Bull. Chem. Soc. Ethiop. **2006**, 20(1), 83-87. ISSN 1011-3924
Printed in Ethiopia 

# SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF INSIGNIN AND ITS 8-METHOXY- AND 6-DEMETHOXY DERIVATIVES

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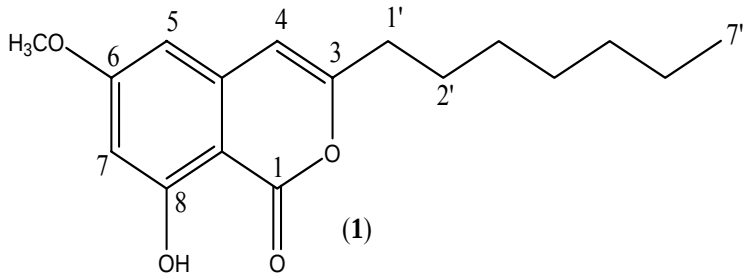
(Received August 19, 2005; revised October 4, 2005)

**ABSTRACT.** A short and efficient synthesis of insignin (8-hydroxy-6-methoxy-3-heptyl-isocoumarin) (**1**), a metabolite of natural lichens, has been described. Reaction of 3,5-dimethoxyhomophthalic anhydride (**2**) with octanoyl chloride in the presence of 1,1,3,3-tetramethylguanidine (TMG) and triethyl amine afforded the 6,8-dimethoxy-3-heptylisocoumarin (**3**) in high yield. Regioselective demethylation of the latter using anhydrous aluminum chloride furnished the 8-hydroxy-6-methoxy-3-heptylisocoumarin (**1**) whereas, the complete demethylation of (**3**) yielded 6,8-dihydroxy-3-heptylisocoumarin (**4**). The isocoumarins (**1**, **3**, and **4**) were examined *in vitro* for antibacterial activity and were shown to exhibit moderate activity.

**KEY WORDS:** Isocoumarin, Insignin, Antibacterial, *Porpidia glaucophaea, Haematomma pachycarpum,* 3,5-Dimethoxyhomophthalic anhydride

## INTRODUCTION

Culberson and coworkers [1] during microchemical studies of the extracts of the natural lichens, *Porpidia glaucophaea* (Propidiaceae) and the crustose Japanese ascolichen *Haematomma pachycarpum* (Haematommataceae) using two-dimensional thin-layer chromatography and HPLC, isolated and identified a mixture of homologous depsides: 2'-*O*-methylsuperphyllinic acid, glaucophaeic acid, superconfluentic acid, 4-*O*-demethylsuperconnuentic acid, and a new isocoumarin insignin. The structure of isocoumarin was revealed as 8-hydroxy-6-methoxy-3-heptylisocoumarin (**1**) (Figure 1) by spectroscopic data and later on confirmed by an unambiguous synthesis by Elix *et al.* [2]. The reported synthesis though elegant, involved at least eight steps with less than 10 % overall yield. Herein, we wish to report an efficient synthesis of 8-hydroxy-6-methoxy-3-heptylisocoumarin as a continuation of our interest towards this class of natural products [3-5] and for evaluation of its bioactivity.




(**1**)

Figure 1. Insignin (8-Hydroxy-6-methoxy-3-heptylisocoumarin).

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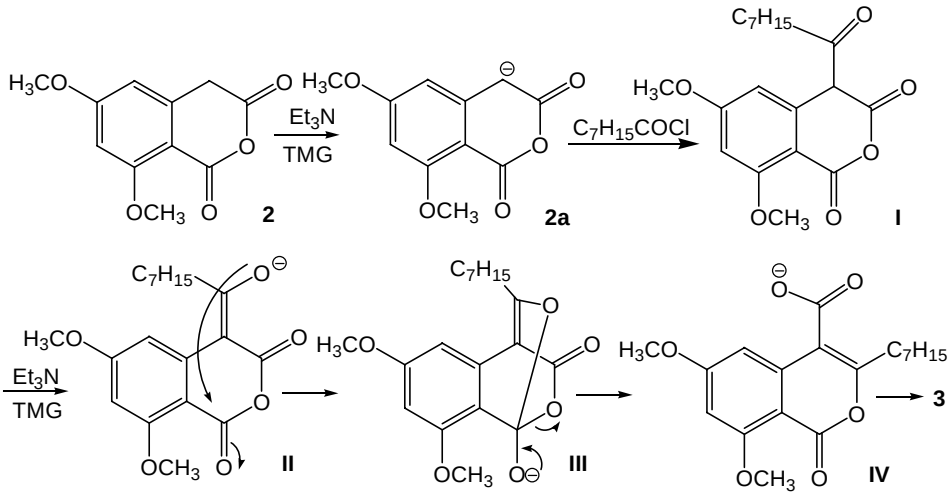
## RESULTS AND DISCUSSION

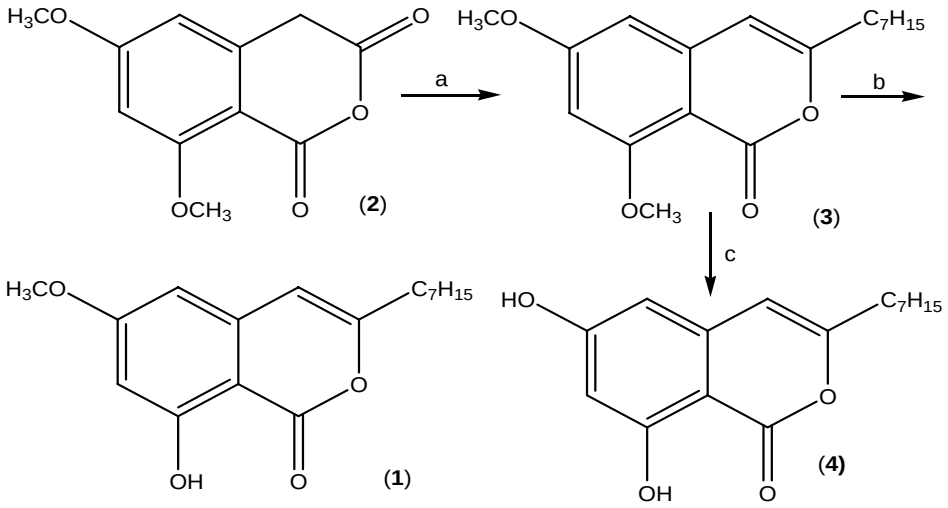
3,5-Dimethoxyhomophthalic anhydride (**2**) was obtained from 3,5-dimethoxyhomophthalic acid [6] by refluxing with acetic anhydride in dry toluene. A solution of the anhydride **2** in acetonitrile was added to a solution of 1,1,3,3-tetramethylguanidine (TMG) in the same solvent at or below 0°C followed by addition of triethyl amine. Treatment of the reaction mixture with octanoyl chloride afforded 6,8-dimethoxy-3-heptylisocoumarin (**3**) in 81 % yield [7]. Isocoumarin **3** showed the singlet for H-4 olefinic proton at δ 6.09, triplet for $CH_2$-1' at δ 2.46 ($J$ = 7.3 Hz) and carbon signals at δ 155.7 and 103.4 for C-4 and C-3 respectively. The δ-lactonic carbonyl absorption in IR spectrum appeared at δ 1725 $cm^{-1}$. The plausible mechanism of formation of isocoumarin **3** is shown in Scheme 1. The loss of benzylic proton affords the enolate **2a** which attacks the octanoyl chloride to give the 4-octanoyl-6,8-dimethoxyisochroman-1,3-dione intermediate I. Abstraction of the ring proton flanked by two carbonyls in the latter, leads to stable enolate species II which on intramolecular transannular *O*-acylation [7] furnishes the tricyclic intermediate III. Ring opening to afford IV followed decarboxylation to provide the isocoumarin **3**.




Scheme 1. Mechanism of isocoumarin formation.

Regioselective demethylation of the 6,8-dimethoxy-3-methylisocoumarin (**3**) using anhydrous aluminum chloride in dry nitrobenzene at 50-60°C [8] furnished the 8-hydroxy-6-methoxy-3-heptylisocoumarin (**1**). The singlet for H-4 proton and the triplet for $CH_2$-1' shifted slightly downfield at δ 6.16 and 2.49 ($J$ = 7.6 Hz), respectively. The carbon signals for C-4 and C-3 appeared at δ 104.0 and 159.7, respectively. The lactonic carbonyl absorption was also lowered to 1685 $cm^{-1}$ due to chelation with 8-hydroxyl which appeared at 3450 $cm^{-1}$.

Complete demethylation of the 6,8-dimethoxy-3-methylisocoumarin (**3**) was achieved in refluxing hydroiodic acid and glacial acetic acid [9] to furnish the 6,8-dihydroxy-3-heptylisocoumarin (**4**).




**Reagents and Conditions**

a: i) TMG/MeCN, 0°C, ii) $Et_3N$, iii) $C_7H_{15}COCl$ (81 %); b: $AlCl_3/C_6H_5NO_2$, 55-60°C, 6h (61 %); c: HI (55%) / HOAc, 4h reflux (67 %)

Scheme 2. Synthesis of insignin.

*Antibacterial activities*

The isocoumarins (**1**), (**2**) and (**3**) were screened *in vitro* for antibacterial activity against five different bacterial strains using agar well diffusion technique [10]. The activity was determined *via* the growth inhibition of microorganism and the zone of inhibition was measured in millimeters. The results indicated that the isocoumarins exhibit weak to moderate activity compared to roxrithromycin used as the standard drug (Table 1). It is evident from the table that the isocoumarin **1** having phenolic hydroxyl shows more bactericidal activity compared to its 8-*O*-methyl ether derivative **3** indicating that a free 8-hydroxyl group being periplanar to lactonic carbonyl plays an important role in activity [11, 12]. However, there was a little change in activity in going from **3** to **4** when the 6-methoxy group of **1** was also demethylated.

Table 1. Antibacterial activity.

| Compound | *Staphylococcus aureus* | *Bacillus subtilis* | *Borsella septica* | *Pseudomonas piktsi* | *Enterobacter aeruginosa* |
|---|---|---|---|---|---|
| Roxrithromycin | 40.0 | 49.5 | 30.4 | 32.4 | 31.4 |
| (**3**) | 8.5 | 16.3 | 10.3 | 9.4 | 13.4 |
| (**1**) | 14.6 | 25.2 | 16.8 | 21.3 | 17.7 |
| (**4**) | 14.7 | 24.8 | 18.7 | 20.6 | 23.4 |

Zone of inhibition (mm); concentration used: 1 mg /1 mL of DMSO).

## CONCLUSION

A facile synthesis of a natural isocoumarin insignin **1**, its 8-*O*-methyl ether derivative **2**, and 6-demethoxy derivative **3** has been achieved. A plausible pathway for formation of isocoumarin **3** from anhydride **2** has also been proposed and the results of *in vitro* antibacterial activity described.

## EXPERIMENTAL

$^1$H and $^{13}$C NMR spectra were recorded in $CDCl_3$ at 400 MHz and 100 MHz, respectively, using a Bruker AM-400 machine. FTIR spectra were recorded on an FTS 3000 MX spectrophotometer; Mass Spectra (EI, 70eV) on a MAT 312 instrument and elemental analyses were conducted using the CHN-Rapid Heräus. All compounds were purified by thick layer chromatography using silica gel 60 HF from Merck.

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

A solution of 3,5-dimethoxyhomophthalic anhydride (**2**, 1.0 g, 4.50 mmol) in acetonitrile (30 mL) was added slowly to a solution of TMG (0.62 mL, 4.95 mmol) in acetonitrile (12 mL), while maintaining the internal temperature ≤ 0 °C. Triethyl amine (1.0 mL, 9.0 mmol) was added in a single portion followed by dropwise addition of octanoyl chloride (1.23 mL, 7.20 mmol). The reaction mixture was further stirred for 20 min, allowed to warm to ambient temperature and then quenched by addition of 1 M HCl (15 mL). The organic layer was separated, washed with saturated brine, dried and concentrated. The crude compound was purified by thick layer chromatography followed by recrystallization from methanol to yield isocoumarin **3** (1.1 g, 3.64 mmol, 81 %). M.p. 69 °C; IR (KBr): ν = 2913, 2849, 1725, 1605, 1575, 1510, 860, 835, 810 cm$^{-1}$; $^1$H NMR ($CDCl_3$, 400 MHz) δ: 0.87 (t, *J* = 7.12, 3H, $CH_3$-7'), 1.25 (m, 8H, $CH_3$'-$H_6$'), 1.62-1.68 (quintet, *J* = 7.5 Hz, 2H, $CH_2$-2'), 2.46 (2H, t, *J* = 7.3 Hz, $CH_2$-1'), 3.85 (s, 3H, MeO), 3.97 (s, 3H, MeO), 6.09 (s, 1H, H 4), 6.39 (d, *J* = 2.2, 1H, H-7), 6.47 (d, *J* = 2.1, 1H, H-5) ppm; $^{13}$C NMR ($CDCl_3$, 100 MHz,) δ: 168.1 (C=O), 167.3 (C6), 163.7 (C8), 155.7 (C3), 141.8 (C9), 103.4 (C4), 102.2 (C5), 100.9 (C7), 99.9 (C10), 55.3 (MeO), 56.6 (MeO), 33.3 (C1'), 31.1 (C3'), 29.74, 29.4 (C4'-C5'), 26.4 (C2'), 22.3 (C6'), 14.1 (C7'); MS: *m/z* (rel. int.): 304 (19), 290 (43), 219 (17), 206 (52), 191 (15), 177 (27), 165 (14), 164 (98). Anal. calcd. for $C_{18}H_{24}O_4$: C, 71.03; H, 7.95 %; found: C, 70.97; H, 7.89 %.

*6-Methoxy-8-hydroxy-3-heptylisocoumarin* **(1)**

Aluminum chloride (0.83 g, 6.24 mmol) was added to a stirred solution of **3** (0.95 g, 3.12 mmol) in freshly distilled dry nitrobenzene (10 mL). The reaction mixture was stirred at 50-60 °C for 6 h, then poured into ice-water and acidified with dil. hydrochloric acid. The acidic solution was stirred for 10 min and extracted with ether (3 x 50 mL). The layers were separated and the aqueous layer extracted with dichloromethane (2 x 50 mL) and then the combined extracts were washed with 10 % sodium hydroxide (2 x 60 mL). The basic solution was extracted with ether, acidified and the aqueous phase again extracted with ether. The last extract was evaporated and residue purified by thick layer chromatography (petroleum ether:ethyl acetate, 8:2) to afford (**1**) (0.47 g, 2.30 mmol, 61 %). M.p. 76-78 °C (Lit. [2] 64 °C). IR (KBr) $\nu_{max}$: 3450, 2913, 2849, 1685, 1625, 1575, 1510, 860, 835, 810 cm$^{-1}$; $^1$H NMR ($CDCl_3$, 400 MHz) δ: 0.89 (t, *J* = 7.12, 3H, H-7'), 1.25 (m, 6H, H3'-H5'), 1.62-1.68 (quintet, *J* = 7.5 Hz, 2H, H2'), 2.49 (t, *J* = 7.61 Hz, 3H, $CH_2$-1'), 3.85 (s, 3H, MeO), 6.18 (s, 1H, H4), 6.39 (d, *J* = 2.2, 1H, H 7), 6.47 (d, *J* = 2.2 Hz, 1H, H5), 11.2 (1H, br s, OH); $^{13}$C NMR ($CDCl_3$, 100 MHz,) δ: 166.5 (C=O), 167.4 (C8), 163.7

(C6), 159.7 (C3), 142.4 (C9), 104.0 (C4), 102.9 (C5), 100.3 (C7), 98.7 (C10), 56.3 (MeO), 33.3 (C1'), 31.1 (C3'), 29.74, 29.4 (C4'-C5'), 26.4 (C2'), 22.3 (C6'), 13.9 (C7'); MS: *m/z* (rel. int.): 290 (37), 219 (19), 206 (52), 191 (15), 177 (27), 165 (14), 164 (100), 149 (29). Anal. calcd. for $C_{17}H_{22}O_4$: C, 70.32; H, 7.64 %; found: C, 70.10; H, 7.58 %.

*6,8-Dihydroxy-3-heptylisocoumarin* **(4)**

Freshly distilled hydroiodic acid (55 %, 30 mL) was added to a stirred solution of isocoumarin (**3**) (0.5 g, 1.64 mmol) in glacial acetic acid (30 mL). Reaction mixture was refluxed for 4 h, cooled and poured onto crushed ice, treated with solid sodium carbonate to adjust the pH to 7 and extracted with ethyl acetate (2 x 15 mL), the combined organic layer was dried ($Na_2SO_4$) and evaporated. Recrystallized from methanol to give 6,8-dihydroxy-3-heptylisocoumarin (**4**) (0.30 g, 1.02 mmol, 67 %). M.p. 98-99 °C. IR (KBr) $\nu_{max}$: 3340, 2956, 1665, 1627, 1271, 1161, 1072, 741 $cm^{-1}$; $^1$H NMR ($CDCl_3$, 400 MHz) δ: 0.91 (t, *J* = 7.12, 3H, $CH_3$-7'), 1.25 (m, 6H, $CH_2$3'-$CH_2$5'), 1.68 (quintet, *J* = 7.5 Hz, 2H, $CH_2$-2'), 2.49 (t, *J* = 7.32 Hz, 2H, $CH_2$-1'), 6.17 (s, 1H, H 4), 6.39 (d, *J* = 2.2, 1H, H 7), 6.47 (d, *J* = 2.2 Hz, 1H, H5); $^{13}$C NMR ($CDCl_3$, 100 MHz,) δ: 166.4 (C=O), 164.6 (C6), 162.8 (C8), 159.6 (C3), 141.8 (C9), 103.9 (C4), 102.2 (C5), 100.9 (C7), 99.9 (C10), 33.9 (C1'), 33.3 (C3'), 31.1 (C3'), 29.74, 29.4 (C4'-C5'), 26.4 (C2'), 22.3 (C6'), 13.9 (C7'); MS: *m/z* (rel. int.): 276 (38), 219 (11), 206 (58), 191 (19), 177 (27), 165 (14), 164 (100), Anal. calcd. for $C_{16}H_{20}O_4$: C, 69.54; H, 7.30 %; found: C, 69.44; H, 7.36 %.

## ACKNOWLEDGEMENT

The author gratefully acknowledges the research grant from Quaid-I-Azam University Islamabad, Pakistan.

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