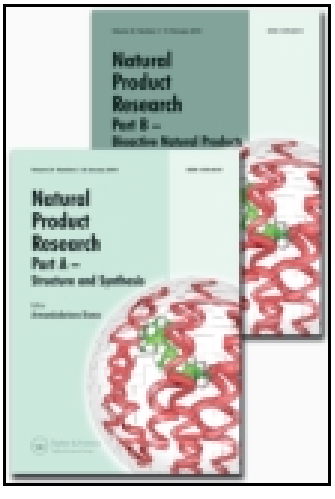

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Total synthesis of cytotoxic metabolite (±)-desmethyldiaportinol from Ampelomyces sp.

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Total synthesis of cytotoxic metabolite (±)-desmethyldiaportinol from *Ampelomyces* sp.

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A concise total synthesis of (±)-desmethyldiaportinol isolated from *Ampelomyces* sp. is described. Microwave-assisted cyclocondensation of 3,5-dimethoxyhomopthalic acid with 3,4-dibromobutanoyl chloride afforded the 3-(2,3-dibromopropyl)-6,8-dimethoxyisocoumarin in 2–3 min as the pivotal step. The 3,4-dibromobutanoyl chloride was itself synthesised from 3-butenoic acid via bromination in carbon tetrachloride at room temperature to yield 3,4-dibromobutanoic acid followed by reaction with thionyl chloride. The replacement of bromo- by hydroxyl substituent was achieved under mild conditions involving the refluxing in a mixture of acetone and water to provide (±)-3-(2,3-dihydroxypropyl)-6,8-dimethoxyisocoumarin which on complete demethylation furnished the title natural product.

Keywords: desmethyldiaportinol; isocoumarin; *Ampelomyces* sp

1. Introduction

Isocoumarins (1*H*-2-benzopyran-1-ones) are the positional isomers of coumarins with an inverted lactone moiety. More than 300 isocoumarins have been isolated so far from a wide range of natural sources (microbes, plants, insects and marine organisms) (Kutschera et al. 2003; Engelmeier et al. 2004). Several biological activities have been reported for these metabolites including cytotoxic, antimicrobial, antiallergic, algicidal, gastroprotective, protease inhibitor and antifungal (Krohn et al. 2001; Özcan et al. 2007; Guimaraes et al. 2008; Oliveira et al. 2009) as well as plant growth regulatory activities (Kuramata et al. 2007). In addition, nephratoxic, protease inhibitor, antifungal, cytotoxic, immunomodulatory, antiallergic and antimalarial activities have also been attributed to this class of natural products (Krohn et al. 2001; Di Stasi et al. 2004; Kostova et al. 2005; Devienne et al. 2007; Zhang et al. 2008). Most of the natural isocoumarins posses a 3-alkyl chain (C_1–C_{17}) or a 3-(un) substituted phenyl ring and 6,8-dioxygenation pattern due to their typical biosynthetic origin (Hill 1986).

Fungal endophytes have been proved to be a source of new and biologically active natural products for specific medicinal and agrochemical uses (Strobel 2003). Thus, Proksch and coworkers isolated a phenolic isocoumarin metabolite from the ethyl acetate extract of fungal endophyte *Ampelomyces* sp. obtained from the flowers of *Urospermum picroides*. The structure of was established unambiguously by spectroscopic techniques as 3-(2,3-dihydroxypropyl)-6,8-dihydroxy-1*H*-isochromen-1-one which displayed potent *in vitro* cytotoxic activity against L5178Y cells of mouse (Aly et al. 2008). The determination of configuration at C-3 proved it to be the R-enantiomer (Figure 1).

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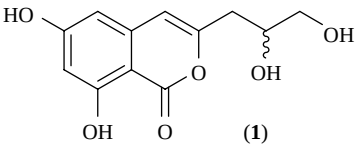


Figure 1. (±)-3-(2,3-Dihydroxypropyl)-6,8-dihydroxyisocoumarin.

As a continuance of our focus on the synthesis and bioevaluation of this important class of secondary metabolites (Saeed 2003, 2004, 2013; Saeed & Ehsan 2005; Saeed & Ashraf 2008; Saeed et al. 2011), herein we report a simple and efficient total synthesis of the title compound as a racemic mixture. The synthesis not only confirms the structural assignment but also makes it accessible for comprehensive evaluation of its bioactivity.

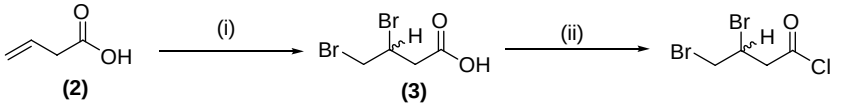
2. Results and discussion

An efficient synthesis of (±)-3-(2,3-dihydroxypropyl)-6,8-dihydroxy-1*H*-isochromen-1-one (**1**) was carried out according to the synthetic route revealed in Schemes 1 and 2. Accordingly, 3-butenoic acid (**2**) was treated with bromine in carbon tetrachloride at room temperature to provide (±)-3,4-dibromobutanoic acid (**3**) which was converted to 3,4-dibromobutanoyl chloride on treatment with thionyl chloride as a viscous yellow oil which was used in the following step. The completion of the reaction and purity of the compound were checked by analytical thin layer chromatography (TLC) where the spot characteristic of acid chlorides was observed (Scheme 1).

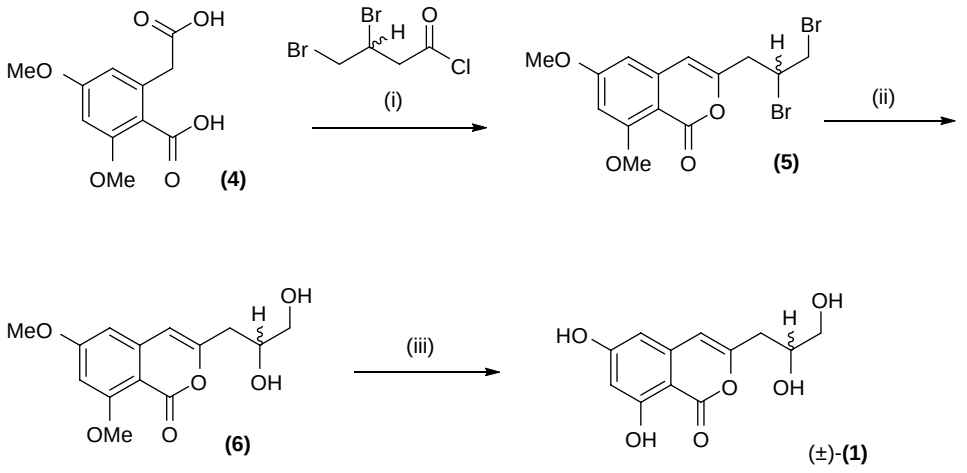
The key intermediate 3,5-dimethoxyhomophthalic acid (**4**) was prepared starting from 3,5-dihydroxybenzoic acid according to the procedure reported earlier (Saeed et al. 2003). Cyclocondensation of 3,4-dibromobutanoyl chloride with **4** afforded 3-(2,3-dibromopropyl)-6,8-dimethoxyisocoumarin (**5**) under microwave irradiation in 2–3 min in high yield, while the same reaction takes 4–8 h under the conventional heating method. Isocoumarin (**6**) was separated by preparative thin layer chromatography (PTLC) using silica gel and petroleum ether:ethyl acetate (2:1) as an eluent and further purified by recrystallisation from methanol. In the FT-IR spectrum, the δ-lactonic carbonyl stretching appeared as a strong absorption at 1724 cm^{-1}, besides those at 3126 cm^{-1} for (C═C—H) and 1570 cm^{-1} for the aromatic moiety. Isocoumarin **5** exhibited the characteristic singlet for H-4 olefinic proton at δ 6.57, the triplet for H-1′ at δ 2.46 ($J = 7.5$ Hz) and the characteristic carbon signals at δ 159.3 C-3, 103.5 for C-4 and 168.1 for lactonic carbon, respectively.

The replacement of bromo- substituents in **5** with hydroxyls was achieved by refluxing with a mixture of acetone and water in the absence of any base to provide 3-(2,3-dihydroxypropyl)-6,8-dimethoxyisocoumarin (**6**). In the ^{1}H NMR spectrum, the singlets at δ 3.6, 4.39 ppm and 3.95 confirmed the presence of hydroxy and methoxy protons respectively and the double doublets at δ 2.1 and 2.3 with $^{1,2}J = 9.0$ Hz, $^{1,3}J = 12.0$ Hz those of the methylenic protons. The signals at δ 165.6 (C-1), 157.2 (C-3), 104.9 (C-4) and 56.17 (2-OMe) were observed in the ^{13}C NMR spectrum.

Complete demethylation of (±)-3-(2,3-dihydroxypropyl)-6,8-dimethoxyisocoumarin (**6**) was achieved by refluxing with hydrobromic acid in glacial acetic acid (Zou et al. 2008) to afford



Scheme 1. Synthesis of (±)-3,4-dibromobutanoyl chloride.



Scheme 2. Synthesis of (±)-desmethyldiaportinol.

(±)-desmethyldiaportinol (**1**) (Scheme 2). In the FT-IR spectrum, the characteristic (O—H) stretching appeared at 3421 cm^{-1} and in the mass spectrum, base peak was observed at *m/z* 206. In the ^{1}H NMR spectrum of isocoumarin (**1**), besides the disappearance of C-6 and C-8 methoxyls, a characteristic broad singlet for —OH protons appeared at δ 4.84 ppm. Furthermore, H-3′ proton appeared as a multiplet at δ 5.63 and a characteristic downfield double doublets were detected for H-4 proton at δ 3.42 and 3.28 ppm. In the ^{13}C NMR spectrum, carbonyl carbon (C═O) was observed at δ 169.6 and an upfield signal for C-3 appeared at δ 88.4 ppm. In addition, two downfield carbons C-6 and C-8 were observed at δ 160.7 and 160.7 ppm, respectively. The product was further characterised by the close agreement of its physicospectral data with that of the natural isocoumarin.

3. Experimental

3.1. *General methods*

All chemicals used in the synthesis were purchased from Sigma-Aldrich and were used as such. Thin layer chromatography was used to monitor the progress of the reactions. All of the compounds were purified over silica gel column. Solvents were distilled before use for purification purposes. Melting points were recorded using a digital Gallenkamp (SANYO, Loughborough, UK) model MPD BM 3.5 apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded using Bruker AM-300 spectrophotometer (Billerica, Middlesex Massachusetts, USA) at 300 MHz and 75.5 MHz, respectively, using TMS as an internal standard. The chemical shift values are recorded on δ scale and the coupling constants (*J*) are in Hz. FT-IR spectra were recorded using Bio Rad Excalibur FTS 3000 MX spectrophotometer (Madison, WI, USA). R_f values are reported in the following solvent system: petroleum ether and ethyl acetate (2:1).

3.1.1. *(±)-3,4-Dibromobutanoic acid (**3**)*

3-Butenoic acid (**2**) (6 ml, 0.0025 mol) was dissolved in dry CCl_4 (35 ml) and treated drop wise with bromine (4.5 ml) and the reaction mixture was stirred at room temperature for 3 h. On completion (TLC control), the reaction mixture was rotary evaporated and then poured into water. Extracted with ethyl acetate, followed by separation, drying over anhydrous sodium

sulphate and rotary evaporation yielded (±)-3,4-dibromobutanoic acid as a viscous brown oil. Yield 72%. R_f 0.35. IR (neat) ν (cm^{-1}); 1722 (C═O acid), 875 (–Br). MS (70 eV) *m/z* (%): 245.87, 243.87 $[M]^{+\cdot}$; elemental analysis: found: C, 19.49%; H, 2.51%; calcd. for $C_4H_6Br_2O_2$: C, 19.54%; H, 2.46%.

3.1.2. *(±)-3,4-Dibromobutanoyl chloride*

(±)-3,4-Dibromobutanoic acid (**3**) (2 ml, 0.0011 mol) was refluxed with thionyl chloride (1 ml) for 2 h to yield (±)-3,4-dibromobutanoyl chloride as a viscous yellow oil which was used in the following step.

3.1.3. *(±)-3-(2,3-Dibromopropyl)-6,8-dimethoxyisocoumarin (**5**)*

A homogeneous mixture of 3,5-dimethoxyhomothalic acid (**4**) (1.9 g, 0.0012 mol) and (±)-3,4-dibromobutanoyl chloride (0.0024 mol) was irradiated for 2–3 min in an alumina bath inside a microwave oven with intermittent cooling. The progress of the reaction was followed by TLC examination using hexane ethyl acetate (2:1). On completion, the reaction mixture was dissolved in ethyl acetate and purified by PTLC using petroleum ether and ethyl acetate (2:1) to afford (**5**) as a brown solid. Yield 85%. R_f 0.32. m.p. 118–120°C. IR (neat) ν (cm^{-1}); 1724 (C═O ester), 3126 (C═C—H), 835 (C—Br). ^{1}H NMR ($CDCl_3$): δ 6.6–7.30 (2H, Ar–H), 6.56 (1H, s, C═C—H), 3.95 (6H, s, OMe), 3.6 (3H, m, —CH_2—Br), 1.9, 2.0 (2H, dd, $^{1,2}J = 8$ Hz, $^{1,3}J = 11$ Hz, CH_2). ^{13}C NMR ($CDCl_3$): δ 165.6 (C-1), 163.4 (C-6), 160.4 (C-8), 157.2 (C-3), 143.9 (C-9), 112.3 (C-10), 104.9 (C-4), 102.3 (C-5), 100.7 (C-7), 56.17 (2-OMe), 44.45 (C-3′), 30.0 (C-4′) 26.1 (C-5′). MS (70 eV) *m/z* (%): 405.92, 403.93 $[M]^{+\cdot}$; elemental analysis: found: C, 41.39%; H, 3.51%; calcd. for $C_{14}H_{14}Br_2O_4$: C, 41.41%; H, 3.48%.

3.1.4. *(±)-3-(2,3-Dihydroxypropyl)-6,8-dimethoxyisocoumarin (**6**)*

3-(2,3-Dibromopropyl)-6,8-dimethoxyisocoumarin (**5**) (0.18 g, 0.00032 mol) was refluxed with an acetone–water mixture (1:5) for 3 h. On completion, the reaction mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with 5% sodium bicarbonate and then dried over anhydrous sodium sulphate and rotary evaporated to leave a solid which was recrystallised from methanol to afford isocoumarin (**6**) as a light yellow solid. Yield 70%. R_f 0.22. m.p. 130–133°C. IR (neat) ν (cm^{-1}); 1722 (C═O ester), 3145 (C═C—H), ^{1}H NMR ($CDCl_3$): δ 6.6–7.30 (2H, Ar—H), 6.57 (1H, s, C═C—H), 4.39 (3H, m, CH_2-OH), 3.95 (6H, s, —OMe), 3.6 (2H, s, —OH), 2.1, 2.3 (2H, dd, $^{1,2}J = 9$ Hz, $^{1,3}J = 12$ Hz, CH_2). ^{13}C NMR ($CDCl_3$): δ 165.6 (C-1), 163.4 (C-6), 160.4 (C-8), 157.2 (C-3), 143.9 (C-9), 112.3 (C-10), 104.9 (C-4), 102.3 (C-5), 100.7 (C-7), 56.17 (2-OMe), 44.45 (C-3′), 57.2 (C-4′) 67.1 (C-5′). MS (70 eV) *m/z* (%): 280.1 $[M]^{+\cdot}$; elemental analysis: found: C, 60.09%; H, 5.77%; calcd. for $C_{14}H_{16}O_6$: C, 59.99%; H, 5.75;%.

3.1.5. *(±)-Desmethyldiaportinol (**1**)*

3-(2,3-Dihydroxypropyl)-6,8-dimethoxyisocoumarin (**6**) (0.1 g, 0.000034 mol) was refluxed with 33% hydrobromic acid in glacial acetic acid (6 ml) for 2 h. The reaction mixture was poured into ice 50 g and then solid sodium carbonate was added to manage to pH 6. The compound was extracted with ethyl acetate and dried and rotary evaporated to give a solid recrystallised from methanol to afford racemic desmethyldiaportinol (**1**) as an yellowish powder. Yield 68%. R_f 0.22. m.p. 178°C. IR (neat) ν (cm^{-1}); 1722 (C═O ester), 3145 (C═C—H), 3455 (—OH). ^{1}H NMR ($CDCl_3$): δ 6.6–7.30 (2H, Ar–H), 6.57 (1H, s, C═C—H), 5.2 (2-OH—Ar), 4.39 (3H, m, —CH_2—OH), 3.95 (6H, s, —OMe), 3.6 (2H, s, OH), 1.9, 2.0 (2H, dd, $^{1,2}J = 9.0$ Hz, $^{1,3}J = 12$ Hz,

CH_2). ^{13}C NMR ($CDCl_3$): δ 165.6 (C-1), 165.4 (C-6), 163.4 (C-8), 157.2 (C-3), 143.9 (C-9), 112.3 (C-10), 104.9 (C-4), 102.3 (C-5), 100.7 (C-7), 56.17 (2-OMe), 44.45 (C-3′), 57.2 (C-4′) 67.1 (C-5′). MS (70 eV) *m/z* (%): 252 $[M]^{+\cdot}$; elemental analysis: found: C, 57.21%; H, 4.78%; calcd. for $C_{12}H_{12}O_6$: C, 57.14%; H, 4.80%.

4. Conclusions

The first total non-enantioselective synthesis of recently isolated natural cytotoxic metabolite desmethyldiaportinol was achieved. The pivotal steps include the condensation of acid chloride with homopthalic acid under microwave irradiation, which drastically reduced the reaction time and improved the yield, and successful substitution of bromo- with hydroxyls under mild conditions using acetone and water in the absence of any base to avoid ring opening.

Supplementary material

Supplementary material relating to this article is available online, alongside Figures S1 and S2.

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