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 thiamethoxam (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 presence work we have studied the synthesis of camalexin according to this biosynthetic scheme.

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 δ= 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-benzoquinone (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.

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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 deuterochloroform, 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\textsubscript{18}H\textsubscript{22}N\textsubscript{2}O\textsubscript{4}S (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); \textsuperscript{1}H-NMR (ppm): 1.66 s, 9H [(CH\textsubscript{3})\textsubscript{3}], 2.96 s 1H (NH), 3.09-4.33 m, 3H (SCH\textsubscript{2}CH), 3.79 s, 3H (OCH\textsubscript{3}), 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 MnO\textsubscript{2} [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 o 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 diethyl ether/hexane. Yield 175 mg (44%), M.p. 128-130 o C; For C\textsubscript{18}H\textsubscript{18}N\textsubscript{2}O\textsubscript{4}S (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); \textsuperscript{1}H-NMR (ppm): 1.66 s, 9H [(CH\textsubscript{3})\textsubscript{3}], 3.79 s, 3H (OCH\textsubscript{3}), 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 Na\textsubscript{2}SO\textsubscript{4}, the solvent evaporated and the product crystallized from a mixture of diethyl ether/hexane. Yield 64 mg (59%), M.p. 166-168 o C; For C\textsubscript{13}H\textsubscript{10}N\textsubscript{2}O\textsubscript{2}S (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); \textsuperscript{1}H-NMR (ppm): 3.79 s, 3H (OCH\textsubscript{3}), 8.10 d, 1H (OCH\textsubscript{3}), 8,10 1H (H-2), 10,85 s, 1H (NH). \textsuperscript{13}C NMR (ppm): 52.32 (CH\textsubscript{3}), 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\textsuperscript{+}, 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, NaHCO\textsubscript{3} (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 diethyl ether/hexane yielded 10 mg (12%); M.p. 140-141 o
C; For C$_{11}$H$_8$N$_2$S (200.10) calculated: 66.00% C, 4.00% H, 14.00% N; found: 65.80% C, 4.00% H, 13.50% N; IR, $^1$H-, $^{13}$C-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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