H, s).

*4-Methyl-3-nitroanisole (**10**).*

4-Methyl-3-nitrophenol (**9**) was methylated as previously described.[8]

*6-Methoxyindole (**12**).*

4-Methyl-3-nitroanisole (**10**) (200 µl, 1.45 mmol), pyrrolidine (145 µl, 1.74 mmol), and dimethylformamide dimethyl acetal (94%, 250 µl, 1.77 mmol) were stirred in dry dimethylformamide (1.0 ml) under argon and heated in a sand bath at 100-110°C for 3 h. The red solution of the intermediate β-pyrrolidinostyrene (**11**) was then cooled to room temperature and transferred to a solution of 4M aqueous ammonium acetate (5 ml) in DMF (5 ml), using additional DMF (5 ml) to rinse the β-pyrrolidinostyrene (**11**) solution into the reaction flask. The red solution was stirred at room temperature while a 20% w/v aqueous solution of titanous chloride (5.5 mL, 7.1 mmol) was added dropwise (slightly exothermic). The dark grey suspension was stirred for an additional 15 min then rinsed into a separatory funnel using water, and made basic with 1M sodium hydroxide and extracted with diethyl ether (3 x 50 ml). Evaporation of the dried extracts gave a brown oil which was chromatographed on silica gel, eluting with hexanes-acetone (4:1) to give 6-methoxyindole (**12**) (155 mg, 73%) as an off-white solid. Distillation (0.5 mm, 110°C) gave an oil which

crystallised on cooling, m.p. 90-91°C (lit.[9] m.p. 91-92°C); ^{1}H nmr ($CDCl_3$): δ 8.02 (1H, br s, NH), 7.52 (1H, d, J = 8.5 Hz, H4), 7.09 (1H, dd, J = 2.7, 2.7 Hz, H3), 6.89 (1H, br d, J = 2.0 Hz, H7), 6.81 (1H, dd, J = 8.5, 2.5 Hz, H 5), 6.49 (1H, m, H2), 3.86 (3H, s, OCH_3).

6-Bromoindole (14).

A solution of 4-bromo-2-nitrotoluene (**13**) (1.00g, 4.65 mmol), dimethylformamide dimethyl acetal (0.79 ml, 5.60 mmol) and pyrrolidine (0.465 ml, 5.58 mmol) was stirred in dimethylformamide (10 ml) under argon and heated in a sand bath at 100-110°C for 2 h. The red solution of the intermediate β-pyrrolidinostyrene was cooled and a solution of 4 M ammonium acetate (20 ml) and dimethylformamide (40 ml) was added. The red solution was stirred while 20% w/v titanous chloride (17 ml, 22.0 mmol) was added dropwise, and the resulting grey suspension was worked up as for the preparation of 6-methoxyindole (**12**). Chromatography of the crude product on silica gel, eluting with hexanes-acetone (4:1) gave 6-bromoindole (**14**) as a pale brown solid (0.69 g, 76%), m.p. 92-92°C (lit.[6] m.p. 95-96°C); ^{1}H nmr ($CDCl_3$): δ 8.12 (1H, v br s, NH), 7.55 (1H, br s, H7), 7.51 (1H, d, J = 8.5 Hz, H4), 7.23 (1H, dd, J = 8.5, 2.0 Hz, H5), 7.18 (1H, dd, J = 3.0, 3.0 Hz, H3), 6.54 (1H, m, H2).

6-Methoxycamalexin (2).

Methylmagnesium iodide was prepared as above from magnesium (60 mg) and methyl iodide (0.3 ml). To a solution of the methylmagnesium iodide in benzene (8 ml) was added a solution of 6-methoxyindole (**12**) (0.15 g, 1.02 mmol) in benzene (4 ml) followed by 2-bromothiazole (4) (0.18 ml, 2.00 mmol). The mixture was refluxed under argon for 20 h then cooled and poured into saturated aqueous ammonium chloride and extracted with ethyl acetate. The extracts were worked up as above and the crude product was chromatographed on silica gel. Elution with ethyl acetate-hexanes (1:1) gave 6-methoxycamalexin (**2**) (0.182 g, 77%) as a pale brown solid. Recrystallisation from dichloromethane-ethyl acetate gave pale brown needles, m.p. 151.5-152.5°C. Sublimation at reduced pressure (145-150°C, 0.5 torr) gave small colorless prisms, m.p. 159-160°C (lit.[1] m.p. 157-159°C, from methanol-hexanes); ^{1}H nmr ($CDCl_3$): δ 8.60 (1H, br s, NH), 8.12 (1H, d, J = 8.8 Hz, H4), 7.82 (1H, d, J = 3.4 Hz, H4' or 5'), 7.74 (1H, d, J = 2.6 Hz, H2), 7.23 (1H, d, J = 3.4 Hz, H4' or 5'), 6.94 (1H, dd, J = 8.8, 2.2 Hz, H5), 6.87 (1H, d, J = 2.2 Hz, H7), 3.84 (3H, s, OCH_3).

5-Methoxycamalexin (16).

Methylmagnesium iodide was prepared as above from magnesium (90 mg) and methyl iodide (0.3 ml). To a solution of the methylmagnesium iodide in benzene (10 ml) was added a solution of 5-methoxyindole (**15**) (0.50 g, 3.40 mmol) in benzene (4 ml) followed by 2-bromothiazole (**4**) (0.61 ml, 6.77 mmol). The mixture was refluxed under argon for 43 h then cooled and poured into saturated aqueous ammonium chloride and extracted with ethyl acetate. The extracts were worked up as above and the crude product was chromatographed on silica gel. Elution with hexanes-acetone (5:1 then 3:1) gave successively unreacted 2-bromothiazole (**4**), unreacted 5-methoxyindole (**15**) (0.249 g, 50%), and 5-methoxycamalexin (**16**) [0.383 g, 98% based on recovered 5-methoxyindole (**15**)] which crystallised from dichloromethane-hexanes as a pale yellow powder, m.p. 112-112.5°C; Ftir ($CHCl_3$ cast): 3400, 3150 (NH), 1625, 1590, 1544, 1478, 1439, 1257, 1214, 805 cm^{-1}; uv (CH_3OH) λ_{max} (ε): 213 (20200), 275 (11700), 321 nm (14900); ^{1}H nmr ($CDCl_3$): δ 10.04 (1H, s, NH), 7.84 (1H, d, J = 3.5 Hz, H4' or H5'), 7.77 (1H, d, J = 2.3 Hz, H4), 7.69 (1H, d, J = 3.1 Hz, H2), 7.20 (1H, d, J = 3.5 Hz, H4' or H5'), 7.19 (1H, d, J = 8.9 Hz, H7), 6.91 (1H, dd, J = 8.9, 2.5 Hz, H6), 3.88 (3H, s, OCH_3); ^{13}C nmr ($CDCl_3$, APT): δ 163.8 (C2'), 155.2 (C5), 142.0 (C2), 131.6 (C7a), 125.5 (C4'), 125.0 (C3a), 115.6 (C5'), 113.2 (C6*), 112.5 (C4*), 111.5 (C3), 102.1 (C7), 55.7 (OCH_3) [* assignments interchangeable]; hreims: m/z calc'd for $C_{12}H_{10}N_2OS$ (M^+): 230.0513; found 230.0512 (100%), 215 (19), 201 (10), 200 (9), 187 (39), 129 (10); Anal. Calcd for $C_{12}H_{10}N_2OS$: C, 62.6; H, 4.4; N, 12.2; S, 13.9. Found: C, 62.5; H, 4.5; N, 12.3; S, 13.6.

Preparation of camalexin (1) from indole-3-carboxamide (17).

Indole-3-carboxamide (**17**) (0.61 g, prepared from indole by the method of Mehta and Dhar[10]) and phosphorus pentasulfide (0.67 g) were heated under reflux in benzene (20 ml) for 3 h. Chloroacetaldehyde diethyl acetal (2.03 g) in anhydrous ethanol (15 ml) was added and the resulting mixture was heated under reflux for 15 h. The solvent was removed and the residue chromatographed on silica gel eluting with hexanes-acetone (7:3), to give camalexin (**1**) (0.27 g), m.p. 145-146°C; identical (Ftir, ^{1}H nmr) with that prepared above.

3-(2'-Oxazolyl)indole (20).

A solution of indole-3-carboxamide (0.32 g) and chloroacetaldehyde diethyl acetal (0.4 g) in anhydrous ethanol (10 ml) was heated under reflux for 15 h. The solvent was removed *in vacuo* and the residue purified by chromatography on silica gel. Elution with hexanes-acetone (7:3) gave **20**, m.p. 130-132°C (from chloroform); ^{1}H nmr ($CDCl_3$): δ 8.91 (1H, br s, NH), 8.31 (1H, m), 7.85 (1H, d, J = 3.0 Hz), 7.69 (1H, d, J = 1.0 Hz), 7.46 (1H, m), 7.32 (2H, m), 7.26 (1H, s); ^{13}C nmr [$CDCl_3$-$(CD_3)_2CO$, APT]: δ 159.8 (s), 136.6 (s), 136.0 (d), 127.1 (d), 125.4 (d), 124.3 (s), 122.3 (d), 120.6 (d), 120.5 (d), 111.3 (d), 104.8 (s); hreims: m/z calc'd for $C_{11}H_8N_2O$ (M^+): 184.0637; found 184.0638 (100%), 155 (11), 144 (8), 129 (48), 128 (14); Anal. Calcd for $C_{11}H_8N_2O$: C, 71.7; H, 4.4; N, 15.1. Found: C, 71.3; H, 4.3; N, 15.1.

Bioautography of thiabendazole (21), camalexin (1), and related compounds.

Solutions of each of the test compounds (10 ppm and 100 ppm) were prepared in methanol and the required amount of the solution was spotted using a micropipette onto Whatman precoated glass plates (10 x 20 cm) of silica gel (10 test samples per plate). After allowing the methanol to evaporate the plates were sprayed with a spore suspension of *Cladosporium sp.* in Czapek-Dox broth (Difco, 7 g per 200 ml distilled water). The silica gel plates were placed face down in a plastic tray lined on the bottom with moist tissues (the silica gel plate was supported approximatley 2 cm above the tissues by plastic supports under each corner). The plastic tray was covered and the fungus allowed to grow at room temperature for 3-4 days before being examined for zones of inhibition.

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