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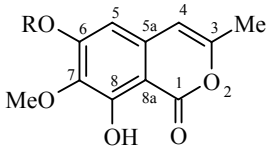
AN EFFICIENT SYNTHESIS OF 8-HYDROXY-6,7-DIMETHOXY-3-METHYLISOCOUMARIN (6-O-METHYLRETICULOL)

Aamer Saeed and Somia Ehsan

An efficient synthesis of 8-hydroxy-6,7-dimethoxy-3-methylisocoumarin (6-O-methylreticulol), a metabolite of several fungal species possessing phosphodiesterase inhibitor, topoisomerase I inhibitor activities and antitumor efficacy, has been described. 3,4,5-Trimethoxyhomophthalic acid was refluxed with acetic anhydride in dry pyridine and the resulting 2,3,4-trimethoxy-6-(2-oxopropyl)benzoic acid was smoothly cyclodehydrated to 6,7,8-trimethoxy-3-methylisocoumarin using acetic anhydride. Regioselective demethylation of the latter yielded the 6-O-methylreticulol.

Keywords: endophytic fungus, isocoumarin, reticulol, 3,4,5-trimethoxyhomophthalic acid.

6-O-Methylreticulol (8-hydroxy-6,7-dimethoxy-3-methylisocoumarin) (**1a**) is a naturally occurring isocoumarin isolated from an endophytic fungus growing on the mangrove plant *Avicennia marina* in the Pearl River Estuary of Southern China [1]. It had also been reported as a metabolite of fungus *Streptomyces mobaraensis* [2, 3], S*treptoverticillium* [4], and liverwort *Wettsteinia schusterana* [5-7] and *W. inversa* [8]. Reticulol itself (6,8-dihydroxy-7-methoxy-3-methylisocoumarin **1b**) was first isolated from *Streptomyces rubrireticulae* [9] and later from *Streptomyces mobaraensis* [10]. Reticulol has been reported as an inhibitor of cyclic adenosine 3',5'-monophosphate phosphodiesterase [11, 12]. Reticulol along with its 6-O-methyl, 8-O-methyl, and 9-hydroxy derivatives have recently been isolated from a culture of *Actinomyces sp. Stamm K 17/9* [13].



1a,**b**

a R = Me (6-O-Methylreticulol), **b** R = H (Reticulol)

It has been found that the culture broth of a strain of *Streptoverticillium* sp. NA-4803 produced an antitumor antibiotic revealed to be reticulol [2, 3]. The phosphodiesterase inhibitor activity of reticulol has long been known. The antitumor efficacy of reticulol in a tumor metastasis model, melanoma B16F10, and its ability to inhibit topoisomerase I (Topo I), an enzyme involved in the melanoma metastasis mechanism [14], was investigated. Reticulol showed effective antitumor activity against melanoma B16F10, particularly when

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Department of Chemistry, Quaid-I-Azam University 45320 Islamabad, Pakistan; e-mail: aamersaeed@yahoo.com. Published in Khimiya Geterotsiklicheskikh Soedinenii, No. 11, pp. 1644-1648, November, 2005. Original article submitted August 6, 2005.

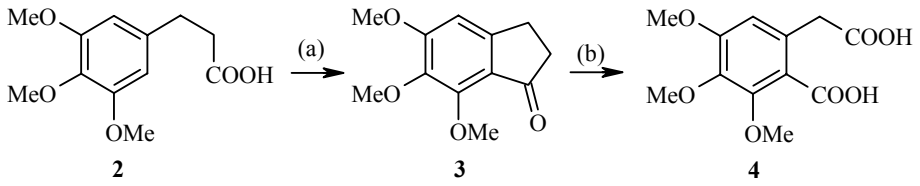
combined with an anthracycline antibiotic, Adriamycin, which when used alone has shown some adverse side effects. Reticulol blocked the replication and/or transcription of DNA by inactivating Topo I, involved in tumor metastasis, showing an inhibitory effect similar to that of camptothecin, which is known as a Topo I inhibitor. In addition, the *in vivo* antitumor efficiacy of reticulol was revealed to be excellent at a concentration of approximately 10 mg/kg, and in particular, a mixture of 5 mg/kg reticulol and 1 mg/kg Adriamycin exhibited strong antitumor efficacy, which was not shown at a concentration of 1 mg/kg Adriamycin alone. Consequently, reticulol and its derivatives proved to be potential antitumor agents.

RESULTS AND DISCUSSION

The reaction of hydroxyphthalides with *p*-toluenesulfonic acid gave a mixture of 8-hydroxy-6,7-dimethoxy-3-methylisocoumarin and 6,7,8-trimethoxy-3-methylisocoumarin [15]. In view of the widespread occurrence and biological activities, an efficient synthesis of **1a** was desired. Herein, we report a facile and high yield synthesis of the title compound.

We envisaged the synthesis of **1a** using 3,4,5-trimethoxyhomophthalic acid (**4**) as a key intermediate. 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 [16]. Thus, cyclodehydration of **2** using polyphosphoric acid gave 5,6,7-trimethoxy-1-indanone **3** in 76% yield. A toluene solution of indanone **3** and diethyl oxalate was treated with a suspension of sodium methoxide in toluene. This followed the addition of potassium hydroxide and 30% H_2O_2 to furnish **4** in 76% yield.

Scheme 1. Synthesis of 3,4,5-trimethoxyhomophthalic acid **4**



2 **3** **4**

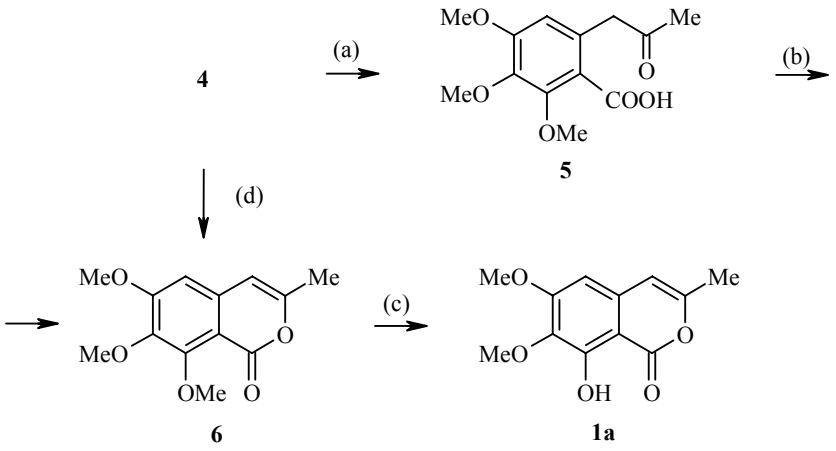
Reagents and conditions: (a) PPA, 90°C, 2 h, 76%; (b) i) $(EtO_2C)_2$, NaOMe, PhMe; ii) 30% H_2O_2, KOH, MeOH, 72%

The synthesis of compound **1a** was accomplished starting from acid **4** as shown in the Scheme 2. Reaction of homophthalic acid **4** with acetic anhydride in dry pyridine afforded the 2,3,4-trimethoxy-6-(2-oxopropyl)benzoic acid **5** [17]. The keto acid showed the signal for benzylic protons at δ 3.91 and that for carbon at δ 77.7 ppm, a characteristic $[M^+–H_2O]$ ion at *m/z* 250, and the carboxylic and ketonic carbonyl absorptions at 1683 and 1716 cm^{-1} respectively.

Cyclodehydration of the keto acid **5** was achieved simply by refluxing with acetic anhydride to yield 6,7,8-trimethoxy-3-methylisocoumarin (**6**). It is noteworthy that the attempted cyclization with a mixture of perchloric acid and acetic anhydride [18] resulted in the concurrent demethylation and acetylation of the 8-methoxyl group to afford 8-acetoxy-6,7-dimethoxy-3-methylisocoumarin. Isocoumarin **6** showed the doublet for the H-4 olefinic proton at δ 6.09 and that for C-3 methyl protons at δ 2.20, carbon signals at δ 104.1 (C-4) and 147.9 ppm (C-3), and the δ-lactonic carbonyl absorption at 1721 cm^{-1}.

Regioselective demethylation of isocoumarin **6** using boron tribromide under mild conditions furnished 8-hydroxy-6,7-dimethoxy-3-methylisocoumarin **1a**. Signals for the H-4 shifted slightly downfield at δ 6.16, and carbon signals at δ 104.6 (C-4) and 153.5 (C-3) were observed. The lactonic carbonyl absorption was lowered to 1685 cm^{-1} due to chelation. Alternatively, 6,7,8-trimethoxy-3-methylisocoumarin (**6**) was also obtained by direct condensation [18] of 3,4,5-trimethoxyhomophthalic acid **4** with acetyl chloride but with poor yield.

Scheme 2. Synthesis of 6-O-methylreticulol **1a**



Reagents and conditions: (a) Ac_2O, py, dry ether, overnight, r. t., 81.7%; (b); a) Ac_2O, 2 h reflux, 76%; (c) BBr_3, dry CH_2Cl_2, -78°C (20 min) r. t. 30 min, 75%, (d) CH_3COCl, 200°C, 10 h, 30%

CONCLUSION

In summary, a simple and efficient synthesis of 8-hydroxy-6,7-dimethoxy-3-methylisocoumarin (6-O-methylreticulol) (**1a**), a natural isocoumarin meta-bolite of several fungal species, possessing enzyme inhibitor activities and antitumor efficacy has been achieved.

EXPERIMENTAL

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

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 (4 × 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 evaporated to dryness. Recrystallization using ethyl acetate gave **3** as light brown crystals (0.758 g, 76%); mp 107-108°C, R_f 0.461 (petroleum ether–ethyl acetate, 6:4). IR spectrum, ν_{max}, cm^{-1}: 1685, 1590. ^1H NMR spectrum, δ, ppm: 2.6 (2H, m, H-3); 3.1 (2H, m, H-2); 3.87 (6H, s, OCH_3); 3.89 (3H, s, OCH_3); 6.41 (1H, s, H-4). Mass-spectrum (EI), *m/z* (*I*, %): 222 $[M]^+$ (26), 194 (53), 180 (70), 179 (82). Found, %: C 64.78; H 6.25. $C_{12}H_{14}O_4$. Calculated, %: C 64.85; H 6.35.

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 added portionwise slowly, keeping the temperature below 50°C; then H_2O_2 (30%, 2.5 ml) was added slowly, keeping

the temperature below 64°C, and the mixture was stirred further at ambient temperature for 16 h. The mixture was filtered and the filtrate was partially reduced *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 M HCl until 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 compound **4** (0.143 g, 72%); mp 151-153°C, R_f 0.08 (petroleum ether–ethyl acetate, 6:4). IR spectrum, ν_{max}, cm^{-1}: 3350, 2600, 1720, 1685, 1590. ^1H NMR spectrum, δ, ppm: 3.75 (2H, s, $ArCH_2$); 3.86 (6H, s, OCH_3); 3.89 (3H, s, OCH_3); 6.47 (1H, s, H-2). ^{13}C NMR spectrum, δ, ppm: 171.7 (COOH); 168.1 (COOH); 161.2 (C-3); 159.9 (C-5); 137.6 (C-1); 116.5 (C-2); 109.1 (C-6); 56.0 (OCH_3); 55.4 (OCH_3); 39.2 ($ArCH_2$). Mass-spectrum (70 eV), *m/z* (*I*, %): 286 $[M]^+$ (30), 268 (38), 242 (53), 198 (90), 184 (35). Found, %: C 55.51; H 6.39. $C_{13}H_{18}O_7$. Calculated, %: C 55.54; H 6.34.

2,3,4-Trimethoxy-6-(2-oxopropyl)benzoic Acid (5). To a stirred mixture of **4** (0.70 mmol, 0.2 g) in acetic anhydride (0.80 ml), pyridine (0.44 ml) was added under argon. The solid was dissolved instantly and after 2 min precipitates were produced, which solidified. 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. To it was added dropwise 10% sodium hydroxide solution until it was completely dissolved and pH 11 was established. It was acidified with dilute hydrochloric acid until pH 2 was attained, extracted using ethyl acetate (4 × 100 ml), dried ($MgSO_4$), and concentrated. The residue was purified by preparative thin layer chromatography using petroleum ether–ethyl acetate (6:4) as eluent and finally by recrystallization using ethyl acetate to afford **5** (0.164 g, 81.7%) as white scales; mp 176-180°C, R_f 0.04 (petroleum ether–ethyl acetate, 6:4). IR spectrum, ν_{max}, cm^{-1}: 3194, 1730, 1594, 1695, 1244, 1047. ^1H NMR spectrum, δ, ppm (*J*, Hz): 2.29 (3H, s, CH_3); 3.86 (6H, s, CH_3O); 3.89 (3H, s, CH_3O); 3.99 (2H, s, CH_2); 6.45 (1H, d, J = 2.2, H-5); 11.2 (1H, br. s, COOH). ^{13}C NMR spectrum, δ, ppm: 195.5 (C-2'); 168.1 (COOH); 132.7 (C-5); 131.8 (C-6); 127.4 (C-7); 77.7 (C-1'); 55.9 (CH_3O); 55.6 (CH_3O × 2); 42.9 (C-3'). Mass-spectrum (EI), *m/z* (*I*, %): 268 $[M]^+$ (30), 250 (41), 196 (53), 178 (70), 150 (32). Found, %: C 58.25; H 6.0. $C_{13}H_{16}O_6$. Calculated, %: C 58.20; H 6.01.

6,7,8-Trimethoxy-3-methylisocoumarin (6). The keto acid **5** (0.523 mmol, 0.14 g) was refluxed with acetic anhydride (3.26 ml) for 2 h. The reaction mixture was then poured into ice water (100 ml) and extracted with ethyl acetate (4 × 100 ml). The extracts were combined, washed with 5% sodium bicarbonate solution, and finally with water. The organic layers were collected, dried ($MgSO_4$), and rotary evaporated. The residue was purified by preparative thin layer chromatography using petroleum ether–ethyl acetate (7:3) as eluent and finally by recrystallization using ethyl acetate (0.107 g, 76%). R_f 0.77 (petroleum ether–ethyl acetate, 8:2); mp 116-118°C (lit. [5] 118°C). IR spectrum, ν_{max}, cm^{-1}: 2956, 1720, 1657, 1271, 1161, 1072, 741. ^1H NMR spectrum, δ, ppm (*J*, Hz): 2.20 (3H, d, J = 0.9, CH_3); 3.85 (3H, s, CH_3O-6); 3.95 (3H, s, CH_3O-8); 3.98 (3H, s, CH_3O-7); 6.09 (1H, d, J = 1.0, H-4); 6.47 (1H, s, H-5). ^{13}C NMR spectrum, δ, ppm: 19.2 (CH_3-3); 56.1 (6-OCH_3); 56.6 (8-OCH_3); 60.5 (7-OCH_3); 98.0 (C-5); 100.7 (C-8a); 104.3 (C-4); 134.4 (C-4a); 139.0 (C-7); 152.9 (C-3); 154.6 (C-8); 159.9 (C-6); 167.3 (C-1). Mass-spectrum (EI), *m/z* (*I*, %): 250 $[M]^+$ (63), 191 (81), 177 (31), 163 (19), 149 (36), 135 (46). Found, %: C 62.21; H 5.79. $C_{13}H_{14}O_5$. Calculated, %: C 62.39; H 5.64.

8-Hydroxy-6,7-dimethoxy-3-methylisocoumarin (6-O-Methylreticulol) (1a). A 1 M solution of boron tribromide in dry dichloromethane (0.44 ml) was injected into a stirred solution of **6** (0.08 g, 0.32 mmol) in dry dichloromethane (3 ml) at -78°C under argon. The mixture was stirred for 20 min and then poured into ice water followed by a further 10 min of stirring. The two layers were separated and the aqueous layer was extracted successively with dichloromethane and ethyl acetate. The combined organic layers were dried ($MgSO_4$) and concentrated. The residue was purified by preparative thin layer chromatography on silica gel using (CH_2Cl_2–MeOH, 19:1) as eluent to afford **1a** as white solid (0.06 g, 75%). R_f 0.86 (petroleum ether–ethyl acetate, 8:2); mp 197-199°C (lit. [1] 198-200°C). IR spectrum, ν_{max}, cm^{-1}: 3425, 1681, 1639, 1561, 1522, 1459, 1443, 1423, 1378, 1356, 1290, 1265, 1212, 1168, 1105, 1025, 1004. ^1H NMR spectrum, δ ppm (*J*, Hz): 2.25 (3H, s, 3-CH_3); 3.89 (3H, s, 6-OCH_3); 3.94 (3H, s, 7-OCH_3); 6.16 (1H, d, J = 0.8, H-4); 6.31 (1H, s, H-5); 11.04

(1H, br. s, 8-OH). ^{13}C NMR spectrum, δ, ppm: 19.3 (3-CH_3); 56.1 (6-OCH_3); 60.7 (7-OCH_3); 98.0 (C-5); 100.7 (C-8a); 104.6 (C-4); 134.4 (C-4a); 135.0 (C-7); 153.5 (C-3); 154.6 (C-8); 159.9 (C-6); 166.3 (C-1). Mass-spectrum, *m/z* (*I*, %): 236 $[M]^+$ (100), 221 $[M–CH_3]^+$ (96), 193 $[M–CH_3–CO]^+$ (65). Found, %: C 59.87; H 5.27. $C_{12}H_{12}O_5$. Calculated, %: C 61.01; H. 5.12.

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