

Synthesis of Camalexin

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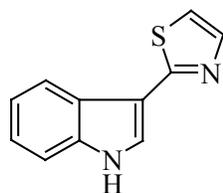
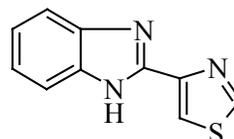
Abstract.- In this paper we describe a new method for the synthesis of camalexin (**1**) based on the reaction of 1-(tert-butoxycarbonyl)indole-3-carboxaldehyde with methyl L-cysteinate hydrochloride, followed by oxidation and decarboxylation. Compounds **1**, and intermediates **5-7** were identified by elemental analysis, ¹H NMR, ¹³C NMR and mass spectroscopy.

Keywords: Camalexin, phytoalexins, indoles

Introduction

Camalexin [3-(2'-thiazolyl)indole] (**1**) is a natural phytoalexin, isolated for the first time from the leaves of *Camelina sativa* and elicited by the fungus *Alternaria brassicae* [1]. Camalexin is also the principal phytoalexin found in *Arabidopsis thaliana* [2]. It exhibits antifungal activity similar to the systemic fungicide thiabendazole (**2**) [1,3] and also has antitumor activity [4]. In the literature there are described four methods for synthesis of camalexin, based on the reaction of indolylmagnesium iodide with 2-bromothiazole [3], heating of indole-3-carboxamide with P₂S₅ and chloroacetaldehyde diethyl acetal in ethanol [5], reductive cyclization of 2-formamidophenyl-2'-thiazolylketone upon heating with

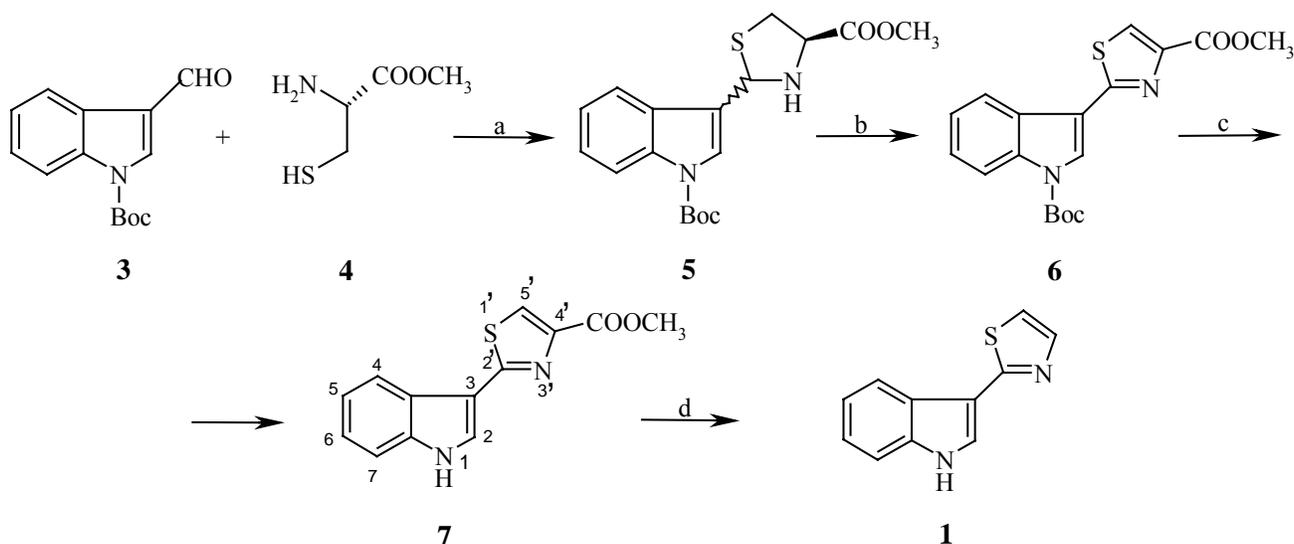
TiCl₃ and zinc dust [6] and reaction of 1-sulfonyl-3-iodoindole with active zinc and following Pd catalyzed arylation with 2-iodothiazole [7]. Recently, it has been suggested that the biosynthesis of camalexin involves the condensation of indole-3-carboxaldehyde with cysteine followed by a two-step oxidation and decarboxylation [8,9]. In the present work we have studied the synthesis of camalexin according to this biosynthetic scheme.

Camalexin **1**Thiabendazole **2**

Results and Discussion

As the first step in the investigated synthesis of camalexin, we have examined the cyclocondensation of indole-3-carboxaldehyde with methyl L-cysteinate. The product of this reaction appeared to be unstable and therefore it was decided to use the 1-Boc protected aldehyde **3**. The cyclocondensation of 1-(tert-butoxycarbonyl)indole-3-carboxaldehyde with methyl L-cysteinate (**4**) hydrochloride afforded 4'-methoxycarbonyl-1-(tert-butoxycarbonyl)-3-thiazolidine-2'-yl)indole (**5**) as a mixture of diastereoisomers in 85% yield (**Scheme 1**). The ratio of diastereoisomers was determined to be 57:43 by integration of the signals of proton H-2' at $\delta = 5,78$ and 6,01 ppm in the ¹H-NMR spectrum of the crude product.

Scheme 1



a) 1:2 methanol/benzene, (C₂H₅)₃N, 25° C, 3 h. (85%); b) MnO₂, benzene/pyridine, 55° C, 1.5 h., (44%); c) CH₃ONa, methanol, 25° C, 20 min. (59%); d) NaOH, NaHCO₃, 25° C, 2 h., (12%).

Oxidation of thiazolidine **5** to thiazole **6** by oxidizing reagents such as pyridinium chlorochromate (PCC), p-chloranil, 2,3-dichloro-5,6-dicyano-p-benzochinone (DDQ) and FeCl₃ lead to decomposition products. The desired oxidation was achieved by using 25 equivalents of activated MnO₂ in dry benzene [10]. Elimination of the tert-butoxycarbonyl protective group was realized using 16 equivalents of sodium methoxide in methanol at ambient temperature. Subsequent hydrolysis and decarboxylation of the resulting 4'-methoxycarbonyl-3-(thiazole-2'-yl)indole (**7**) with an aqueous solution of NaOH and NaHCO₃ gave camalexin (**1**) in 12% yield. The spectral data and melting point of **1** are identical with the literature data [1,3,6].

Conclusions

In this contribution we report a biomimetic synthesis of camalexin (**1**) according to the proposed biosynthetic scheme. The formation of the thiazole ring involves only one oxidation step followed by decarboxylation to camalexin.

Acknowledgments

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Experimental

General

Melting points were measured on a Koffler hot stage apparatus and are uncorrected. Purity of compounds was confirmed by elemental analysis on a Perkin-Elmer, model 2400 analyzer. The reaction course was monitored by TLC on Silufol (Kavalier) and Alumina 60 F₂₅₄ neutral (Merck) TLC plates. Preparative column chromatography was performed on Kavalier 40/100 μm silica gel and Merck Kieselgel 60 F25. The infrared absorption spectra of compounds **1**, and **5-7** were measured in CHCl₃ on an IR75 (Zeiss Jena) spectrometer in the region 400-4000 cm⁻¹. The ¹H-NMR spectrum of **5** was measured on a TESLA BS 487 A (80 MHz), ¹H- and ¹³C-NMR spectra of compounds **1**, **6**, **7** on a Varian Gemini 2000 (300 MHz) in deuteriochloroform, using tetramethylsilane as an internal standard. The electron impact mass spectra of **7** were recorded on a Finnigan SSQ 700 spectrometer at an ionization energy of 70 eV. Methyl L-cysteinate hydrochloride, pyridinium chlorochromate (PCC), p-chloranil and 2,3-dichloro-5,6-dicyano-p-benzoquinone (DDQ) from Fluka, Merck and Avocado were used as obtained without further purification. 1-(tert-Butoxycarbonyl)indole-3-carboxaldehyde (**3**) was prepared according to the described procedure [11].

1-(tert-Butoxycarbonyl)-4'-methoxycarbonyl-3-(thiazolidin-2'-yl)indole (5).

To a suspension of methyl L-cysteinate hydrochloride (595 mg, 3.47 mmol) in 1:2 methanol/benzene (6mL) was added 1-(tert-butoxycarbonyl)indole-3-carboxaldehyde (490 mg, 2 mmol) and triethylamine (672 mg, 6.6 mmol). The reaction mixture was stirred for 3 hours at room temperature, the solvent was evaporated and the oily residue purified by column chromatography, using a mixture of cyclohexane/acetone (2:1) as eluent. Yield 615 mg (85%), yellow oil; For $C_{18}H_{22}N_2O_4S$ (362.50) calculated: 59.65% C, 6.12% H, 7.73% N; found: 59.60% C, 6.07% H, 7.65% N; IR: 3323 (NH), 1720 a 1730 (C=O); 1H -NMR (ppm): 1,66 s, 9H [(CH₃)₃], 2,96 s 1H (NH), 3,09-4,33 m, 3H (SCH₂CH), 3,79 s, 3H (OCH₃), 5,78 s a 6,01 s 57:43, 1H (CH), 7,13-8,22 m, 5H (H-arom.).

1-(tert-Butoxycarbonyl)-4'-methoxycarbonyl-3-(thiazol-2'-yl) indole (6).

To a stirred suspension of activated MnO₂ [10] (1000 mg, 11.5 mmol) in a mixture of dry benzene (10mL) and pyridine (0.050 mL) was added a solution of thiazolidine **5** (400 mg, 1.10 mmol) in dry benzene (2 mL). The reaction mixture was stirred for 1.5 hour at 55° C. After cooling the insoluble material was removed by filtration, washed with benzene, the solvent was evaporated and the solid residue crystallized from a mixture of diethylether/hexane. Yield 175 mg (44%), M.p. 128-130° C; For $C_{18}H_{18}N_2O_4S$ (359.48) calculated: 60.14% C, 5.05% H, 7.79% N; found: 60.23% C, 5.21% H, 7.29% N; IR: 3323 (NH), 1720 a 1730 (C=O); 1H -NMR (ppm): 1,66 s, 9H [(CH₃)₃], 3,79 s, 3H (OCH₃), 8,10 s, 1H (H-2), 8,25 s, 1H (H-5'), 7,13-8,22 m, 4H (H-arom.).

4'-Methoxycarbonyl-3-(thiazol-2'-yl) indole (7).

To a suspension of thiazole **6** (150 mg, 0.42 mmol) in dry methanol (12mL) was added sodium methoxide (330 mg, 6.11 mmol) during 5 min. The reaction mixture was poured into cold water (60 mL), extracted with chloroform (3x10 mL), dried over Na₂SO₄, the solvent evaporated and the product crystallized from a mixture of diethylether/hexane. Yield 64 mg (59%), M.p. 166-168° C; For $C_{13}H_{10}N_2O_2S$ (259.50) calculated: 60.17% C, 3.88% H, 10.80% N; found: 60.28% C, 3.99% H, 10.95% N; IR: 3200 (NH), 1720 a 1730 (C=O); 1H -NMR (ppm): 3,79 s, 3H (OCH₃), 8,10 d, 1H (H-2), J=2,55 Hz, 8,25 s, 1H (H-5'), 7,13-8,22 m, 4H (H-arom.), 10,85 s, 1H (NH). ^{13}C NMR (ppm): 52,32 (CH₃), 113.05, 121.66, 122.04, 123.83, 125.50, 125.72, 127.41, 127.58, 137.99, 147.78 (C arom.), 162.61 (C=N), 164.75 (C=O). MS, m/z (%), : 258 (M⁺, 100), 200 (24), 160 (24), 142 (24), 115 (12), 57 (12).

Camalexin (1).

To a solution of NaOH (22.0 mg, 5.6 mmol) in water (2 mL) was added a solution of thiazole **7** (120 mg, 0.46 mmol) in methanol (2 mL) and the reaction mixture was refluxed for 1 hour. After cooling and evaporation of the methanol, NaHCO₃ (660 mg, 7.86 mmol) was added and the reaction mixture was refluxed for 1 hour. The product separated after cooling and was collected on filter paper and dried. Crystallization from a mixture of diethylether/hexane yielded 10 mg (12%); M.p. 140-141°

C; For C₁₁H₈N₂S (200.10) calculated: 66.00% C, 4.00% H, 14.00% N; found: 65.80% C, 4.00% H, 13.50% N; IR, ¹H-, ¹³C-NMR and mass spectra were identical with previously described data for camalexin [1, 3, 6].

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Sample availability: Samples of compounds **1**, **5** and **6** are available from the authors.

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