Synthesis of 8-Desoxythunberginol A and (±)-8-Desoxy-3,4-dihydrothunberginol A

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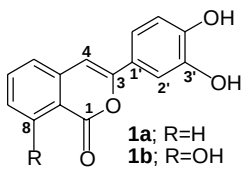
A two step synthesis of title isocoumarin isolated from *Homalium longifolium* and its conversion into corresponding 3,4-dihydroisocoumarin has been described. 3,4-Dimethoxybenzoyl chloride on condensation with homophthalic acid afforded 3-(3′,4′-dimethoxyphenyl)isocoumarin which was demethylated to furnish the 8-desoxythunberginol A, whereas its sequential saponification, reduction and demethylation yielded the (±)-8-desoxy-3,4-dihydrothunberginol A. The synthesized compounds were examined *in vitro* for antibacterial activity.

Keywords: *Homalium longifolium*; *Hydrangea macrophylla*; Thunberginol A; Isocoumarins; Antibacterial.

INTRODUCTION

In 1995, K. Shaari isolated 3-(3′,4′-dihydroxyphenyl)isocoumarin (**1a**; R=H) from the stem of *Homalium longifolium* Benth., a rain forest tree found in the southern part of the Malay Peninsula.[1] Earlier in 1992, M. Yoshikawa had isolated its 8-hydroxy derivative: thunberginol A (**1b**; R=OH) along with thunberginol B, and 3,4-dihydro- derivatives: thunberginols C, D, E and G from the Hydrangeae Dulcis Folium (amacha or sweet tea) a natural medicine indigenous to Japan, prepared from the leaves of *Hydrangea macrophylla* SERINGE var. *thunbergii* MAKINO (Saxifragaceae).[2,3] This natural medicine is listed in the Japanese Pharmacopoeia XII and is extensively used in confectionary, drinks and food as an oral refrigerant and a sweetener. The methanolic extract showed potent antiallergic, antibacterial, antioxidative, antiulcer and cholagoic activities. Two isocoumarins (thunberginol A and B), four dihydroisocoumarins (thunberginol C, D, E and G) and some related compounds were recognized as the antiallergic and antibacterial principles of this natural medicine.[4] Phyllodulcin and hydrangenol had already been identified as its principal constituents and sweetening agents.[5] Thunberginol A, B inhibited the histamine release from rat peritoneal mast cells induced by compound 48/80, calcium ionophore A23187, with IC_{50} values of less than 100 μM. Structure-activity studies have shown that isocoumarins such as thunberginol A and B are more potent as histamine release inhibitors as compared to the dihydroisocoumarins such as hydrangenol, thunberginol D and E.[6] S. Ohta has reported a synthesis of thunberginol A&B.[7]



1a; R=H
1b; R=OH

Condensation of acid chlorides with homopthhalic acid is useful for the preparation of 3-substituted isocoumarin skeleton.[8,9] Herein a short and efficient synthesis of 8-desoxythunberginol A achieved by using this method and its conversion into corresponding (±)-3,4-dihydroisocoumarin as a model for thunberginols C, D, E, G, hydrangenol and phyllodulcin, and the antibacterial activity of the synthesized compounds are described.

Reaction of commercial homophthalic acid with 3,4-dimethoxybenzoyl chloride at elevated temperature afforded the 3-(3′,4′-dimethoxyphenylisocoumarin (**2**) in 85% yield. The isocoumarin showed the characteristic 1H singlet of isocoumarin moiety (H-4) at δ 6.74 in the ^{1}H NMR and at δ 100.46 (C-4) and 153.49 (C-3) in the ^{13}C NMR spectrum. IR spectrum showed the lactonic carbonyl absorption at 1720 cm^{-1}.

Complete demethylation of (**2**) was proceeded using BBr_3 in dry CH_2Cl_2 in good yield to furnish 3-(3′,4′-dihydroxyphenyl)isocoumarin (**1a**; R=H). The dihydroxy isocoumarin was characterized by complete absence of both MeO singlets and the downfield shift of the characteristic H-4 singlet at δ 7.26 in the ^{1}H NMR and at δ 99.68 (C-4) in the ^{13}C

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NMR spectrum. IR spectrum showed the lactonic carbonyl absorption at 1722 cm^{-1}.

Alkaline hydrolysis of the isocoumarin (**2**) to furnish the 2-(3,4-dimethoxybenzoylmethyl)benzoic acid (**3**) was accomplished in 80% yield. The keto acid showed the characteristic 2H singlet at δ 4.73 (H-4, $ArCH_2$) in the ^1H NMR and that at δ 44.02 (C-4) in ^{13}C NMR spectrum.[10] DEPT 90° and 135° experiments confirmed these assignments. The ketonic and carboxylic carbonyl absorptions were observed in IR spectrum at 1716 and 1685 cm^{-1}, respectively.

Sodium borohydride reduction of the keto acid (**3**) afforded the corresponding racemic hydroxy acid (**4a**) which underwent spontaneous cyclodehydration on standing for a few minutes (as monitored by TLC) to (±)-3-(3′,4′-dimethoxyphenyl)-3,4-dihydroisocoumarin (**4**) without any dehydrating agent.[11] The methylenes protons (C4) adjacent to the newly generated chiral centre (C3) in dihydroisocoumarin (**4**) showed the diastereotopic effect. Thus, typical ABX splitting[12] (dddd) of the three 3,4-hydrogens, ($ArCH_2$, AB) and H3 (X) in ^1H NMR at δ 3.08-3.39 ppm was observed. Each hydrogen was split by the other nearly to the same extent and unequally by the adjacent methine proton. The double doublet of the hydrogen *cis* to phenyl ring is located slightly upfield at 3.13-3.08 ppm (J_{gem} = 16.3 Hz, J_{cis} = 3.14 Hz) and that of *trans* hydrogen is located slightly downfield at 3.39-3.32 ppm (J_{gem} = 16.54, J_{trans} = 12.16 Hz). The H-3 appeared as a double doublet at 5.52-5.48 ppm with vicinal coupling constant to the *trans* H4 of 12.04 Hz and to the *cis* H4 of 3.26 Hz due to coupling with each of the unequivalent C-4 protons. ^{13}C NMR spectrum showed signals at δ 80.03 and 35.76 for C-3 and C-4, respectively. The δ-lactonic carbonyl absorption appeared at 1721 cm^{-1} in the IR spectrum.

The final deprotection to unveil the 8-desoxy-3,4-dihydrothunberginol A (**5**), was carried out using boron tribromide. Absence of both MeO singlets and the slight downfield shift of the characteristic signals in the ^1H and ^{13}C NMR spectra confirmed the complete demethylation.

Biological Activity

The isocoumarins (**1a**), (**2**), keto acid (**3**) and the dihydroisocoumarins (**4**) and (**5**) were screened *in vitro* for antibacterial activity[13] against various bacterial strains and were shown to exhibit weak or moderate activity compared to the standard drug (Table 1). The activity was determined *via* the growth inhibition of microorganisms i.e. the zone of inhibition was measured in millimeters. It is evident from the table that the dihydroisocoumarin **4** and **5** show more bactericidal activity compared to corresponding isocoumarin **1a** and **2** and the 3′ and 4′ hydroxyl groups are important for activity as the corresponding dimethyl ethers are less active.

It is interesting to note that whereas *d*-phyllodulcin and *dl*-hydrangenol possess antifungal activities,[14] our compounds were inactive as antifungal agents indicating that 8-hydroxyl group is essential for such activity.

EXPERIMENTAL

Melting points of the compounds were determined using a Gallenkemp melting point apparatus and are uncorrected. Commercial ethyl acetate and petroleum ether (60-80 °C) were distilled before use; CH_2Cl_2 was dried over CaH_2 ^1H NMR and ^{13}C NMR spectra were recorded respectively on 400 MHz (Bruker, AM-400) and 100 MHz (Bruker, AM-100) as $CDCl_3$ or DMSO-d_6 solutions. Coupling constants *J* were measured in Hz. IR spectra were recorded on a Bruker Vector 22 and Mass Spectra (EI, 70 eV) on a MAT 312 instrument. Flash column chromatography was carried out on Merck Kieselgel 60.

3-(3′,4′-Dimethoxyphenyl)isocoumarin (2)

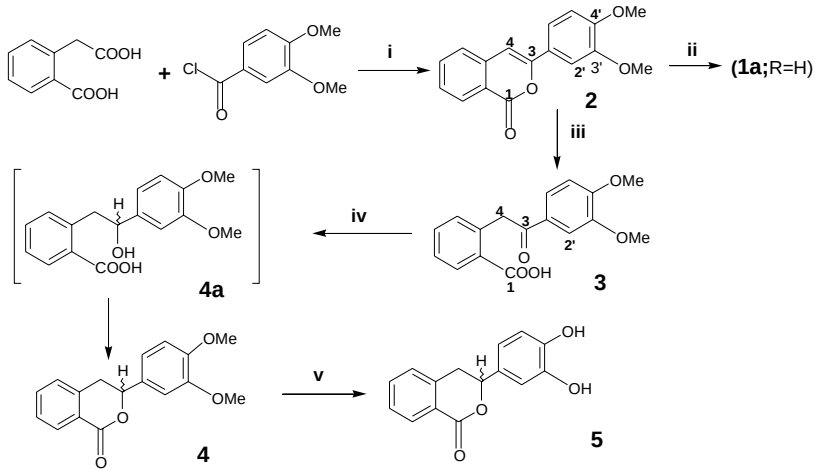
A stirred mixture of homophthalic acid (0.5 g, 2.77 mmol) and 3,4-dimethoxybenzoyl chloride (2.19 g, 9.7 mmol) was heated in an oil bath at 200 °C for 4 h. Flash chromatography of the residue (petroleum ether: ethyl acetate

Table 1.

Microorganism	**2**	**1a**	**3**	**4**	**5**	Standard drug (Imipenem)
Escherichia coli	11	13	10	12	13	30
Bacillus subtilis	00	12	8	00	10	31
Shigella flexenari	00	10	8	00	8	35
Staphlococcus aureus	00	12	10	12	11	45
Pseudomonas aeruginosa	10	13	13	12	17	29
Salmonella typhi	00	11	9	00	8	40

(Concentration Used 100 μg/100 mL of DMSO) Zone of Inhibition (mm)

Scheme I



Reagents and conditions:
(i) 200 °C, 4 h (85%); (ii) BBr_3/CH_2Cl_2, -78 °C, R.T., overnight (70%); (iii) 5% KOH / EtOH, 4 h reflux (80%); (iv) $NaBH_4$ / EtOH, 2 h, R.T. (85%); (v) BBr_3, CH_2Cl_2, -78 °, R.T., overnight (75%)

8:3) followed by recrystallization from MeOH-H_2O gave the isocoumarin **2** (0.66 g, 2.35 mmol, 85%) as orange yellow needles. m.p. 110 °C; EIMS *m/z* (%): 282 (M^+, 30), 165 (28), 137 (19.8), 117 (100); IR (film, ν, cm^{-1}) 2913, 2849, 1720, 1694, 1598, 1572, 1471, 1151, 832 cm^{-1}; ^1H NMR ($CDCl_3$, δ, ppm) 3.85 (s, 3H, MeO-3′) 3.90 (s, 3H, MeO-4′), 6.74 (s, 1H, H-4), 7.26 (d, *J* = 7.6, 1H, H-5), 7.36 (td, *J* = 2.12, 1H, H-7), 7.57-7.62 (dt, *J* = 2.12, 1H, H-6), 8.17 (d, *J* = 7.6, 1H, H-8); ^{13}C NMR (CDCl3, δ, ppm): 162.7 (C1, C=O), 153.4 (C3), 100.4 (C4), 137.7 (C4a), 125.6 (C5), 134.7 (C6), 127.6 (C7), 129.4 (C8), 119.9 (C8a), 124.6 (C1′), 111.9 (C2′), 149.0 (C3′), 150.6 (C4′), 108.0 (C5′), 118.4 (C6′), 56.0 (MeO-4), 55.9 (MeO-3). HREIMS: *m/z* 282.0902 (calcd. for $C_{17}H_{14}O_4$ 282.0892).

3-(3′,4′-Dihydroxyphenyl)isocoumarin (1a; R=H)

A 1M solution of BBr_3 in CH_2Cl_2 (3.52 mL, 3.52 mmol) was injected to a stirred solution of **2** (0.25 g, 0.88 mmol) at -78 °C in dry CH_2Cl_2 (40 mL) under Ar. The reaction mixture was allowed to warm to room temperature and stirred overnight. The reaction mixture was poured into ice-water (50 mL) and the layers separated. The aqueous layer was extracted with CH_2Cl_2 (2 × 50 mL) and then EtOAc (50 mL). The combined organic phases were dried ($MgSO_4$) and concentrated. Flash chromatography (Petroleum ether:ethyl acetate 7:3) followed by recrystallization from MeOH-H_2O afforded **1a** as intense yellow needles (0.15 g, 0.61 mmol, 70%). m.p. 229-230 °C (lit[1]. 234-235 °C); EIMS: *m/z* (%): 254 (M^+, 19.4), 252 (14.1), 226 (19), 170 (37), 137 (19.8), 117 (100); IR (film, ν, cm^{-1}) 3538, 3278, 1697, 1631, 1618, 1605, 1524, 1297, 1175; ^1H NMR (DMSO-d_6, δ, ppm): 6.92 (d, 1H, *J* = 7.8, H-5), 7.26 (s, 1H, H-4), 7.32 (dd, *J* = 8.4, 2.24, 1H, H-2′), 7.37 (d, *J* = 2.24, 1H, H-6′), 7.60 (dt, *J* = 1.84, 1.0, 1H, H-7′), 7.71 (t, *J* = 7.8, 1H, H-5′), 7.88 (dt, *J* = 6.4, 1.64, 1H, H-6′), 8.19 (d, *J* = 7.76, 1H, H-8); ^{13}C NMR (DMSO-d_6, δ, ppm): 161.53 (C1, C=O), 153.2 (C3), 99.7 (C4), 137.9 (4a), 126.2 (C5), 135.2 (C6), 127.8 (C7), 128.7 (C8), 119.2 (C8a), 122.9 (C1′), 112.2 (C2′), 145.6 (C3′), 147.6 (C4′), 116.0 (C5′), 116.9 (C6′). HREIMS: *m/z* 254.0571 (calcd. for $C_{15}H_{10}O_4$ 254.0579).

2-(3′,4′-Dimethoxybenzoylmethyl)benzoic acid (3)

A stirred solution of 3-(3′,4′-dimethoxyphenyl)isocoumarin **1a** (0.4 g, 1.42 mmol) in ethanol (20 mL) was treated with 5% KOH (40 mL) and the mixture refluxed for 4 hr. After cooling the reaction mixture, most of the ethanol was rotary evaporated. Cold water (20 mL) was added and the mixture acidified with dil. hydrochloric acid when the yellow solid was precipitated. Filtration followed by drying under vacuum afforded **3** as a yellow solid. Recrystallized from MeOH (0.34 g, 1.13 mmol, 80%). m.p 182-184 °C; EIMS *m/z* (%): 300 [M^+] (11.5), 282 (37.0), 256 (11.6), 178 (100); IR (film, ν, cm^{-1}): 2915, 2849, 1713, 1694, 1601, 1202, 1162; (DMSO-d_6, δ, ppm): 3.81 (s, 3H, MeO-3′), 3.85 (s, 3H, MeO-4′), 4.73 (s, 2H, Ar-CH_2, H4), 7.11-7.0 (d, *J* = 8.28, 1H, H-5), 7.34 (dd, *J* = 7.72, 2.0, 1H, H-4′), 7.42 (dt, *J* = 7.28, 1.26, 1H, H-6), 7.52 (d, *J* = 7.21, 1H, H-5′), 7.56 (dt, *J* = 7.4, 1H, H-7), 7.77 (dd, *J* = 8.52, 2.0, 1H, H-2′), 7.95 (dd, *J* = 7.76,

1.38, 1H, H-8); ^{13}C NMR (DMSO-d_6, δ, ppm): 168.3 (C1, COOH), 195.5 (C=O, C3), 44.0 ($ArCH_2$, C4), 148.5 (4a), 132.6 (C5), 131.8 (C6), 130.8 (C7), 130.4 (C8), 152.9 (C8a), 137.3 (C1′), 129.8 (C2′), 126.8 (C3′), 122.5 (C4′), 110.9 (C5′), 110.3 (C6′), 55.7 (MeO-4), 55.5 (MeO-3). For direct comparison and to avoid confusion, the C/H numbering of isocoumarins **1a, 2** was maintained in the keto acid **3**) HREIMS: *m/z* 300.502 (calcd. for $C_{17}H_{16}O_5$ 300.0998).

(±)-3-(3′,4′-Dimethoxyphenyl)-3,4-dihydroisocoumarin (4)

Sodium borohydride (0.67 g, 18 mmol) was added portion wise to a stirred solution of **3** (0.2 g, 0.66 mmol) in ethanol (25 mL) and water (75 mL). The reaction mixture was stirred for 2 h at room temperature, diluted with water (150 mL), acidified with conc. HCl and stirred for a further 2 h. It was then saturated with ammonium sulfate, and extracted with EtOAc (3 × 100 mL). The layers were separated and the organic layer dried ($MgSO_4$) and concentrated. Flash chromatography (Petroleum ether:ethyl acetate 7:2) afforded **4** as yellow prisms (0.16 g, 0.56 mmol, 85%). m.p. 56-58 °C EIMS *m/z* (%): 284 (M^+, 56), 147 (14), 118 (100), 90 (59), 89 (15%); IR (film, ν cm^{-1}) 2933, 2849, 1684, 1606, 1517, 1460, 1263, 1117, 1026; ^{1}H NMR ($CDCl_3$, δ, ppm): 3.13-3.08 (dd, 1H J_{gem} = 16.3 Hz, J_{cis} = 3.14 Hz, H-4), 3.39-3.32 (dd, 1H, J_{gem} 16.54, J_{trans} = 12.16, H-4), 3.91 (s, 3H, MeO-3′), 3.89 (s, 3H, MeO-4′), 5.52-5.48 (dd, *J* = 12.04, 3.26 H-3), 6.88 (dd, *J* = 7.16, 1H, H-5), 6.99 (dd, *J* = 7.76, 1H, H-5′), 7.29 (m, 2H, H-6′, H-2′), 7.74 (dt, *J* = 7.4, 0.5, 1H, H-6*), 7.58 (dt, *J* = 7.28, *J* = 2.28, 1H, H-7*), 8.15 (d, *J* = 7.28, 1H, H-8); ^{13}C NMR (CDCl3, δ, ppm): 165.5 (C1), 80.0 (C3), 35.7 (C4), 149.5 (4a), 131.2 (C5), 133.9 (C6), 129.5 (C7), 129.0 (C8), 118.8 (C8a), 139.2 (C1′), 130.6 (C2′), 127.9 (C3′), 125.3 (C4′), 109.6 (C5′), 111.1 (C6′), 56.1 (MeO-4), 55.0 (MeO-3). HREIMS: *m/z* 284.1031 (calcd. for $C_{17}H_{16}O_4$ 284.1049).

(±)-3-(3′,4′-Dihydroxyphenyl)-3,4-dihydroisocoumarin (5)

A 1M solution of BBr_3 in CH_2Cl_2 (1.10 mL, 1.1 mmol) was injected to a stirred solution of (0.1 g, 0.35 mmol) in at -78 °C in dry CH_2Cl_2 (4 mL) under Ar. The reaction mixture was allowed to warm to room temperature and stirred overnight. The layers were separated and the aqueous layer was extracted with EtOAc (3 × 50 mL). The combined organic phases were dried ($MgSO_4$) and concentrated. Flash chromatography (Petroleum ether: ethyl acetate 7:3) followed by recrystallization from MeOH afforded **5** as yellow prisms (0.063 g, 0.24 mmol, 75%). m.p. 146-47 °C EIMS *m/z* (%): 256 (M^+), 254 (18.6), 138 (19.8), 118 (100), 90 (22.4); IR (film, ν, cm^{-1}) 2913, 2849, 1720, 1694, 1598, 1572, 1471, 1151, 832; ^{1}H NMR ($CDCl_3$ δ, ppm): 2.94-3.02 (dd, 1H, J_{gem} = 16.3 Hz, J_{cis} = 3.14 Hz, H-4), 3.23-3.21 (dd, 1H, J_{gem} = 16.54, J_{trans} = 12.16, H-4), 5.52-5.48 (dd, *J* = 12.04, 3.26, H-3), 6.88 (dd, *J* = 7.16, 1H, H-5), 6.99 (dd, *J* = 7.76, 1H, H-5′), 7.29 (m, 2H, H-6′, H-2′), 7.74 (dt, *J* = 7.4, *J* = 0.5, 1H, H-6*), 7.58 (dt, *J* = 7.28, *J* = 2.28, 1H, H-7*), 8.15 (d, *J* = 7.28, 1H, H-8); ^{13}C NMR ($CDCl_3$, δ, ppm): 169.2 (C1), 80.0 (C3), 35.6 (C4), 142.1 (4a), 131.3 (C5), 135.9 (C6), 129.5 (C7), 129.0 (C8), 118.8 (C8a), 139.0 (C1′), 130.2 (C2′), 126.9 (C3′), 127.3 (C4′) (*interchangeable assignments) HREIMS: *m/z* 256.0534 (calcd. for $C_{15}H_{12}O_4$ 256.0736).

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