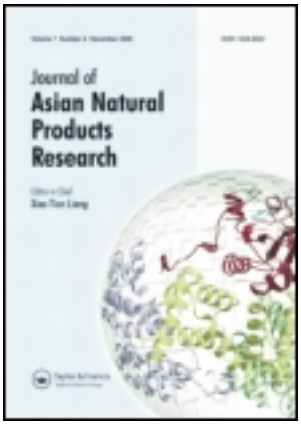

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Synthesis of (±)-6-O-methyl and 7-demethylannulatomarin

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ORIGINAL ARTICLE

Synthesis of (±)-6-*O*-methyl and 7-demethylannulatomarin

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Synthesis of 6-*O*-methyl and 7-demethyl derivatives of annulatomarin (**1**) (6,8-dihydroxy-7-methoxy-3-phenyl-3,4-dihydroisocoumarin) isolated from the aerial parts of *Hypericum annulatum* is described. The key step involves an efficient, microwave-accelerated solvent and catalyst-free condensation of methyl benzoate with 3,4,5-trimethoxyhomophthalic acid (**2**) to afford 6,7,8-trimethoxy-3-phenylisocoumarin (**3**). Saponification of the latter to keto acid (**4**) followed reduction to furnish 6,7,8-trimethoxy-3-phenyl-3,4-dihydroisocoumarin (**5**), which was either regioselectively demethylated to (±)-6-*O*-methylannulatomarin (**6**) or completely demethylated to (±)-7-demethylannulatomarin (**7**).

Keywords: annulatomarin; microwave-accelerated; dihydroisocoumarin; synthesis

1. Introduction

Nedialkov *et al.* [1] reported the isolation and structural elucidation of a new natural isocoumarin named as annulatomarin (**1**) (Figure 1), together with the known physcion and β-sitosterol from the aerial parts of *Hypericum annulatum*, a herbaceous plant growing on the Balkan Peninsula. In Bulgarian folk medicine, the aerial parts of *Hypericum* are used for the treatment of gastric and liver disorders, and this was the first isocoumarin found in *Hypericum* species [2]. The structure of the new compound was established unambiguously as 6,8-dihydroxy-7-methoxy-3-phenyl-3,4-dihydroisocoumarin using 2D NMR techniques: COSY, long-range COSY, NOESY, HETCOR, and COLOC; the multiplicities were revealed by DEPT experiments. The configuration at C-3 was not defined, but an *S*-configuration was more likely on the basis of similarity in structure and the sign of optical rotations with montroumarin isolated from *Montrouziera sphaeroidea* [3]. Annulatomarin was shown to exhibit a modest growth inhibitory activity *in vitro* against human chronic myeloid leukemia LAMA-84 cells with an IC_{50} value of 111 μM.

A synthesis of annulatomarin derivatives was undertaken as a continuation of our previous efforts toward the synthesis of naturally occurring isocoumarins and dihydroisocoumarins and their nitrogen sulfur analogs [4–8]. The limited quantities obtainable from the natural source, together with the prospect of preparing analogs with enhanced biological activities, show the crucial need for the total synthesis. Herein, the synthesis of 6-*O*-methylannulatomarin and 7-demethylannulatomarin is described, which not

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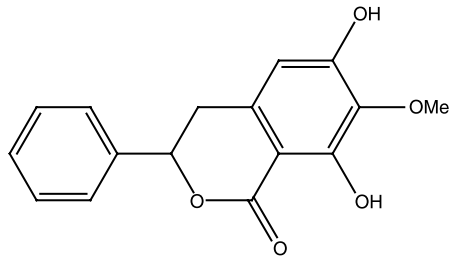


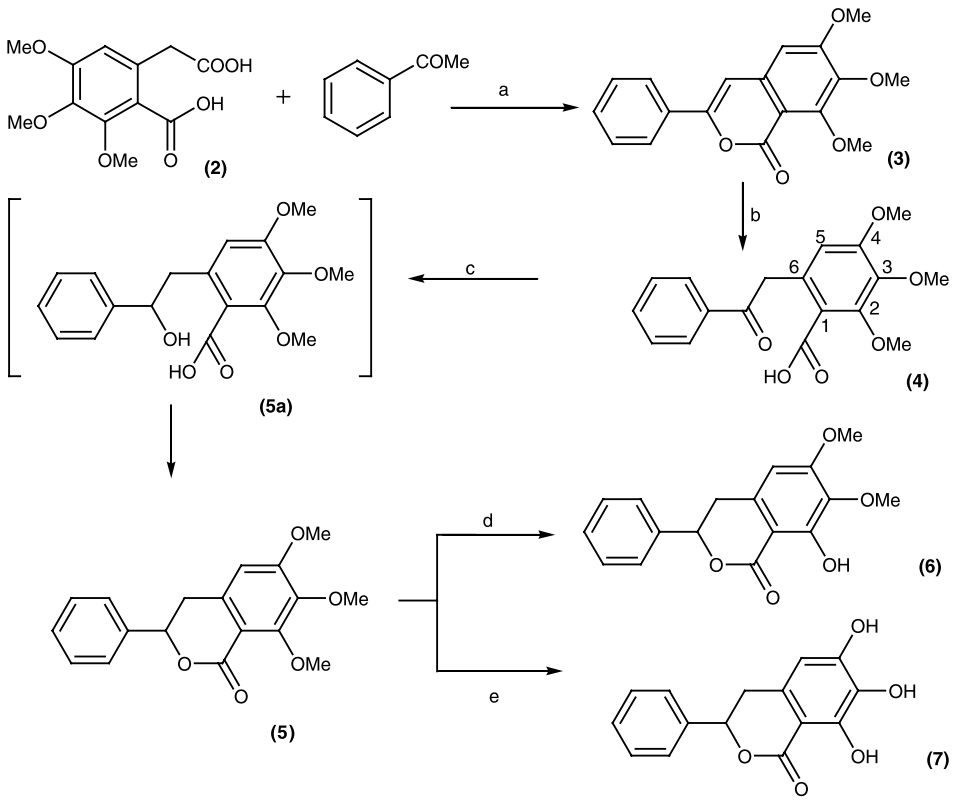
(1)

Figure 1. Structure of compound **1**.

only confirms the structural assignments but also makes it accessible for biological evaluation.

2. Results and discussion

The synthetic route to annulatomarin derivatives is outlined in Scheme 1. We envisaged the synthesis of annulatomarin derivatives using 3,4,5-trimethoxyhomophthalic acid (**2**) as a key starting material. The requisite acid (**2**) was prepared in two steps in good yield from the commercially available 3-(3,4,5-trimethoxyphenyl)propionic acid according to the method reported earlier [9]. Numerous synthetic routes to the isocoumarin scaffold have been reported in the literature. Condensation of acid chlorides with homophthalic acids is a traditional but reliable route toward a 3-substituted isocoumarin skeleton [10,11]. The key feature of the current synthesis, which prefers it over such reported synthesis [12], is the use of microwave irradiation which considerably reduces the reaction time from 3 to 4 h under conventional mode of heating to a few minutes and the replacement of hazardous acid chloride with the corresponding ester



Scheme 1. Synthesis of the derivatives of annulatomarin: (a) MW, 5–6 min, 74%; (b) 5% KOH, EtOH, 4 h reflux, 72%; (c) $NaBH_4$, EtOH, 72%; (d) $AlCl_3$, Et_2O, 1 h, 71%; (e) $AlCl_3$, EtSH, 65%.

which offers a cleaner, safer, and environmentally friendly reagent. Accordingly, an intimate mixture of the 3,4,5-trimethoxyhomophthalic acid and methyl benzoate was irradiated for 5–6 min to afford 6,7,8-trimethoxy-3-phenylisocoumarin (**3**) in good yield. This compound showed the characteristic singlet of the isocoumarin moiety at δ 6.72 (H-4) in the ^{1}H NMR spectrum and signals at δ 100.2 (C-4) and 145.5 (C-3) in the ^{13}C NMR spectrum. The IR spectrum showed the lactonic carbonyl absorption at 1725 cm^{-1}.

Alkaline hydrolysis of isocoumarin **3** furnished 2,3,4-trimethoxy-6-(benzoylmethyl)benzoic acid (**4**). The keto acid showed a characteristic 2H broad singlet of the benzylic methylene ($ArCH_2$) at δ 4.27 in the ^{1}H NMR spectrum, and the corresponding signal in the ^{13}C NMR spectrum was observed at δ 45.4, in addition to those at δ 170.8 and 196.5 for carboxylic and ketonic carbons, respectively. The carboxylic and the ketonic carbonyl absorptions in the IR spectrum appeared at 1685 and 1720 cm^{-1}, respectively.

Reduction of the prochiral keto acid **4** using sodium boronhydride afforded the corresponding racemic hydroxy acid **5a**, which underwent spontaneous cyclodehydration on standing (as monitored by TLC) to afford (±)-6,7,8-trimethoxy-3-phenyl-3,4-dihydroisocoumarin (**5**) without any dehydrating agent [13]. The methylene protons (C-4) adjacent to a newly generated chiral center in dihydroisocoumarin **5** showed the typical diastereotopic effect [14,15]. Therefore, the typical ABX splitting (dddd) of the three 3,4-hydrogens was observed [12]. The double doublet of the hydrogen *cis* to the phenyl ring is shifted slightly upfield at δ 3.13–3.08 ($J_{gem} = 16.5$ Hz, $J_{cis} = 3.4$ Hz), and that of the *trans* hydrogen is shifted slightly downfield at δ 3.39–3.32 ($J_{gem} = 16.5$ Hz, $J_{trans} = 12.1$ Hz). H-3 showed a double doublet at δ 5.52–5.48 with vicinal coupling constant to the *cis* H-4 of 3.4 Hz and to the *trans* H-4 of 12.1 Hz due to coupling with each of the unequivalent C-4 protons. The ^{13}C NMR spectrum showed signals at δ 171.4, 80.0 and 35.7 for C-1, C-3, and C-4, respectively. The δ-lactonic carbonyl absorption appeared at 1721 cm^{-1} in the IR spectrum.

The following order of the ease of cleavage of aromatic methoxyls of the isocoumarin system was observed: $C_8 > C_7 > C_6$. As a result, C_8 methoxyl, which is periplanar to the lactonic carbonyl, is most easily cleaved due to chelation of the resulting phenolic proton with the carbonyl group. Of the methoxyls at C_7 and C_6, the former possessing a more electron-rich ethereal oxygen is cleaved more readily than the latter; consequently, regioselective demethylation of C-7 methoxyl in **5** to afford the natural product annulatomarin (**1**) was difficult to achieve. Therefore, either demethylation of C-8 methoxy to **6** or complete demethylation to **7** was carried out. Thus, using anhydrous $AlCl_3$ in dry ether at reflux temperature, 8-hydroxy-6,7-dimethoxy-3-phenyl-3,4-dihydroisocoumarin (**6**) was obtained. The IR spectrum showed the hydroxyl at 3380 cm^{-1} and the lactonic carbonyl absorption at 1665 cm^{-1} due to internal chelation. In the ^{1}H NMR spectrum, the disappearance of signals for 8-MeO at δ 3.93 and the appearance of a broad singlet at δ 11.3 were noted. Complete demethylation was achieved using $AlCl_3$ in ethane thiol to afford 6,7,8-trihydroxy-3-phenyl-3,4-dihydroisocoumarin (**7**) characterized by the total lack of MeO singlets and the slight downfield shift of the characteristic signals in the ^{1}H NMR and ^{13}C NMR spectra.

The formation of isocoumarin **3** from homophthalic acid **2** may be visualized by the attack of hydroxyl of aliphatic carboxyl function to methyl benzoate to furnish the 4-benzoyl-6,7,8-trimethoxyisochroman-1,3-dione intermediate which undergoes decarboxylative rearrangement to ketone followed by cyclization and dehydration.

In summary, an efficient synthesis of annulatomarin derivatives has been accomplished, which unequivocally establishes the structural assignments and makes it available for biological evaluation.

2.1 General experimental procedures

Melting points were recorded using a digital Gallenkamp (SANYO, Leicestershire, UK) model MPD BM 3.5 apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were determined in $CDCl_3$ or acetone-d_6 solutions at 300 and 75 MHz, respectively, using a Bruker AM-300 spectrophotometer. FT-IR spectra were taken using an FTS 3000 MX spectrophotometer. Mass spectra (EI, 70 eV) were recorded on a GC-MS instrument, Agilent Technologies, and elemental analyses with a LECO-183 CHNS analyzer. The first step was carried out in a microwave oven (MW 900 W, frequency 2450 MHz, power level 1; Dawlance, Pakistan). All compounds were purified by thick layer chromatography using silica gel from Merck (Darmstadt, Germany).

2.2 6,7,8-Trimethoxy-3-phenylisocoumarin (3)

A homogenized mixture of 3,4,5-trimethoxyhomophthalic acid (**2**) (0.5 g, 1.97 mmol) and methyl benzoate (0.97 ml, 7.87 mmol) was irradiated for 5–6 min in an alumina bath. On completion of the reaction, followed by TLC examination, the reaction mixture was diluted with ethyl acetate and subjected directly to thick layer chromatography using petroleum ether–ethyl acetate (3:1, v/v) to afford pure isocoumarin **3** (0.45 g, 1.45 mmol, 74%). EI-MS *m/z* (%): 312 $[M]^{+\cdot}$ (30), 165 (28), 137 (19.8), 117 (100); IR (film, ν, cm^{-1}): 2913, 2849, 1725, 1694, 1598, 1572, 1471, 1151, 832; ^{1}H NMR ($CDCl_3$, δ, ppm): 3.87 (3H, s, MeO-7), 3.88 (3H, s, MeO-6), 3.95 (3H, s, MeO-8), 6.31 (1H, s, H-5), 6.72 (1H, s, H-4), 7.25 (1H, t, $J = 7.2$ Hz, H-4′), 7.30 (2H, t, $J = 7.2$ Hz, H-3′, 5′), 7.44 (2H, d, $J = 7.2$ Hz, H-2′, 6′); ^{13}C NMR (100 MHz, $CDCl_3$): 163.7 (C-1), 145.5 (C-3), 100.2 (C-4), 137.7 (C-4a), 99.8 (C-5), 165.5 (C-6), 164.5 (C-8), 100.9 (C-8a), 135.2 (C-1′), 126.8 (C-2′, 6′), 129.5 (C-3′, 5′), 128.0 (C-4′), 139.4 (C-7), 57.2 (MeO-7), 56.4 (MeO-6), 55.7 (MeO-8); Elemental analysis: Found: C, 69.01%, H, 5.23%; calcd for $C_{18}H_{16}O_5$: C, 69.22%, H, 5.16%.

2.3 2,3,4-Trimethoxy-6-(benzoylmethyl)benzoic acid (4)

A stirred solution of 6,7,8-trimethoxy-3-phenylisocoumarin (**3**) (0.42 g, 1.48 mmol) in ethanol (20 ml) was treated with 5% KOH (40 ml) and the mixture was refluxed for 4 h. After cooling the reaction mixture, most of the ethanol was rotary evaporated. Cold water (20 ml) was added and the mixture was acidified with dilute hydrochloric acid when the solid was precipitated. Filtration followed by drying under vacuum afforded **4** as a light yellow solid which was recrystallized from MeOH (0.35 g, 1.07 mmol, 80%). EI-MS *m/z* (%): 330 $[M]^{+\cdot}$ (11.45), 282 (37.09), 256 (11.62), 178 (100); IR (film, ν, cm^{-1}): 2915, 2849, 1720, 1685, 1601, 1202, 1162; ^{1}H NMR (acetone-d_6, δ, ppm): 11.22 (1H, br s, COOH), 8.31 (2H, d, $J = 7.2$ Hz, H-2′, H-6′), 7.42 (2H, t, $J = 7.2$ Hz, H-3′, H-5′), 7.36 (1H, t, $J = 7.5$ Hz, H-4′), 6.41 (1H, s, H-5), 4.02 (2H, s, Ar-CH_2), 3.82 (3H, s, MeO-4), 3.84 (3H, s, MeO-3), 3.88 (3H, s, MeO-2); ^{13}C NMR (acetone-d_6, δ, ppm): 197.5 (C=O), 170.8 (COOH), 139.2 (C-6), 99.8 (C-5), 165.5 (C-4), 139.4 (C-3), 164.5 (C-2), 135.2 (C-1′), 129.9 (C-3′, 5′), 129.3 (C-2′, 6′), 128.0 (C-4′), 109.4 (C-1), 45.3 ($ArCH_2$), 56.3 (MeO-4), 56.4 (MeO-3), 55.7 (MeO-2).

2.4 6,7,8-Trimethoxy-3-phenyl-3,4-dihydroisocoumarin (5)

Sodium boronhydride (0.96 g, 20 mmol) was added portionwise to a stirred solution

of **4** (0.3 g, 0.91 mmol) in ethanol (30 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 was dried ($MgSO_4$) and concentrated. The racemic hydroxy acid **5a** produced *in situ* underwent spontaneous cyclization to **5** on standing for some time (TLC; 0.2 g, 0.063 mmol, 72%). EI-MS *m/z* (%): 314 $[M]^{+\cdot}$ (56), 178 (100), 147 (14), 118 (42), 90 (59), 89 (15); IR (film): 2850, 1730, 1710, 1604, 1583, 1572, 1464, 1198, 832 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ: 3.13–3.08 (1H, dd, J_{gem} = 16.5 Hz, J_{cis} = 3.4 Hz, H-4), 3.26 (1H, dd, J_{gem} = 16.5 Hz, J_{trans} = 12.1 Hz, H-4), 3.85 (3H, s, MeO-7), 3.89 (3H, s, MeO-6), 3.94 (3H, s, MeO-8), 5.42 (1H, dd, J = 12.1, 3.4 Hz, H-3), 6.45 (1H, s, H-5), 7.21 (1H, t, J = 7.8 Hz, H-4′), 7.30 (2H, t, J = 7.2 Hz, H-3′, H-5′), 7.47 (2H, d, J = 7.2 Hz, H-2′, H-6′) ppm; ^{13}C NMR ($CDCl_3$) δ: 171.3 (C-1), 163.0 (C-8), 162.8 (C-6), 144.0 (C-4a), 107.2 (C-8a), 103.9 (C-5), 134.4 (C-7), 78.6 (C-3), 35.6 (C-4), 141.2 (C-1′), 126.7 (C-2′, 6′), 129.3 9 (C-3′, 5′), 128.9 (C-4′), 55.7 (MeO-7), 56.7 (MeO-6), 59.4 (MeO-8); Elemental analysis: Found: C, 68.91%, H, 5.63%; calcd for $C_{18}H_{18}O_5$: C, 68.78%, H, 5.77%.

2.5 8-Hydroxy-6,7-dimethoxy-3-phenyl-3,4-dihydroisocoumarin [(±)-6-O-methylannulatomarin] (6)

Anhydrous $AlCl_3$ was added to a stirred solution of **5** (100 mg, 0.32 mmol) in dry ether (1.2 ml, 1.2 mmol) under nitrogen. The reaction mixture was refluxed for 1 h. The ether was evaporated and the reaction mixture was poured into ice water (20 ml) and stirred for 10 min. The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (2× 50 ml). The combined organic phase was dried ($MgSO_4$) and concentrated to afford **6** (68 mg, 0.22 mmol, 71%). IR (film, ν, cm^{-1}): 3600, 3375, 2924, 1667, 1622, 1604, 1581, 1464, 1198, 832; EI-MS *m/z* (%): 300 $[M^+]$ (11.45), 238 (37.09), 181 (100), 165 (72.3); ^1H NMR (acetone-d_6, δ, ppm): 3.20 (1H, dd, J = 16.3, 3.2 Hz, H-4), 3.32 (1H, dd, J = 16.3, 11.7 Hz, H-4), 3.77 (3H, s, MeO-7), 3.90 (3H, s, MeO-6), 5.65 (1H, dd, J = 11.7, 3.2 Hz, H-3), 6.37 (1H, s, H-5), 7.29 (1H, t, J = 7.8 Hz, H-4′), 7.39 (2H, t, J = 8.2 Hz, H-3′, 5′), 7.51 (2H, d, J = 8.2 Hz, H-2′, 6′); ^{13}C NMR (acetone-d_6, δ, ppm): 169.8 (C-1), 164.8 (C-6), 158.6 (C-8), 144.0 (C-4a), 107.2 (C-8a), 105.9 (C-5), 136.1 (C-7), 79.8 (C-3), 35.7 (C-4), 140.2 (C-1′), 126.7 (C-2′, 6′), 129.0 (C-4′), 129.9 (C-3′, 5′), 59.7 (MeO-6), 56.8 (MeO-7); Elemental analysis: Found: C, 67.89%, H, 5.31%; calcd for $C_{17}H_{16}O_5$: C, 67.99%, H, 5.37%.

2.6 6,7,8-Trihydroxy-3-phenyl-3,4-dihydroisocoumarin [(±)-7-demethylannulatomarin] (7)

Anhydrous $AlCl_3$ was added portionwise to a stirred solution of **5** (80 mg, 0.22 mmol) in ethane thiol (1.2 ml, 1.2 mmol) at 0°C and the reaction mixture was stirred further for 1 h. The reaction mixture was poured into ice water (20 ml) and extracted with EtOAc (3× 50 ml). The combined organic phase was dried ($MgSO_4$) and concentrated to afford **7** (44 mg, 0.16 mmol, 65%). IR (film, ν, cm^{-1}): 3600, 2924, 1663, 1619, 1604, 1578, 1464, 1198, 831; EI-MS *m/z* (%): 272 $[M^+]$ (11.45), 238 (37.09), 181 (100), 165 (72.3); ^1H NMR (acetone-d_6, δ, ppm): 3.20 (1H, dd, J = 16.0, 2.9 Hz, H-4), 3.32 (1H, dd, J = 16.0, 11.7 Hz, H-4), 5.65 (1H, dd, J = 11.7, 2.9 Hz, H-3), 6.45 (1H, s, H-5), 7.29 (1H, t, J = 7.2 Hz, H-4′), 7.39 (2H, t, J = 7.2 Hz, H-3′, 5′), 7.51 (2H, d, J = 7.2 Hz, H-2′, 6′) ppm; ^{13}C NMR (acetone-d_6, δ, ppm): 169.6 (C-1), 156.4 (C-6), 157.8 (C-8), 144.0 (C-4a), 107.2 (C-8a), 105.9 (C-5), 134.2 (C-7), 79.8 (C-3), 35.7 (C-4), 140.21 (C-1′), 126.7 (C-2′,

6′), 129.0 (C-4′), 129.9 (C-3′, 5′); Elemental analysis: Found: C, 66.22%, H, 4.41%; calcd for $C_{15}H_{12}O_5$: C, 66.17%, H, 4.44%.

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