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Article

Synthesis of (±)-Kigelin

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Descreve-se a síntese da 8-hidróxi-6,7-dimetóxi-3-metil-3,4-diidroisocumarina (±) kigelina, um metabólito bioativo encontrado em *Aspergillus terreus* e *Kigelia pinnata*. O ácido 3,4,5-trimetóxi-homoftálico após tratamento com nidrido acético na presença de piridina, sob refluxo, forneceu o ácido 2,3,4-trimetóxi-6-(2-oxopropril)benzóico. Redução deste último, seguido de ciclodesidratação resultou na formação da 6,7,8-trimetóxi-3-metil-3,4-diidroisocumarina, que sofreu desmetilação regiosseletiva fornecendo a (±) kigelina.

A simple synthesis of racemic 8-hydroxy-6,7-dimethoxy-3-methyl-3,4-dihydroisocoumarin or kigelin, a metabolite of *Aspergillus terreus* and *Kigelia pinnata*, exhibiting several bioactivities, is described. 3,4,5-Trimethoxyhomophthalic acid was refluxed with acetic anhydride in dry pyridine to afford 2,3,4-trimethoxy-6-(2-oxopropyl)benzoic acid. Reduction of the latter followed by cyclodehydration yielded 6,7,8-trimethoxy-3-methyl-3,4-dihydroisocoumarin which on regioselective demethylation furnished the (±)-kigelin.

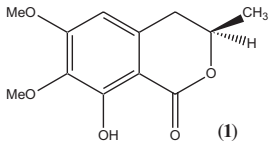
Keywords: dihydroisocoumarin, *Kigelia pinnata, Aspergillus terreus*

Introduction

Kigelia pinnata (Syn. *Kigelia africana*) is a highly variable mono-specific genus of the family Bignoniaceae. *K. pinnata* (also known as the 'sausage tree' or Worsboom due to its large fruits) is cultivated in many parts of India as an ornamental and roadside tree[1] and in Africa where it grows as an endemic species in many areas and finds a variety of medicinal uses.[2] The plant has deep chocolate red flowers and gourd like fruit.

Govindachari *et al.* isolated kigelin[3] as the major constituent of plant from the root heartwood of *Kigelia pinnata* DC. The structure of kigelin was established by chemical methods and spectroscopic techniques as 8-hydroxy-6,7-dimethoxy-3-methyl-3,4-dihydroisocoumarin (**1**) and it was concluded that the absolute configuration at C-3 was *R* on the basis of CD spectral analysis (Figure 1).

Chaudary *et al.* have recently reported [4] the isolation of (3*R*)-kigelin from the extract of fungus *Aspergillus terreus* culture medium. Kigelin was shown to display significant activity against human pathogenic dermatophytes, *Microsporum canis* and *Trichophyton longifusus* and also exhibited potent xanthine oxidase (XO) inhibitor activity even better than those of 3-*t*-butyl-4-hydroxyanisole (BHA) and propylgallate (PG).



(**1**)

Figure 1. Kigelin.

Kigelin is an active ingredient of cosmetics and skin lotions and is likely to be responsible for skin healing properties. It is used in the removal of solar keratosis lesions on the skin, particularly the face, neck, hands and arms.[2,5,6.] Kigelia cream containing kigelin as major constituent is used successfully against melanoma. Kigelin is known to cure certain types of eczemas and is also an active constituent of Bust Force Multi Toner Serum.[7]

The great biological and commercial importance of kigelin together with its simple structure has made kigelin an attractive target for synthesis.[8-14] The aim of present study was to devise an efficient synthesis of commercially important and bioactive dihydroisocoumarin **1**.

Results and Discussion

We envisaged the synthesis of **1** using 3,4,5-trimethoxyhomophthalic acid (**4**) as a key starting material.

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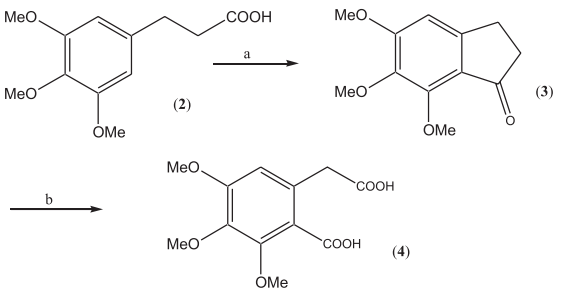
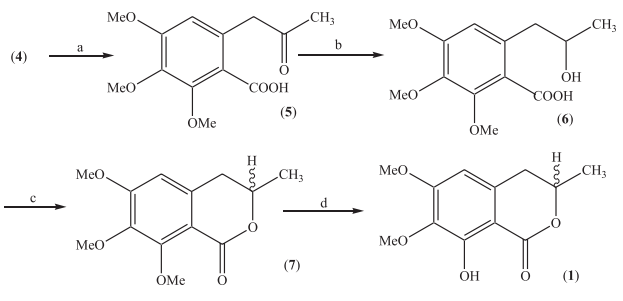
The requisite acid **4** was prepared in two steps in good yield from the commercially available 3-(3,4,5-trimethoxyphenyl)propionic acid (**2**) using the method of Bauta *et al.*[15] Thus, cyclodehydration of **2** using polyphosphoric acid gave 5,6,7-trimethoxy-1-indanone (**3**). A toluene solution of indanone **3** and diethyl oxalate was treated with a suspension of sodium methoxide in toluene followed by the addition of potassium hydroxide and 30% H_2O_2 to furnish **4** (Scheme1).



Scheme 1. Reagents and conditions: a) PPA, 90 ºC, 2h, 76%; b) *i*) $(EtO_2C)_2$, NaOMe, Ph-Me; *ii*) 30% H_2O_2 KOH, MeOH, 54%.

Reaction of **4** with acetic anhydride in dry pyridine afforded 2,3,4-trimethoxy-6-(2-oxopropyl)benzoic acid (**5**).[16] Spectral data for **5** include the signal for benzylic protons at δ 3.99 and that for benzylic carbon at δ 43.7, characteristic M^+-H_2O ion at *m/z* 250, and carboxylic and ketonic carbonyl absorptions at 1685 and 1720 cm^{-1}, respectively.

Reduction of **5** with sodium borohydride gave the racemic hydroxy acid (**6**) which was cyclodehydrated using refluxing acetic anhydride to furnish 6,7,8-trimethoxy-3-methyl-3,4-dihydroisocoumarin (**7**). In the IR spectrum of **7**, the lactonic carbonyl absorption was observed at 1725 cm^{-1}. The protons of the C-4 methylene group adjacent to the stereogenic centre (C3) exhibited the diastereotopic effect characteristic of an ABX system.[17] The hydrogen *cis* to side chain methyl resonated slightly upfield at δ 2.81 and the *trans* hydrogen slightly downfield, at δ 2.95 respectively. The H-3 (X) showed a multiplet at δ 4.56.The carbon signals for C-3 and C-4 appeared at δ 80.2 and 68.1.



Scheme 2. Reangents and conditions: a) Ac_2O, py., dry ether, overnight, r.t., 82%; b) $NaBH_4$, 4 h reflux, 75%; c) Ac_2O, reflux, 2h, 80%; d) BBr_3, dry CH_2Cl_2, -78 °C, 20 min then r.t. 30 min, 75%.

Regioselective demethylation of trimethoxy-dihydroisocoumarin **7** was attained using boron tribromide under mild conditions (-78 °C, 20 min) to furnish kigelin **1**. The presence of phenolic hydroxyl was confirmed by purple ferric chloride test and solubility in dilute aqueous sodium hydroxide. The lactonic carbonyl absorption was lowered to 1665 cm^{-1} due to chelation with 8-hydroxyl which appeared at 3440 cm^{-1}.

In summary, an efficient synthesis of racemic kigelin, a natural bioactive dihydroisocoumarin has been accomplished.

Experimental

Melting points were recorded using a MEL TEMP MP-D apparatus and are uncorrected. ^1H NMR and the ^{13}C NMR spectra were determined as $CDCl_3$ or CD_3OCD_3 solutions at 400 MHz (Bruker AM-400) and 100 MHz (Bruker AM-100), respectively. FT-IR spectra were recorded on an FTS 3000 MX spectrophotometer; mass spectra (EI, 70 eV) on a MAT 312 instrument and elemental analyses were conducted using a CHN-Rapid Heräus apparatus. All compounds were purified by thin layer chromatography using silica gel from Merck having a fluorescent indicator F254 (Merck) and the spots were detected under ultraviolet light.

*5, 6,7-Trimethoxyindan-1-one (**3**)*

3-(3,4,5-Trimethoxyphenyl)propionic acid (**2**) (1 g, 4.5 mmol) was dissolved in polyphosphoric acid (12.5 g) and the resulting yellow solution was heated along with stirring at 90 °C for 2 h. The cooled solution was added to 200 mL ice-water and extracted with ethyl acetate (4x 100 mL). The combined extracts were washed with 5% sodium bicarbonate solution and then with water until the washings were neutral. The organic layer was dried ($MgSO_4$), filtered and rotary evaporated to dryness. Recrystallization using ethyl acetate gave (**3**) as light brown crystals. (0.758 g, 76%); mp 107-108 °C; R_f = 0.46 (pet. ether-ethyl acetate 6:4); IR (KBr) ν_{max}/cm^{-1}: 1685, 1590; ^1H NMR (400 MHz, $CDCl_3$): δ 2.65 (m, 2H, H-3), 3.0 (m, 2H, H-2), 3.86 (s, 6H, OMe) 3.89 (s, 3H, OMe), 6.45 (s, 1H, H-4); MS (70 eV): *m/z* (rel. int.): 222 (M^+, 26), 194 (53), 180 (70), 179 (82). Anal. Calc. for $C_{12}H_{14}O_4$: C 64.85, H 6.35; Found: C 64.78, H 6.25%.

*3,4,5-Trimethoxyhomophthalic acid (**4**)*

A solution of indanone (**3**) (0.49 g, 2.20 mmol) and

diethyl oxalate (0.52 mL) in toluene (5.3 mL) was added slowly to a suspension of NaOMe (0.27 g) in toluene (0.3 mL) at 0 °C. After addition was complete, the mixture was stirred for 1 h at ambient temperature. The solvent was removed *in vacuo* and the residue was suspended in MeOH (13 mL). Solid KOH (85%, 1.3 g) was slowly added portion wise keeping the temperature below 50 °C; then H_2O_2 (30%, 2.5 mL) was added slowly, keeping the temperature below 64 °C. The mixture was stirred further at ambient temperature for 16 h. The mixture was filtered and the filtrate was partially evaporated *in vacuo* to remove MeOH. The remaining aqueous filtrate was washed with ether and the organic layer was discarded. The aqueous layer was acidified with 12 mol L^{-1} HCI to pH < 2. The acidic aqueous layer was extracted with ethyl acetate and the combined organic portions were dried ($MgSO_4$). The solvent was removed *in vacuo* and the residue was crystallized to give (**4**) (0.143 g, 72%); mp 151-153 °C; R_f =0.08 (pet. ether-ethyl acetate 6:4); IR (KBr) ν_{max}/cm^{-1}: 3350, 2600, 1720, 1685, 1590; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.77 (s, 2H, $ArCH_2$), 3.86 (s, 6H, OMe), 3.89 (s, 3H, OMe), 6.47 (s, 1H, H-2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.7 (COOH), 168.1 (COOH), 162.6 (C3), 159.9 (C5), 137.6 (C1), 116.2 (C2), 109.3 (C6), 56.2 (OMe) 55.6 (OMe), 39.7 ($ArCH_2$); MS (70 eV): 286 (M^+, 30), 268 (38), 242 (53), 198 (90), 184 (35). Anal. Calc. for $C_{13}H_{18}O_7$: C 55.54, H 6.34; Found: C, 55.51, H 6.39%.

*2,3,4-Trimethoxy-6-(2-oxopropyl)benzoic acid (**5**)*

To a stirred mixture of 3,4,5-trimethoxyhomophthalic acid **4** (0.2 g, 0.70 mmol) in acetic anhydride (0.80 mL), pyridine (0.44 mL) was added under argon. The solid was dissolved instantly and after 2 min. precipitate was observed. Dry ether (6.5 mL) was added to facilitate the stirring. The solid was filtered and washed with ether after being stirred overnight. It was then suspended in water (8 mL) and heated at 60 °C. 10% aqueous sodium hydroxide solution was added dropwise until it was completely dissolved and pH 11 was established. Next, the reaction mixture was treated with diluted hydrochloric acid until pH 2 was attained; then extracted with ethyl acetate (4x100 mL), followed by drying of the extracts ($MgSO_4$) and concentration. The residue was purified by preparative thin layer chromatography using pet. ether and ethyl acetate (6:4) as eluent and finally by recrystallization from ethyl acetate to afford **5** (0.164 g, 82%) as white scales. mp 176-180 °C; R_f =0.13 (pet. ether-ethyl acetate 6:4); IR (KBr) ν_{max}/cm^{-1}: 3194, 1730, 1594, 1695, 1244, 1047; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.29 (s, 3H, CH_3), 3.86 (s, 6H, MeO), 3.89 (s, 3H, MeO), 3.99 (s, 2H, CH_2), 6.45 (d, *J* 2.2 Hz, 1H, H5), 11.2 (1H, br s, COOH); ^{13}C NMR (100 MHz, CDCl3): δ 195.5 (C2'), 168.1 (COOH), 132.7 (C5), 131.8 (C6), 127.4 (C7), 77.7 (C1'), 55.9 (MeO), 55.6 (MeO x2), 42.9 (C3'); EIMS: *m/z* (rel.int.): 268 (M^+, 30), 250 (41), 196 (53), 178 (70), 150 (32). Anal. Calc. for $C_{13}H_{16}O_6$: C, 58.20; H, 6.01; Found: C, 58.14; H 6.13%.

*(±)-2,3,4-Trimethoxy-6-(2-hydroxypropyl) benzoic acid (**6**)*

Keto acid (**5**) (0.634 mmol, 0.17 g) was heated under reflux with sodium borohydride (0.15 g) in absolute ethanol (15.2 mL) for 4 h. Ethanol was then rotary evaporated, the residue diluted with cold water and acidified with diluted sulfuric acid to give a precipitate, which was extracted with ethyl acetate. Finally the solvent was rotary evaporated to afford **6** as a semi solid (0.13 g, 0.475 mmol, 75%).

*(±)-6,7,8-Trimethoxy-3-methyl-3,4-dihydroisocoumarin (**7**)*

The hydroxy acid (**6**) (0.12 g, 0.44 mmol) was dissolved in acetic anhydride (1 mL) and heated under reflux for 2 h. The reaction mixture was then cooled and water (16 mL) was added. The oil, which separated on stirring, was extracted with dichloromethane. The extracts were combined, treated with 5% aqueous sodium bicarbonate solution washed with water, dried over sodium sulphate anhydrous and filtered. The solvent was stripped off on rotary evaporator to leave **7** (0.088 g, 80%) as a colorless crystalline solid mp 103 – 104 °C (lit.[10] 105 °C). IR (KBr) ν_{max}/cm^{-1}: 2924, 2852, 1725, 1595, 1426,1 235, 1121; ^{1}H NMR (CD_3OD): δ 6.54 (1H, s, H-5), 4.56 (IH, m, H-3), 3.87 (3H, s, 6-OMe), 3.93 (3H, s, 8-OMe), 3.90 (3H, s, 7-OMe), 2.94 (1H, dd, *J* 16.1 Hz, *J* 3.4 Hz, H-4), 2.81 (1H, dd, *J* 16.1 Hz, *J* 11.1 Hz, H-4), 1.46 (3H, d, *J* 6.3 Hz, 3-Me); ^{13}C NMR (CD_3OD): 170.9 (C1), 80.2 (C3), 68.1 (C4), 139.3 (4a), 104.3 (C5), 160.2 (C6), 134.2 (C7), 156.7 (C8), 102.9 (C8a), 60.9 (7 OMe), 56.8 (6 OMe), 16.3 (3 Me); EIMS: *m/z* (rel. int.): 252 (76) $[M]^+$, 223 (25), 209 (16), 195 (34), 191 (21), 179 (46), 177 (40), 151 (37). Anal. Calc. for $C_{13}H_{16}O_5$: C 61.90, H 6.39; Found: C 61.86, H 6.46%.

*(±)-8-Hydroxy-6,7-dimethoxy-3-methyl-3,4-dihydroisocoumarin (**1**)*

A 1 mol L^{-1} solution of boron tribromide in dry dichloromethane (0.55 mL) was injected into a stirred solution of (**7**) (0.1 g, 0.4 mmol) in dry dichloromethane (4 mL) at -78 ºC under argon. After stirring for 20 min., the mixture was poured into ice water (20 mL) and stirred for

30 min. The layers were separated and the aqueous layer was extracted with dichloromethane and then with ethyl acetate. The combined organic phases were dried using anhydrous sodium sulphate and concentrated. It was further purified by preparative TLC using petroleum ether and ethyl acetate (8:2) as eluent and finally by recrystallization from ethyl acetate to afford (**1**) (0.071 g, 75%) as a colorless crystalline solid. mp 141-143 ºC (lit. [5] 144 ºC). IR ($CHCl_3$) ν_{max}/cm^{-1}: 2924, 2852, 1663, 1426, 1269, 1121; ^{1}H NMR (CD_3OD): δ 6.51 (1H, s, H 5), 4.71 (IH, m, H 3), 3.77 (3H, s, 6 OMe), 3.90 (3H, s, 7 OMe), 2.96 (1H, dd, *J* 16.2 Hz, J 3.4 Hz, H-4), 2.87 (1H, dd, *J* 16.2 Hz, J 11.1 Hz, H-4), 1.47 (3H, d, *J* 6.3, 3-Me); ^{13}C NMR (CD_3OD): δ 170.9 (C1), 79.8 (C3), 67.7 (C4), 139.3 (4a), 104.3 (C5), 160.2 (C6), 134.2 (C7), 156.7 (C8), 102.9 (8a), 60.9 (7 OMe), 56.8 (6 OMe), 16.3 (3 Me); EIMS: *m/z* (rel. int.): 238 (M^+, 100), 223 (25), 179 (46), 209 (16), 195 (34), 177 (40), 151 (37), 136 (15), 106 (19), 77 (27), 51 (62). Anal. Calc. for $C_{12}H_{14}O_5$: C 60.50, H 5.92; Found: C 60.47, H 6.05%.

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