A Convenient Synthesis of Isothiazolo[5,4-*b*]indole (Brassilexin) via a Polyphosphoric Acid Initiated Ring Closure

Michel Devys, Michel Barbier*

Institut de Chimie des Substances naturelles, CNRS, Avenue de la Terrasse, F-91198 Gif sur Yvette Cedex, France

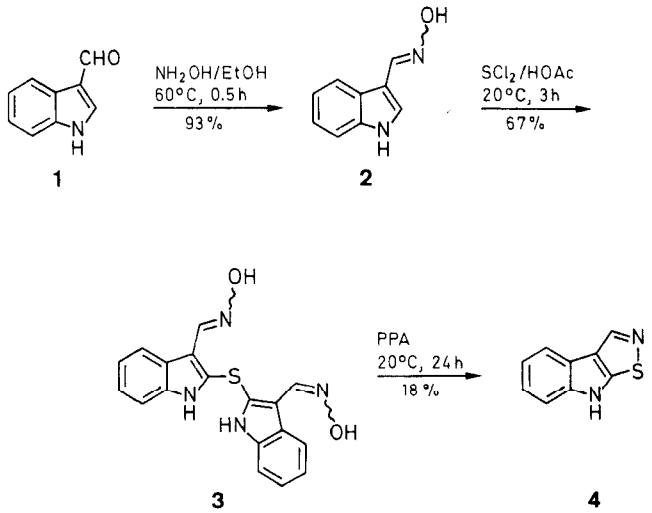
Brassilexin (**4**), an antifungal compound previously isolated from *Brassica juncea L.* (Cruciferae), has been synthesized with an overall yield of 11% starting from 3-indolecarbaldehyde (**1**).

Brassilexin (**4**) was previously reported as a phytoalexin from *Brassica juncea* L. (Cruciferae), and its structure was established.[1,2] This substance is a new member of a family of sulfur-containing indoles, which were recently[3] isolated from Cruciferae. Brassilexin (**4**) is of particular interest as it represents the so far only known isothiazolo[5,4-*b*]indole. It was proposed as a hypothesis[2] that its biosynthesis should proceed through 3-indolecarbaldehyde (**1**), a known metabolite of plant origin.

In the present publication, the synthesis of brassilexin (**4**) starting from 3-indolecarbaldehyde (**1**) is reported. The oxime **2** (93% from **1** according to a known method[4]) was used to prepare the corresponding monosulfide **3** by action of sulfur chloride in acetic acid[5] in 67% yield. This sulfide cyclizes into brassilexin quite easily when treated with polyphosphoric acid (PPA) at room temperature, a reaction described for the synthesis of fused heterocycles from suitably 1,2-substituted aromatic structures, leading for example to thiocoumarins[6] or benzisothiazoles.[7] The facile preparation of the symmetrical sulfide **3** represents, however, in the case of brassilexin (**4**) a particularly interesting simplification of the method. The final product was obtained by column chromatography and preparative TLC on silica gel followed by crystallization. The synthetic brassilexin (**4**) proved to be identical with the natural product through direct comparison of the physico chemical data (control TLC, mp, MS, HRMS, and ^{1}H-NMR; yield: 18%, overall yield from **1**: 11%.)

Attempts to carry out the cyclization with polyphosphoric acid at 100°C as reported[6,7] or by thermal rearrangement[8] did not give better yields due to the difficulties of the separation from the numerous side-products. The same reaction carried out at 20°C with polyphosphoric acid and the disulfide obtained by reaction of the oxime **2** with sulfuryl chloride instead of sulfur dichloride also furnished lower yields of the expected **4** and was consequently abandoned.

Determination of the cytotoxicity of brassilexin (**4**) on cultures of human cancer cells KB showed a 100% inhibition at a concentration of 20 µg/mL with an LD_{50} at 8 µg/mL, which is now under more detailed study.



The reagents 3-indolecarbaldehyde (**1**) and SCl_2 were purchased from Fluka AG. The melting points were determined on a Kofler apparatus under the microscope and are corrected. MS was determined on an AEI MS 50 apparatus, ^{1}H-NMR on a Bruker 400 MHz and UV on a Perkin-Elmer spectrophotometer with automatic recorder Lambda-5. Preparative TLC were carried out on Schleicher-Schüll silica gel (20 × 20 mL) fluorescent plates LS254, control TLC on corresponding analytical TLC plates. The authentic sample of brassilexin (**4**) used as a standard was extracted from elicited leaves of the brown mustard *Brassica juncea*.[2]

3-Hydroxyiminomethylene indole (**2**) was prepared according to a reported method;[4] yield: 93%; mp 196–198°C (Lit.[4] mp 197–198°C); TLC: silica gel, hexane/EtOAc (1:1), R_f = 0.3.

MS: m/z = 160 (M^+).

2,2′-Bis(3-hydroxyiminomethyleneindolyl)sulfide (3):

To a stirred solution of the oxime **2** (960 mg, 6 mmol) in HOAc (10 mL) is added dropwise SCl_2 (0.78 mL, 9 mmol) at r.t.[5] After 3 h, the red solution is poured into anhydrous Et_2O (400 mL) affording a brown sticky precipitate. The supernatant liquid is decanted and the precipitate is washed twice with Et_2O, resulting in a thick brown paste. The yield is determined by drying under *vacuo* an aliquot part; yield: 700 mg (67%). Drying of this product results in the formation of red polymers, from which brassilexin is not obtained, so that the crude monosulfide **3** is used directly for the next step.

Brassilexin (Isothiazolo[5,4-*b*]indole 4):

Freshly prepared monosulfide **3** (700 mg, 2 mmol) is stirred with PPA [20 g, prepared from 84% H_3PO_4 (30 g) and P_4O_{10} (10 g)] for 24 h at 20°C. The mixture is cooled in ice, water (50 mL) is added and the pH is made neutral by slow addition of 6N NaOH under stirring. A clear brown precipitate is obtained, which is filtered, washed with water, dissolved in CH_2Cl_2, dried (Na_2SO_4), and concentrated. This solution is applied to a column of silica gel (60 g), and eluted with a gradient of EtOAc in hexane, starting from 1:9. Brassilexin comes out with the 3:7 mixture together with a secondary product, R_f = 0.35 on TLC (CH_2Cl_2), while **4** has a R_f of 0.30 (yield: 190 mg). The final purification is carried out by preparative TLC using CH_2Cl_2 as eluent, extracting the silica gel layer with EtOAc, followed by crystallizations from EtOAc/pentane; yield: 62 mg (18%, overall from **1** 11%); mp 164–167°C (Lit.[2] mp 164–167°C); colorless microcrystals.

HRMS: m/z, C_9H_6NS calc: 174.02517; found: 174.0244.

IR ($CHCl_3$): ν = 3460, 3250, 2980, 2850; (KBr): 860, 740 cm^{-1}.

UV (MeOH): λ_{max} = 218 (ε = 50000), 245 (ε = 14000), 264 (12000) nm.

^{1}H-NMR (CD_3OD/TMS): δ = 7.20 (t, 1 H, $J_{6,7} = J_{5,6}$ = 8 Hz, H-6), 7.32 (t, 1 H, $J_{4,5} = J_{5,6}$ = 8 Hz, H-5), 7.48 (d, 1 H, $J_{6,7}$ = 8 Hz, H-7), 7.90 (d, 1 H, $J_{4,5}$ = 8 Hz), 8.70 (s, 1 H, H-3, this signal integrates for 2 H in $CDCl_3$ as previously noticed[2]).

MS: m/z (%) = 174 (M^+, 100), 147 (5), 146 (6), 142 (12).

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