A Facile Synthesis of Cytogenin (8-Hydroxy-3-hydroxymethyl-6-methoxyisocoumarin)

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A facile and straightforward synthesis of cytogenin **1** (8-hydroxy-3-hydroxymethyl-6-methoxyisocoumarin) metabolite of *Streptoverticillium eurocidicum* and *Certocystis fimbriata* has been achieved. Condensation of chloroacetyl chloride with 3,5-dimethoxyhomophthalic acid **2** directly furnished 3-chloromethyl-6,8-dimethoxyisocoumarin **3** which was hydrolyzed to 6,8-dimethoxy-3-hydroxymethylisocoumarin **4** using 0.05% aqueous sodium hydroxide in THF. Regioselective demethylation of **4** yielded cytogenin **1**. In model experiments, 3-chloromethylisocoumarin was prepared and converted into 3-hydroxymethyl isocoumarin.

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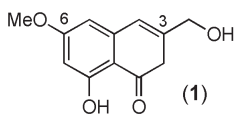
Introduction.

Cytogenin (8-hydroxy-3-hydroxymethyl-6-methoxyisocoumarin) (**1**; Figure 1) is a natural isocoumarin first isolated in 1990 from *Streptoverticillium eurocidicum* MI43-37F11 strain identified as FERM BP-2783 and was given the name of antibiotic MI43-37F11 [1,2]. In 1994, it was reported as a metabolite of *Certocystis fimbriata*, a pathogenic ascomycete that causes black rot in sweet potato along with the related isocoumarin derivatives *e.g.* 6,8-dihydroxy-3-hydroxymethylisocoumarin and 6,8-dihydroxy-3-methylisocoumarin [3]. The latter are also metabolites of blue-stain fungi *C. minor* and *C. ulmi*, responsible for pine beetle disease and Dutch elm disease respectively [4]. Cytogenin is the first natural isocoumarin to exhibit anticancer activity against experimental tumor cells and human cancer cells: Leukemia L1210, mouse Leukemia P388 and Leukemia EL4, mouse IMC carcinoma, human lung cancer LX-1 cells and against Erhlich carcinoma in mice at 6.3 to 100 mg/kg/day, while exhibiting low cytotoxicity. It also possesses an activity to enhance the production of interleukin-1 *in vivo* in a mammalian [1]. The antitumor and antirheumatoid arthritis effects possessed by cytogenin are due to its anti-angiogenic activity. Angiogenesis is the process for generation of new blood vasculature, involved in a variety of normal biological functions, as well as disease states such as rheumatoid arthritis, psoriasis, diabetic retinopathy, cancer and solid tumors [5]. The inhibition of angiogenesis deprives the tumors of nutrients by inhibiting the generation of microvasculature. Cytogenin is a novel oral antiangiogenic agent which suppresses angiogenesis induced by Sarcoma-180 tumor cells [6], reduced antitumor effector activity of spleen cells taken from tumor bearing mice *in vitro* [7] and was found to modify collagen-induced arthritis in mice [8,9]. It activates macrophages to produce monokines such as IL-1 alpha and stimulates proliferation and differentiation of T cells resulting in production of lymphokines such as IFN gamma and GM-CSF. Cytogenin has low cytotoxicity on murine and human tumor cells *in vitro* and a potent inhibitory effect on spontaneous polyarthritis in MR/1 mice and pristine-induced arthritis in DBA/1J mice and its mode of action is different from those of NSAIDs [10]. Various 3-side chain modified analogues of cytogenin have been synthesized and tested among which NM-3 showed the best pharmacological properties [8].

Biosynthetically, the carbon skeleton of cytogenin is derived from pentaketide intermediate due to head to tail condensation of five acetate units and methyl group of the 6-methoxyl is derived from methionine [11].



(**1**)

Figure 1

The great medicinal importance coupled with simple structure makes cytogenin an attractive target for synthesis. The patented synthesis by Hirano *et al.* [12] involved five steps starting from 8-hydroxy-6-methoxy-3-methyl- isocoumarin, a natural product, itself synthesized through a number of steps. Taylor *et al.* [13] have recently reported an improved synthesis of cytogenin. In this approach, the benzylic anion of ethyl 2-ethoxymethoxy-4-methoxy-6-methylbenzoate generated with LDA, was quenched with benzylglycolate. Proton transfer was a competing reaction, and the condensation provided the α-hydroxyketone. TBS protection of the primary hydroxyl followed lactonization under basic conditions. Finally, deprotection with hydrochloric acid provided the cytogenin.

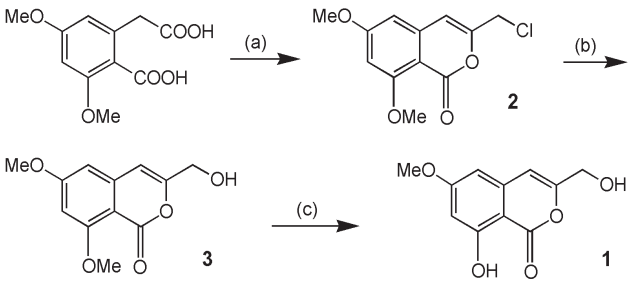
An efficient synthesis of cytogenin was undertaken as a continuation of our interest towards synthesis of antitumor metabolites [14] and naturally occurring isocoumarins [15].

Results and Discussion.

3,5-Dimethoxyhomophthalic acid **2** was prepared by the method reported by us earlier [16]. Condensation of 3,5-dimethoxyhomophthalic acid with chloroacetyl chloride at elevated temperature directly afforded the 3-chloromethyl-6,8-dimethoxyisocoumarin **3** in 45% yield [17]. The characteristic 1H singlet of olefinic proton (H-4) appeared at δ 6.51 and a 2H singlet (CH_2Cl) at δ 4.31 ppm in the ^1H NMR and carbon signals at δ 101.3 (C-4) 144.3 (C-3) and 52.4 (CH_2Cl) in the ^{13}C NMR spectrum. IR spectrum showed the δ-lactonic carbonyl absorption at 1685 cm^{-1}.

Due to presence of base sensitive lactone ring a mild method for nucleophilic substitution of 3-chloromethyl group by 3-hydroxymethyl was required [18]. This needed experimentation on some easily available substrate to optimize the conditions and yield. To achieve this goal 3-chloromethylisocumarin was prepared as a model compound by a similar condensation of commercial homophthalic acid with chloroacetyl chloride and various combinations of solvents and varying concentrations of aqueous sodium hydroxide were tried. Thus for example, refluxing with aqueous dioxane only or in presence of 0.1 % aqueous sodium hydroxide resulted in hydrolytic ring opening to the corresponding keto acid. The successful substitution was achieved by refluxing with 0.05 % aqueous sodium hydroxide in THF for 6 h to afford the 3-hydroxymethylisocoumarin whose physical and spectral data were in close agreement to that reported in literature [19]. After optimization of conditions 3-chloromethyl-6,8-dimethoxylisocoumarin **3** was smoothly converted into 6,8-dimethoxy-3-hydroxymethylisocoumarin **4** in good yield. Thus in addition to the 1H singlet at δ 6.67, a 2H broad singlet (CH_2OH) at δ 4.41 ppm was observed further confirmed by the carbon signals at δ 102.6 (C-4) 160.0 (C-3) and 60.1 (CH_2OH). IR spectrum showed a strong absorption at 3420 cm^{-1} for the hydroxylic function indicating the successful substitution and the lactonic carbonyl absorption at 1730 cm^{-1}.

Scheme 1



Reagents and conditions: (a) $ClCH_2COCl$, 200°, 4h, 65%; (b) 0.05% aq. NaOH (0.1 to 0.05%), THF reflux 4h; (c) $AlCl_3$, $C_6H_5NO_2$, 80°, 78%.

Regioselective cleavage of the C-8 methoxy ether in 6,8-dimethoxy-3-hydroxymethylisocoumarin **4** to unveil cytogenin **1** was achieved using anhydrous aluminum chloride in freshly distilled dry nitrobenzene [20]. ^1H NMR showed a slight deshielding of the characteristic absorptions at δ 6.70 (H-4) and 2H broad singlet or triplet (CH_2OH) at δ 4.47 ppm and carbon signals at δ 163.4 and 103.2, 60.30 for C-3 and C-4 and CH_2OH respectively and the absence of 6-MeO signals. IR spectrum showed absorptions at 3460 and 1685 cm^{-1} the lactonic carbonyl absorption being lowered due to internal chelation.

In summary, an efficient synthesis of natural isocoumarin antibiotic MI43-37F11 (cytogenin) has been accomplished. It involves three linear steps and proceeds with an overall 40 % yield. This route also allows an easy conversion of **4** into dihydrocytogenin *via* hydrolytic ring opening, reduction, cyclization sequence, for comparison of bioactivity. A noteworthy feature of the present synthesis besides its simplicity is that it does not involve expensive reagents nor require difficult to acquire experimental conditions.

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 at 400 MHz (Bruker AM-400) and 100 MHz (Bruker AM-100) instruments respectively. FT IR spectra were recorded on an FTS 3000 MX spectrophotometer; Mass Spectra (EI, 70eV) on a MAT 312 instrument and elemental analyses with CHN-Rapid Hereaus. All compounds were purified by thick layer chromatography using silica gel from Merck.

3-Chloromethyl-6,8-dimethoxyisocoumarin (**3**).

A stirred mixture of 3,5-dimethoxyhomophthalic acid **2** (0.5 g, 2.08 mmol) and chloroacetyl chloride (1.82 g, 8.33 mmol) was heated in an oil bath at 200 °C for 4 h. After cooling, the blackish residue was taken up in ethyl acetate and was filtered. The filtrate was concentrated to leave a brownish solid which on thick layer chromatography (petroleum ether: ethyl acetate =6:4) afforded the 0.24 g isocoumarin (**3**) as light brown powder (45%), mp 209-212°, IR (potassium bromide): 2913, 2849, 1730, 1645, 1625, 1575, 1180, 810 cm^{-1}; ^1H NMR (deuteriochloroform): δ 3.89 (s, 3H, MeO-6) 3.95 (s, 3H, MeO-8), 4.31 (s, 2H, CH_2Cl), 6.40 (d, 1H, *J*=2.3, H-5), 6.51 (s, 1H, H-4), 6.53 (d, 1H, *J*=2.3, H-7); ^{13}C NMR (deuteriochloroform): δ 167.5 (C1), 163.5 (C8), 144.3 (C3), 159.5 (C6), 142.6 (4a), 104.3 (C8a), 102.6 (C4), 99.6 (C5), 98.3 (C7), 56.4 (MeO-8), 55.7 (MeO-6), 52.4 (CH_2Cl); EIMS m/z (%) = 256 [M^++2] (6.3), 254 [M^+] (63), 220 (43), 206 (17), 205 (100), 177 (52), 149 (38).

Anal. Calcd. for $C_{12}H_{11}ClO_4$: C, 56.60; H, 4.35; Cl, 13.92. Found: C, 56.53 H, 4.38 Cl, 13.89.

3-Chloromethylisocoumarin.

Prepared in a manner analogous to **3** from commercial homophthalic acid and chloroacetyl chloride in 75% yield. Thick layer chromatography (petroleum ether: ethyl acetate=8:2) gave

compound as orange crystals. Mp 117-118°. IR (potassium bromide): 2930, 2849, 1730, 1625, 1575, 1170 cm^{-1}; ^1H NMR (deuteriochloroform): δ 4.29 (s, 2H, CH_2Cl), 6.51 (s, 1H, H-4), 7.37 (d, *J*=7.6, 1H, H-5), 7.51 (t, *J*=7.6, 1H, H-6), 7.69 (t, *J*=7.6, 1H, H-7), 8.30 (d, *J*=8.0, 1H, H-8); ^{13}C NMR (deuteriochloroform): δ 162.5 (C1), 153.7 (C3), 137.7 (C4a), 125.6 (C5), 134.7 (C6), 127.6 (C7), 129.4 (C8), 119.9 (C8a), 53.4 (CH_2Cl); EIMS m/z (%) =196 [M^++2] (43), 194 [M^+] (43), 158 (13), 145 (17), 118 (29), 87 (91).

Anal. Calcd. for $C_{10}H_7ClO_2$: C, 61.72 H, 3.63 Cl 18.22. Found: C, 61.28 H, 3.71 Cl 18.38.

3-Hydroxymethylisocoumarin.

Sodium hydroxide (0.05 % aqueous solution) (8 mL) as added to a stirred solution of 3-chloromethylisocoumarin (300 mg, 1.54 mmol) in tetrahydrofuran (10 ml). The reaction mixture turned reddish and was refluxed for 6 h at which time tlc marked the completion of reaction. The reaction mixture was neutralized and extracted with ethyl acetate (3x75 mL dried (Na_2SO_4) and concentrated *in vacuo*. Thick layer chromatography (petroleum ether: ethyl acetate=4:6) yielded the 154 mg product (85%) as yellow amorphous powder. mp 97-98° (lit. [19] 97°). IR (potassium bromide): 3420, 1705, 1645, 1625, 1575, 1160, 835, 760, 695 cm^{-1}; ^1H NMR (deuteriochloroform) δ 4.42 (s, 2H, CH_2OH), 6.51 (s, 1H, H-4), 7.40 (d, *J*=7.6, 1H, H-5), 7.51 (t, *J*=7.6, 1H, H-6), 7.69 (t, *J*=7.6, 1H, H-7), 8.30 (d, *J*=8.0, 1H, H-8); EIMS m/z (%): 176 [M^+] (65), 148 (43), 147 (17), 118 (100), 89 (43).

Anal. Calcd. for $C_{10}H_8O_3$: C, 68.18; H, 4.58. Found: C, 67.94 H, 4.92.

6,8-Dimethoxy-3-hydroxymethylisocoumarin (**4**).

Sodium hydroxide (6 mL, 0.05 % aqueous solution) was added to a stirred solution of 3-chloromethyl-6,8-dimethoxyisocoumarin (**3**) (200 mg, 0.78 mmol) in THF (8 mL). The reaction mixture turned reddish and was refluxed for 6 h. The reaction mixture was neutralized and extracted with ethyl acetate (3x75 mL) dried (Na_2SO_4) and concentrated. Thick layer chromatography (petroleum ether:ethyl acetate =3:7) gave 154 mg **4** as yellow solid (82%) mp 99-102°. IR (potassium bromide) 3450, 2924, 2820, 1730, 1704, 1685, 1602, 1575,1459, 1272, 1074 cm^{-1}; ^1H NMR (deuteriochloroform) δ 3.89 (s, 3H, MeO-6) 3.95 (s, 3H, MeO-8), 4.41 (br s, or t, 2H, CH_2OH), 6.54 (s, 1H, H-4), 6.40 (d, 1H, *J*=2.3, H-5), 6.51 (d, 1H, J=2.3, H-7); ^{13}C NMR (deuteriochloroform) δ 167.5 (C1), 163.5 (C8), 144.3 (C3), 159.5 (C6), 142.6 (4a), 103.1 (C4), 99.6 (C5), 98.3 (C7), 56.4 (MeO-8), 55.7 (MeO-6), 60.1 (CH_2OH); EIMS (70eV): m/z (%) =236 [M^+] (63), 220 (43), 206 (17), 205 (100), 177 (52), 149 (38).

Anal. Calcd. for $C_{12}H_{12}O_5$: C, 61.01 H, 5.12. Found: C, 60.98 H, 5.17.

8-Hydroxy-3-hydroxymethyl-6-methoxyisocoumarin (**1**).

Aluminum chloride (0.11 g, 0.85 mmol) was added to a solution of **4** (100 mg, 0.43 mmol) in freshly distilled dry nitrobenzene (6 mL). The reaction mixture was stirred at 50-60° for 6 h, then poured into ice-water and acidified with dil. hydrochloric acid. The acidic solution was extracted with ether (3 x 50 mL) and stirred for 10 min. The layers were separated and the aqueous layer extracted with dichloromethane (2 x 50 mL) and then the combined extracts with 10% sodium hydroxide (2 x 60 mL). The basic solution was extracted with ether, acidified and again extracted with ether. The last extract was evaporated and residue purified by thick layer chromatography (Petroleum ether:ethyl acetate 8:2) to afforded 74 mg **1** as light yellow powder (78%). mp152°. (lit. [1] 148.5-149.5°). IR (film): 3460, 2950, 1685, 1665, 1580, 1480, 1271, 1162, 741 cm^{-1}; ^1H NMR (deuterioacetone): δ 3.95 (s, 3H, MeO-6), 4.47 (2H,d, *J*=0.9 CH_2OH), 6.54 (d, 1H, *J*=2.76, H-7), 6.67 (d, 1H, *J*=2.64, H-5), 6.70 (1H, s, H-4) ppm; ^{13}C NMR (deuterioacetone): δ 169.3 (C1, CO), 166.9 (C6), 164.7 (C8), 157.9 (C8), 140.2 (C3), 105.3 (C5), 103.2 (C4), 101.9 (C5), 100.1 (C8a), 99.5 (C7), 60.31 , 56.5 (MeO-6); EIMS m/z (%): 222 (M^+, 65), 205 (13), 204 (5), 194 (12), 191 (100), 177 (21).

Anal. Calcd. For $C_{11}H_{10}O_5$: C 59.46, H 4.54; Found C, 59.21, H 4.59.

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