Synthesis of Montroumarin

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Z. Naturforsch. **58c**, 691–696 (2003); received November 12, 2002/April 14, 2003

A simple stereoselective synthesis of montroumarin [(*3S*)-6,8-dihydroxy-3-phenyl-3,4-dihydroisocoumarin] isolated from *Montrouziera sphaeroidea* has been achieved. Condensation of benzoyl chloride with 3,5-dimethoxyhomophthalic acid afforded 6,8-dimethoxy-3-phenylisocoumarin (**3**) which on sequential saponification and esterification yielded the keto ester **5**. Enantioselective reduction of the latter with baker's yeast directly furnished the (3*S*)-6,8-dimethoxy-3-phenyl-3,4-dihydroisocoumarin (**6**) in good enantioselectivity which on demethylation provided montroumarin. All of the synthesized compounds were examined *in vitro* for antifungal activity.

Key words: Montroumarin, Dihydroisocoumarin, Antifungal

Introduction

During phytochemical studies (Ito *et al.*, 2000) on the stem bark of *Montrouziera sphaeroidea* Pancher Ex Planchon et Triana family Guttiferae, a new dihydroisocoumarin, montroumarin, along with a xanthone, montrouxanthone and two known compounds, were isolated from an acetone extract. The structure of the dihydroisocoumarin was determined by modern spectroscopic techniques as 6,8-dihydroxy-3-phenyl-3,4-dihydroisocoumarin (**1**) and the absolute configuration at C-3 was concluded as *S* on the basis of CD spectral analysis. (Fig. 1).

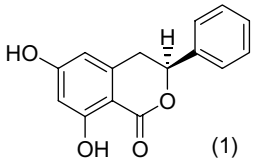

Fig. 1. Structure of monotroumarin: (*3S*)-6,8-dihydroxy-3-phenyl-3,4-dihydroisocoumarin.

Results and Discussion

A synthesis of montroumarin was undertaken as a continuation of our previous efforts towards synthesis of naturally occurring isocoumarins and dihydroisocoumarins (Rama *et al.*, 1993, 1995, 1998; Saeed and Rama, 1996) which exhibit a wide variety of biological activities despite having a relatively simple structure. The limited quantities available from the natural source together with possibility of preparing analogues with improved biological activities show the imperative need for total synthesis. Herein, a short stereoselective total synthesis of montroumarin is described which not only confirms the structural assignments but also makes it available for biological evaluation.

Synthesis

The starting substance 3,5-dimethoxyhomophthalic acid (**2**) was prepared from 3,5-dimethoxybenzyl bromide *via* rhodium-catalyzed direct carbonylation to corresponding phenylacetic acid as a key step (Giroux *et al.*, 2000). 6,8-Dimethoxy-3-phenylisocoumarin (**3**) was prepared by direct condensation of 3,5-dimethoxyhomophthalic acid with benzoyl chloride at elevated temperature in 82 % yield (Kaji *et al.*, 1986; Nozawa *et al.*, 1981). This compound showed the characteristic 1H singlet of isocoumarin moiety at δ 6.72 (H-4) in the ^{1}H NMR and signals at δ 100.2 (C-4) and 145.5 (C-3) in the ^{13}C NMR spectrum, respectively. IR spectrum showed the lactonic carbonyl absorption at 1720 cm^{-1}.

Alkaline hydrolysis of the isocoumarin derivative **3** to furnish the 2,4-dimethoxy-6-(benzoylmethyl)benzoic acid (**4**) was accomplished in 80 % yield. The keto acid showed the characteristic 2H singlet of benzylic methylene ($ArCH_2$) at δ 4.02 in the ^{1}H NMR and at δ 45.31 in ^{13}C NMR spectrum. DEPT 90° and 135° experiments confirmed these assignments. The ketonic and carboxylic carbonyl absorptions were observed in IR spectrum at 1720

and 1685 cm^{-1}, respectively. The esterification of keto acid **4** with methyl iodide in presence of anhydrous potassium carbonate in dry acetone furnished the keto ester **5**. Keto ester showed the 3H singlet at δ 3.66 (COOMe) and 2H singlet at 3.95 ($ArCH_2$) in ^1H NMR, M^+-CH_3OH ion at *m/z* 360 in the mass spectrum and ester and ketonic carbonyl absorptions at 1716 and 1694 cm^{-1}, respectively, in the IR spectrum.

The enantioselective reduction of the prochiral keto ester **5** was carried out using baker's yeast (*Saccharomyces cerevisiae*) to afford (3*S*)-6,8-dimethoxy-3-phenyl-3,4-dihydroisocoumarin (**6**) in 98 % ee (45 % yield) (Krohen *et al.*, 1997; Deshpande *et al.*, 1996; Arnoldi *et al.*, 1992). The enantiomeric excess (ee) was determined by NMR using a chiral shift reagent and the absolute configuration was checked by the sign of optical rotation. The methylene protons (C-4) adjacent to newly generated chiral centre in dihydroisocoumarin **6** showed the diastereotopic effect. Thus the typical ABX splitting (dddd) of the three 3,4-hydrogens was observed (Bovicelli *et al.*, 1999; Uchida *et al.*, 1997; Kendall *et al.*, 1989). 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 slightly downfield at 3.39–3.32 ppm (J_{gem} = 16.54, J_{trans} = 12.16 Hz). The H-3 showed a double doublet at 5.52–5.48 ppm with vicinal coupling constant to the *cis* H-4 of 12.04 Hz and to the *trans* H-4 of 3.26 Hz due to coupling with each of the unequivalent C-4 protons. ^{13}C NMR spectrum showed signals at δ 80.0 and 35.76 for C-3 and C-4, respectively. The δ-lactonic carbonyl absorption appeared at 1721 cm^{-1} in the IR spectrum.

Complete demethylation of **6** was proceeded using BBr_3 in dry CH_2Cl_2 at – 78 °C to unveil the montroumarin [(3*S*)-6,8-dihydroxy-3-phenyl-3,4-dihydroisocoumarin) (**1**)] characterized by the complete absence of both MeO singlets and the downfield shift of the characteristic signals in the ^1H NMR and ^{13}C NMR spectra. IR spectrum showed the lactonic carbonyl absorption at 1665 cm^{-1} due to internal chelation (Fig. 2).

Biological activity

The isocoumarin **3** keto acid **4** keto ester **5**, the dihydroisocoumarins **6** and **1** were screened *in vitro* for antifungal activity against some human, animal and plant pathogenic molds (Atta-ur-Rahman *et al.*, 2001) (Table I). It is evident that keto ester **5** is more potent than keto acid **4** and the dihydroisocoumarin **4** shows more antifungal activity than the corresponding isocoumarin **3**. Among dihydroisocoumarins montroumarin the 6,8-dihydroxy compound, is more active compared

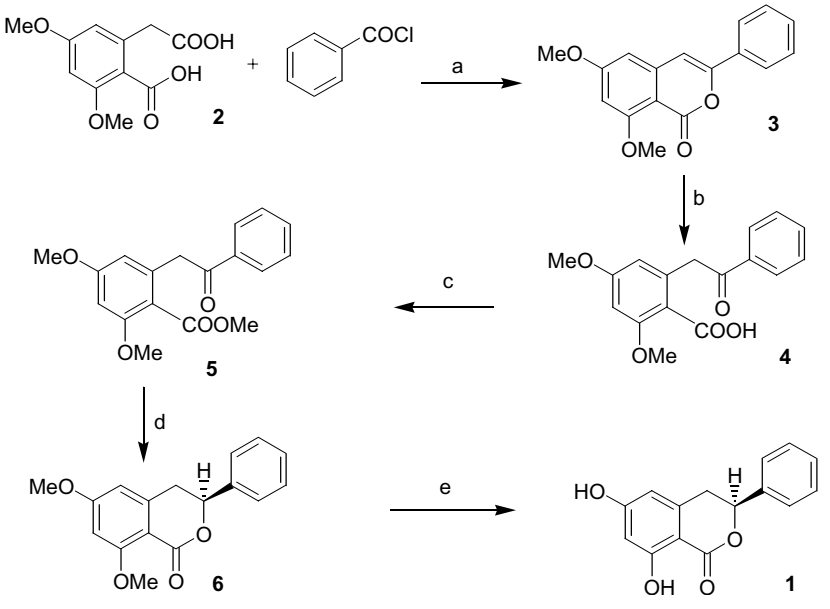

Fig. 2. Synthesis pathway. Reagents and conditions: a: C_6H_5COCl, 200 °C, 4 h, 82 %; b: 5 % KOH, EtOH, 4 h, reflux, 80 %; c: CH_3I, K_2CO_3, dry acetone, 5 h, 91 %; d: Baker's yeast 3 d, 45 %; e: BBr_3, CH_2Cl_2, (– 78 °C)–(+ 4 °C), overnight, 72 %.

Table I. Antifungal activity* determined by agar diffusion test and the results reported as linear growth inhibition in % of control (LGI; the zone of inhibition measured in millimeters) at 400 μg/ml of media SDA.

Compound	M_r	A	B	C	D	E	F
Montroumarin (**1**)	256.0	26.0	54.0	60.0	32.0	15.0	00
Isocoumarin **3**	282.2	20.0	47.9	48.6	24.8	12.5	00
Keto acid **4**	300.3	17.5	46.2	31.0	16.8	6.9	00
Keto ester **5**	314.3	27.3	54.0	55.0	29.0	21.0	00
Dihydroisocoumarin **6**	284.3	19.4	50.0	56.0	34.0	13.2	00
Miconazole	415.3	70.0	20.0	98.4	–	100	–
Ketoconazole	531.4	–	–	–	73.5	–	79

Pathogens: A: *Trichophyton schoenleinii*; B: *Aspergillus niger*; C: *Microsporum canis*; D: *Fusarium solani*; E: *Pseudallescheria boydii*; F: *Candida albicans*.

to dimethoxy compound **4**, possibly due to internal chelation between hydroxyl and lactonic carbonyl moiety (Nozawa *et al.*, 1981). It is also interesting to note that the isomeric 3-(3′,4′-dimethoxyphenyl)isocoumarin, and corresponding dihydroisocoumarin were inactive as antifungal agents indicating that 8-hydroxyl group is important for antifungal activity.

In summary, an efficient stereoselective synthesis of montroumarin (*3S*)-6,8-dihydroxy-3-phenyl-3,4-dihydroisocoumarin isolated from *Montrouziera sphaeroidea* has been accomplished. It involves five linear steps and proceeds with an overall yield of 25 %, which unambiguously confirms the structural assignments and makes it available for biological evaluation.

Experimental

General

^{1}H NMR and ^{13}C NMR spectra were recorded, respectively, on a 400 MHz AM-400 and a 100 MHz Bruker instrument with $CDCl_3$ or acetone-d_6 as solvent. 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. Optical rotations were measured in $CHCl_3$ with a Perkin-Elmer 341 polarimeter. Enantiomeric excesses (ee) were determined by ^{1}H NMR using (–)-[Eu(hfc)$_3$] as optically active shift reagent. Flash column chromatography was carried out on Merck Kieselgel 60 (230–400 mesh).

Antifungal activity (in vitro)

The antifungal activity against six fungal strains was tested by using agar diffusion test (Atta-ur-Rahman *et al.*, 2001). The voucher specimens are deposited at HEJ International Centre for Chemical Sciences Karachi, Pakistan. Miconazole (1-[2,4-dichloro-beta-[(2,4-dichlorobenzyl)oxy]-phene-thyl]-imidazole) and ketoconazole [*cis*-1-acety1–4-[4-[[2-(2,4-dichlorophenyl)-2-(1*H*-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxyl]phenyl]piperazine] were used as standard antifungal drugs. Stock solutions of the pure compounds were prepared in sterile DMSO. Sabouraud dextrose agar (SDA) was prepared by mixing Sabouraud (32.5 g), glucose agar (4 %) and agar-agar (4 g) in 500 ml distilled water followed by steamed dissolution, 4 ml media was dispensed into screw caped tubes and autoclaved at 121 °C for 15 min. Test compounds were added from the stock solution to non-solidified Sabouraud agar media (50 °C). Tubes were allowed to solidify at room temperature and inoculated with 4 mm diameter of inoculums derived from a seven day old fungal culture. For non-mycelial growth, an agar surface streak was employed. The tubes incubated at 27–29 °C for 7–10 days and the growth in the compound-containing media was determined by measuring the linear growth (mm) and growth inhibition with reference to the respective control. The activity was determined *via* the growth inhibition of microorganism (the zone of inhibition measured in millimeters). The zone of inhibition of each compound is presented in Table I.

6,8-Dimethoxy-3-phenylisocoumarin (**3**)

A stirred mixture of 3,5-dimethoxyhomophthalic acid (**2**) (0.5 g, 2.08 mmol) and benzoyl chloride (0.97 ml, 8.33 mmol) was heated on an oil

bath at 200 °C for 4 h. Flash chromatography of the residue (petroleum ether:ethyl acetate = 9:1 v/v) afforded the isocoumarin **3** as colourless oil (0.47 g, 1.68 mmol, 82 %). EIMS: *m/z* (%) = 282 (M^+, 30), 165 (28), 137 (19.8), 117 (100). – IR (film): ν = 2913, 2849, 1720, 1694 ,1598, 1572, 1471, 1151, 832 cm^{-1}. – ^1H NMR ($CDCl_3$ ppm): δ = 3.87 (s, 3H, MeO-6), 3.95 (s, 3H, MeO-8), 6.72 (s, 1H, H-4), 6.30 (s, 1H, H-5), 6.41 (s, 1H, H-7), 7.25 (1H, t, J = 7.5 Hz, H-4′), 7.30 (2H, t, J = 7.2 Hz, H-3′, H-5′), 7.44 (2H, d, J = 7.2 Hz, H-2′, H-6′). – ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.70 (C-1, C=O), 145.49 (C-3), 100.2 (C-4), 137.70 (C-4a), 99.87 (C-5), 165.53 (C-6), 98.37 (C-7), 164.50 (C-8), 100.99 (C-8a), 135.2 (C-1′), 126.8 (C-2′, C-6′), 129.51 (C-3′, C-5′), 128.0 (C-4′), 56.40 (MeO-6), 55.72 (MeO-8). – HREIMS *m/z* 282.0918 (calcd. for $C_{17}H_{14}O_4$, 282.0892).

2,4-Dimethoxy-6-(benzoylmethyl)benzoic acid (**4**)

A stirred solution of 6,8-dimethoxy-3-phenylisocoumarin (**3**) (0.42 g, 1.48 mmol) in ethanol (20 ml) was treated with 5 % KOH (40 ml) and the mixture 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 acidified with dil. hydrochloric acid when the solid was precipitated. Filtration followed by drying under vacuum afforded **3** as a light yellow solid. Recrystallized from MeOH (0.34 g, 1.13 mmol, 80 %). M.p. 92–93 °C. – EIMS *m/z* (%) = 300 [M^+] (11.45), 282 (37.09), 256 (11.62), 178 (100). – IR (film): ν = 2915, 2849, 1720, 1685, 1601, 1202, 1162 cm^{-1}. –^1H NMR (acetone-d_6, ppm) = δ 3.82 (s, 3H, MeO-4), 3.88 (s, 3H, MeO-2), 6.30 (d, J = 2.0 Hz, 1H, H3), 6.41 (d, J = 2.2 Hz, 1H, H-5), 11.22 (1H, br, s, COOH), 4.02 (s, 2H, Ar-CH_2), 7.36 (1H, t, J = 7.5 Hz, H-4′), 7.42 (2H, t, J = 7.2 Hz, H-3′, H-5′), 8.31 (2H, d, J = 7.2 Hz, H-2′, H-6′). – ^{13}C NMR (acetone-d_6, ppm): δ = 170.81 (COOH), 195.54 (C=O, C-3), 45.31 ($ArCH_2$), 139.20 (C-6), 99.87 (C-5), 165.53 (C-4), 98.37 (C-3), 164.50 (C-2), 109.43 (C-8a), 135.2 (C-1′), 129.3 (C-2′, C-6′), 129.9 (C-3′, C-5′), 128.0 (C-4′), 56.40 (MeO-4), 55.72 (MeO-2). – HREIMS: *m/z* = 300.502 (calcd. for $C_{17}H_{16}O_5$ 300.0998).

Methyl 2,4-dimethoxy-6-(benzoylmethyl)benzoate (**5**)

A mixture of 2,4-dimethoxy-6-(benzoylmethyl)-benzoic acid (**4**) (0.30 g, 1.0 mmol), anhydrous potassium carbonate (1.5 g) in dry acetone (30 ml) was treated with methyl iodide (74.1 ml, 1.48 mmol) and refluxed for 5 h. The reaction mixture was filtered while hot, the filter cake washed with warm dry acetone and solvent rotary-evaporated to leave ester **5** as an oil (0.28 g, 0.91 mmol, 91 %). – IR (film): ν = 3011, 2949, 1725, 1710, 1694, 1601, 1162 cm^{-1}. – ^1H NMR (400 MHz, $CDCl_3$): δ = 3.66 (3H, s, COOMe), 3.81 (s, 3H, MeO-4), 3.88 (s, 3H, MeO-2), 3.95 (2H, s, $ArCH_2$), 6.30 (d, J = 2.0, 1H, H-5), 6.41 (d, J = 2.24, 1H, H-3) , 7.36 (1H, t, J = 7.5 Hz, H-4′), 7.42 (2H, t, J = 7.2 Hz, H-3′, H-5′), 8.31 (2H,d, J = 7.2 Hz, H-2′, H-6′). – ^{13}C NMR (acetone-d_6, ppm): δ = 168.30 (COOMe), 195.54 (C=O), 44.02 ($ArCH_2$, C-4), 139.20 (C-6), 99.87 (C-5), 165.53 (C-4), 98.37 (C-3), 164.50 (C-2), 109.43 (C-8a), 135.2 (C-1′), 129.3 (C-2′, C-6′), 129.9 (C-3′, C-5′), 128.0 (C-4′), 56.40 (MeO-4), 55.72 (MeO-2). – EIMS: *m/z* (%) = 314 [M^+] (42.0), 237 (17.80), 220 (53), 209 (43.8), 178 (70). – HREIMS: *m/z* = 314.0136 (calcd. for $C_{18}H_{18}O_5$, 314.1154).

(3S)-6,8-Dimethoxy-3-phenyl-3,4-dihydro-isocoumarin (**6**)

Baker's yeast (1.4 g) was introduced to distilled water (25 ml) and then ethanol (0.06 ml) and glucose (0.4 g, 2.2 mmol) were added and the mixture kept for 3 h at 30 °C. Keto ester **5** (0.25 g, 0.80 mmol) was added to the reaction mixture and further stirred for 5 days when the completion of reaction was marked by TLC. The reaction mixture was filtered through a Celite pad and the filtrate was adjusted by addition of 2 N HCl to pH 1 and extracted with Et_2O (3 × 50 ml). The combined organic layer was dried ($MgSO_4$) and concentrated *in vacuo*. Flash chromatography (petroleum ether:ethyl acetate 7:1 v/v) of the residue afforded **1** as prisms (0.10 g, 0.36 mmol, 45 %). M.p. 51–53 °C. $[\alpha]_D^{25}$ = + 84.6° (*c* 0.06, $CHCl_3$). – EIMS: *m/z* (%) = 284 (M^+, 56), 178 (100), 147 (14), 118 (42), 90 (59), 89 (15). – IR (film): ν = 3600, 3350, 2850, 1730, 1710, 1604, 1583, 1572, 1464, 1198, 832 cm^{-1}. – 1H NMR (400 MHz, $CDCl_3$): δ = 3.13–3.08 (dd, 1H, J_{gem} = 16.3 Hz, J_{cis} = 3.9 Hz,

H-4), 3.26 (dd, 1H, J_{gem} = 16.54 Hz, J_{trans} = 11.2 Hz, H-4), 3.85 (s, 3H, MeO-6), 3.94 (s, 3H, MeO-8), 5.42 (dd, *J* 12.0, 3.65 H-3) , 6.33 (d, *J* = 2.1 Hz, 1H, H-7), 6.45 (d, *J* = 2.2 Hz, 1H, H-5), 7.21 (1H, t, *J* = 7.5 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 (100 MHz, $CDCl_3$): δ = 164.89 (CO), 163.05 (C-8); 162.87 (C-6), 144.08 (C-4a), 107.24 (C-8a), 103.98 (C-5), 97.86 (C-7), 78.61 (C-3), 35.71 (C-4), 141.21 (C-1′), 126.7 (C-2′, C-6′), 129.3 99 (C-3′, C-5′), 128.9 (C-4′), 56.40 (MeO-8), 55.72 (MeO-6). – HREIMS: *m/z* = 284.1031 (calcd. for $C_{17}H_{16}O_4$ 284.1049).

(3S)-6,8-Dihydroxy-3-phenyl-3,4-dihydroisocoumarin (montroumarin) (**1**)

A 1 M solution of BBr_3 in CH_2Cl_2 (1.2 ml, 1.2 mmol) was injected into a stirred solution of **6** (80 mg, 0.22 mmol) in dry CH_2Cl_2 (4 ml) at – 78 °C, under Ar. After stirring for 1 h at – 78 °C, the reaction mixture was warmed to 4 °C and stirred for 24 h. The reaction mixture was poured into ice-water (20 ml) and stirred for 10 min. The layers were separated and the aqueous layer extracted with CH_2Cl_2 (2 × 50 ml). The combined organic phases were dried ($MgSO_4$) and concentrated. Flash chromatography (petroleum ether:ethyl acetate 8:2 v/v) afforded (**1**) as colourless oil (56 mg, 0.22 mmol, 78 %). – $[\alpha]_D^{25}$ = + 66.6° (*c* 0.06, $CHCl_3$). – IR (film): ν = 3600, 3350, 2924, 1665, 1620, 1604, 1572, 1464, 1198, 832 cm^{-1}. – EIMS: *m/z* (%) = 256 [M$^+$] (11.45), 238 (37.09), 181 (100), 165 (72.3). – ^{1}H NMR (400 MHz, acetone-d_6) δ = 3.20 (dd, *J* = 16.0 Hz, 3.2, 1H, H-4), 3.32 (dd, *J* = 16.3 Hz, 11.70, 1H, H-4), 5.65 (dd, *J* = 12.04 Hz, 3.26, H-3), 6.30 (dd, *J* = 2.1 Hz, 1H, H-7), 6.37 (dd, *J* = 2.2 Hz, 1H, H-5), 7.51 (2H, d, *J* = 7.2 Hz, H-2′, H-6′), 7.29 (1H, t, *J* = 7.5 Hz, H-4′), 7.39 (2H, t, *J* = 7.2 Hz, H-3′, H-5′), 7.51 (2H, d, *J* = 7.2 Hz, H-2′, H-6′) ppm. – ^{13}C NMR (acetone-d_6, ppm): δ = 170.1 (CO), 164.8 (C-6), 165.4 (C-8), 144.08 (C-4a), 107.24 (C-8a), 105.98 (C-5), 101.81 (C-7), 79.89 (C-3), 35.71 (C-4), 140.21 (C-1′), 126.7 (C-2′, C-6′), 129.02 (C-4′), 129.9 (C-3′, C-5′), 129.02 (C-4′). – HREIMS: *m/z* = 256.0534 (calcd. for $C_{15}H_{12}O_4$ 256.0736).

Acknowledgements

The author gratefully acknowledges a Georg Forster research fellowship from the Alexander von Humboldt-Foundation, Germany. The author is indebted to Prof. Chihiro Ito, Faculty of Pharmacy, Meijo University Nagoya, Japan for providing the original spectra of montroumarin for comparison.

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