Synthesis and Bioactivity of Xyridin A and B, Metabolites from *Xyris indica*

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A facile synthesis of the title isocoumarins isolated from *Xyris indica* was accomplished. Condensation of butanoyl chloride and 2-oxo-butanoyl chloride with 3,4-methylenedioxyhomophthalic acid afforded xyridin A and xyridin B respectively. Xyridin A was saponified to the corresponding keto acid which on reduction furnished the (±)-3,4-dihydro-6,7-methylenedioxy-3-propylisocoumarin. All of the synthesized compounds were examined *in vitro* for antibacterial and antifungal activities.

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Introduction.

In 1995, Ruangrungsi *et al*. isolated from the non-polar fraction of the chloroform extract of the flowering heads of a weed *Xyris indica*, two new isocoumarins, which they named as xyridin A and B [1]. *Xyris indica* L. (tall yellow-eyed grass) is one of the five species of genus *Xyris* found throughout Thailand and is known locally as 'Kra thin thung'. In Bengal the plant has been used in folklore medicine as a cure for ring worm, itch and leprosy. The structures of xyridin A and B were established by modern spectroscopic techniques as 3-*n*-propyl-6,7-(methylenedioxy-)isocoumarin (**1a**) and 3-(1'-oxopropyl)-6,7-(methylenedioxy)isocoumarin (**1b**), respectively.

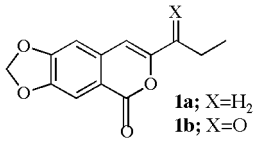


Figure 1

Majority of the naturally occurring isocoumarins are derived biogenetically from acetate *via* the acetate-polymalonate pathway and possess a C-8 or C-6 and C-8 oxygenation [2]. Xyridin A & B with a similar mode of biosynthesis are unique in being 3-alkyl/acyl- substituted isocoumarins with a 6,7-methylene dioxygenation, from a natural source. 6,7-Methylenedioxyisocoumarin and 5,6-, and 6,7-methylenedioxy-3,4-dihydroisocoumarins are known in literature through syntheses along with 3-methyl-6,7-methylenedioxy-3,4-dihydroisocoumarin as the only example of a 3-alkyl substituted 6,7-methylenedioxyisocoumarin [3,4,5]. Important examples of other natural products containing a 6,7-methylenedioxy bearing dihydroisocoumarin moiety are hippeastrine [6], lycoricidin [7], pancratistatin [8] and tazettine [9]. Peshawarine, like dihydroinversin, contains a 3-substituted-7,8-methylenedioxy-3,4-dihydroisocoumarin skeleton [10].

A synthesis of xyridin A and B was undertaken as a continuation of our previous efforts towards synthesis of naturally occurring isocoumarins [11] and dihydroisocoumarins [12] which exhibit a wide variety of bioactivities despite having a simple structure. The limited quantities available from natural source together with possibility of preparing analogues with improved biological activities show the imperative need for total syntheses. Condensation of acid chlorides with homophthalic acids is a reliable route towards 3-substituted isocoumarin skeleton [13]. Herein, the first total synthesis of xyridin A and B accomplished employing this method and conversion of the former into dihydroxyridin A, for comparison of bioactivity are described.

Results and Discussion.

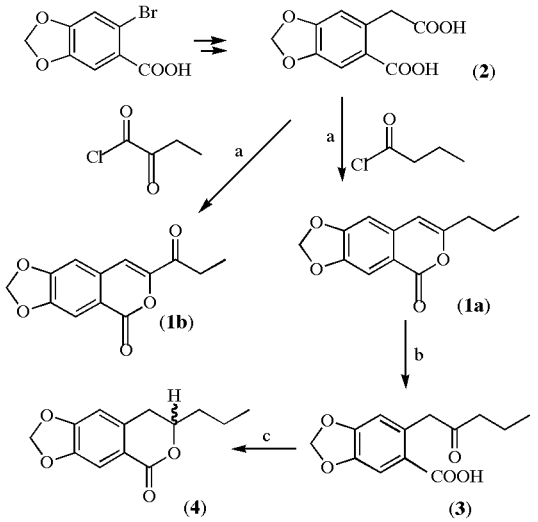
4,5-Methylenedioxyhomophthalic acid (**2**) was prepared in two steps from 2-bromo-4,5-methylenedioxybenzoic acid according to the literature procedure [3]. Commercial 2-oxobutanoic acid was converted into the acid chloride at room temperature by treatment with oxalyl chloride in dry benzene; butanoyl chloride was the commercial product. Initial efforts to condense 4,5-methylenedioxyhomophthalic acid with acid chlorides led to some opening of the methylenedioxy- bridge due to its sensitivity towards acidic environment. Eventually the condensation was successfully achieved in the presence of a few drops of pyridine. Normally, a yield of 60-85% has been obtained in such condensations however, in case of xyridin A due to relatively lower boiling point of butanoyl chloride (102 °C), and sensitivity of methylenedioxy bridge to acid only a moderate yield of 45 % could be obtained. In case of xyridin B the limiting factor was the susceptibility to decomposition of 2-oxobutanoyl chloride resulting in 58% yield. However, the relatively lesser yields are compensated by the remarkable simplicity of one-pot procedure.

Thus, condensation of 4,5-methylenedioxyhomophthalic acid with, butanoyl chloride at elevated temperature afforded the xyridin A (**1a**). The ^{1}H NMR spectrum of **1a** showed the characteristic singlet of the isocoumarin

moiety at δ 6.15 for H-4, and a 2H singlet at 6.09 for the methylenedioxy group. In the ^{13}C NMR spectrum, signals are observed at δ 103.2 (C-4) 157.4 (C-3) and 102.2 (OCH_2O). The IR spectrum showed the δ-lactonic carbonyl absorption at 1722 cm^{-1}. In ^1H NMR spectrum the of xyridin B, due to extension of conjugation, the characteristic singlet (H4) was found further downfield in the aromatic region at δ 7.30 and that of the methylenedioxy group at δ 6.20. In the ^{13}C NMR spectrum, resonances were observed at δ 109.1 (C-4) 149.41 (C-3) and 195.3 (C=O). DEPT 90° and DEPT 135° experiments confirmed these assignments. The lactonic and ketonic carbonyl absorptions were observed at 1728 and 1685 cm^{-1} respectively.

In order to compare the bioactivity of xyridin A with corresponding 3,4-dihydro derivative, it was subjected to alkaline hydrolysis to furnish the 4,5-methylenedioxy-2-(2'-oxopentyl)benzoic acid (**3**). The keto acid existed partially in its cyclic tautomeric lactol form 3-hydroxy-6,7-methylenedioxy-3-propyl-3,4-dihydroisocoumarin as evidenced by the ^1H NMR. Thus, in addition to the 2H singlet at δ 3.94 (H-4, open chain form) each proton of $ArCH_2$ showed a broad signal at δ 2.91 and 3.10 (lactol form). In the ^{13}C NMR spectrum C-4 resonated at δ 51.8 ppm and carbonyl absorptions were observed at 1680 and 1705 cm^{-1} in IR spectrum.

Scheme 1



Reagents and Conditions: a) 200°C, dry pyridine 4-20h, 45-58%; b) 5% KOH/EtOH, 4h reflux; 70% c) $NaBH_4$, EtOH, 4h, r.t. 85%.

Reduction of the keto acid lactol tautomeric mixture (**3**) using sodium borohydride afforded the corresponding racemic hydroxy acid which underwent spontaneous cyclodehydration on standing for a few minutes (as monitored by TLC) to (±)-3-*n*-propyl-6,7-(methylenedioxy)-3,4-dihydroisocoumarin (**4**) without any dehydrating agent [14]. The H-3 methine proton and methylenes protons on either side of the newly created stereogenic center at C3 (C4, C1' C2' corresponding to ABX and ABMNX and $ABMNX_2$ systems respectively) exhibited the characteristic diastereotopic effect [15] in the ^1H NMR spectrum whereas the ^{13}C NMR spectrum showed signals at δ 77.63 and 35.01 for C-3 and C-4 respectively. The lactonic carbonyl absorption appeared at 1720 cm^{-1} in IR spectrum.

Xyridin A (**1a**), xyridin B (**1b**), keto acid (**3**) and dihydroxyridin A (**4**) were screened *in vitro* for antibacterial activity against various Gram positive and Gram negative bacteria (Table-1). The activity was determined *via* the growth inhibition of microorganism (zone of inhibition measured in millimeters). The results indicate that these compounds show moderate activity. Xyridin B is slightly more potent than xyridin A and (±)-dihydroxyridin A is most active in the series.

Table 1

Antibacterial Activity

Microorganism	**1a**	**1b**	**3**	**4**	Standard drug
Escherichia coli	10	13	7	12	30
Bacillus subtilis	7	10	4	13	31
Shigella flexenari	9	10	00	11	35
Staphlococcus aureus	00	00	00	9	45
Pseudomonas aeruginosa	10	12	9	14	29
Salmonella typhi	00	10	00	8	40

(Concentration Used 100 µg/100 ml of DMSO) Zone of Inhibition (mm)
Standard drug: (Imipenem)

All of these compounds were also subjected to fungicidal screening against various human, plant and animal pathogens (Table-2). It may be seen that (±)-dihydroxyridin A is more active as antifungal agent [16].

Table 2

Antifungal Activity

Pathogens	**1a**	**1b**	**3**	**4**	Standard drugs
Trichophyton schoenleinii	21.6	19.2	11	19.8	70
Aspergillus niger	44.9	46.2	14	50	20
Microsporum canis	46.9	31	25	53	98.4
Fusarim solani	14.8	16.8	10	24	73.5
Pseudallescheria boydii	22.5	14	10	23.4	100
Candida albicans	00	00	00	00	79

Antifungal activity determined by agar dilution method and the results reported as linear growth inhibition (LGI) (%) at 400 µg/ml (%) of media SDA, Standard drugs: Miconazole and Ketoconazole.

Thus efficient a synthesis of the natural isocoumarins, xyridin A and B has been achieved which unambiguously

confirms the structural assignments and makes them available for biological evaluation.

EXPERIMENTAL

The commercial starting compounds purchased from Aldrich were 2-oxobutanoic acid and butanoyl chloride. 2-Bromo-4,5-methylenedioxybenzoic acid was prepared from piperonal according to the reported procedure [17]. ^{1}H NMR and the ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker AM-400 at 400 MHz and 100 MHz, respectively. IR spectra were recorded on a Bruker Vector 22; Mass Spectra (EI, 70 eV) on a MAT 312 instrument and elemental analyses with a CHN-Rapid Heräus. Flash column chromatography was carried out on Merck Kieselgel 60 (230-400 mesh).

3-*n*-Propyl-6,7-(methylenedioxy)isocoumarin (**1a; xyridin A**).

A stirred mixture of 4,5-methylenedioxyhomophthalic acid **2** 0.25 g, (1.11 mmol) and butanoyl chloride 0.46 ml (4.46 mmol) containing a few drops of dry pyridine was heated on an oil bath at 200 °C for 20 h. Flash column chromatography of the residue (petroleum ether:ethyl acetate (8:1) afforded **1a**, 0.11 g (45%) as yellow scales; mp 64-65° C (lit. [1] 67-88 °C). IR (KBr): ν = 3072, 2913, 2849, 1722, 1685, 1645, 1575, 1260, 1120, 935, 810 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.95 (3H, t, *J* = 7.2, H-3'), 1.67 (2H, tq, *J* = 7.2, 7.24, H2'), 2.44 (2H, t, J = 7.0, H-1'), 6.09 (2H, s, OCH_2O), 6.15 (1H, s, H-4), 6.70 (1H, s, H-5), 7.59 (1H, s, H-8) ppm;^{13}C NMR ($CDCl_3$) δ: 163.0 (C1), 157.4 (C3), 153.7 (C7), 148.3 (C6), 135.8 (8a), 114.6 (4a), 104.0 (C5), 103.2 (C4), 102.2 (OCH_2O), 35.7 (C1'), 20.8 (C2'), 13.5 (C3') ppm. EIMS (70eV): m/z (%) = 232 [M$^+$] (79), 204 (14), 203 (77), 189(17), 176 (76), 162 (23), 161 (12), 133 (37), 75 (24).

Anal. Calcd. For $C_{13}H_{12}O_4$: C 67.23, H 5.21. Found: C 67.26, H 5.19.

3-(1'-Oxopropyl)-6,7-(methylenedioxy)isocoumarin (**1b: xyridin B**).

A stirred mixture of 4,5-methylenedioxyhomophthalic acid **2** 0.25 g (1.11 mmol) and 2-oxobutanoyl chloride 0.53 g (4.46 mmol) containing a few drops of dry pyridine, was heated on an oil bath at 200 °C for 4 h. Flash chromatography of the residue (petroleum ether:ethyl acetate (15:1) afforded **1b** 0.16 g (58%) as scales; mp 188-190 °C (lit. [1] 198-199°C). IR (KBr): ν = 3084, 2913, 2849, 1728, 1685, 1645, 1575, 1260, 1120, 935, 810 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 1.21(3H, t, *J* = 7.2, H-3'), 3.01 (2H, q, *J* = 7.2, H2'), 6.20 (2H, s, OCH_2O), 7.10 (1H, s, H-5), 7.30 (1H, s, H-4), 7.38 (1H, s, H-8) ppm;^{13}C NMR ($CDCl_3$) δ :195.3 (C1', C=O), 161.0 (C1), 149.4 (C3), 154.0 (C7), 151.0 (C6), 132.8 (8a), 118.6 (4a), 109.1 (C4), 106.4 (C5), 102.8 (OCH_2O); 31.8 (C2'), 7.51 (C3') ppm. EIMS (70eV): m/z (%) = 246 [M$^+$] (68), 189 (20), 162 (19), 161 (16), 133 (100), 75 (24).

Anal. Calcd. For $C_{13}H_{10}O_5$: C 63.42 H 4.09. Found: C 63.29 H 4.16.

4,5-Methylenedioxy-2-(2-oxopentyl)benzoic acid (**3**).

A stirred solution of xyridin A (**1a**) 0.095 g (0.40 mmol) in 10 ml ethanol was treated with 20 ml of 5% potassium hydroxide and the mixture was refluxed for 4 h. After cooling the reaction mixture, 10 ml cold water was added and the mixture acidified with dilute hydrochloric acid and immediately extracted with dichloromethane (2x 30 mL). The organic phase was dried over magnesium sulphate and the solvent evaporated under vacuum to leave (**3**) as a yellowish solid. Recrystallized from petroleum ether-ethyl acetate 66 mg (70%); mp 104-106 °C. IR (KBr): ν = 3011, 2949, 1715, 1694, 1601, 1202, 1162 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 0.87 (3H, t, *J* = 6.02, H-3'), 1.44 (2H, br m, H2'), 1.75 (2H, br m, H1'), 2.38 (2H, br m H-1' open chain), 2.91(1H, br m H-4 lactol), 3.10 (1H, br m H-4 lactol), 3.94 (2H, br m H-4 open chain), 6.17 (s, 2H, OCH_2O), 6.73 (s, 1H, H-5), 7.65 (s, 1H, H-8), 11.22 (1H, br s COOH) ppm; ^{13}C NMR ($CDCl_3$) δ: 195.5 (C3, C=O), 168.3 (COOH), 57.7(C4), 153.9 (C7), 148.3 (C6), 104.4 (8a), 112.9 (C5), 102.2 (OCH_2O), 42.9 (C1') 19.7 (C2'), 13.8 (C3') ppm EIMS: m/z (%) = 250 [M$^+$] (11.4), 232 (80.1), 203 (11.6), 179 (41.4), 134 (100). In order to avoid confusion and for direct comparison C/H numbering is same as in isocoumarins **1a,b**.

Anal. Calcd. For $C_{13}H_{14}O_5$: C 62.39 H 5.64. Found: C 62.23 H 5.67.

(±)-3-*n*-Propyl-6,7-(methylenedioxy)-3,4-dihydroisocoumarin (**4**).

Sodium borohydride 0.67 g (18 mmol) was added portion wise to a stirred solution of (**3**) 0.05 g (0.21 mmol) in 10 ml ethanol and 30 ml water. The reaction mixture was stirred for 4 h at room temperature, diluted with 50 ml water, acidified with conc. hydrochloric acid and stirred for a further 2 h. The reaction mixture was then saturated with ammonium sulphate (solid), and the mixture was extracted with ethyl acetate (3x30 ml). The organic layer was dried over magnesium sulphate and concentrated. Flash chromatography with petroleum ether:ethyl acetate (7:1) afforded **4** as light yellow prisms 0.16 g (85%); mp 54-57 °C. IR (KBr) ν = 2960, 2853, 1720, 1604, 1583, 1464, 1198, 1085, 840 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 0.96 (3H, t, *J*=7.3, H3'), 1.64 (1H, m, $H2_A$'), 1.71 (1H, m, $H2_B$') 1.71(1H, dddd, *J*= 13.5,10.2, 7.4, 5.2 $H1_A$') 1.87 (1H, dddd, *J*= 13.5,10.8, 5.0, 5.0 $H1_B$') 2.85 (1H, dd, J_{gem}= 16.3 J_{trans}= 4.0 Hz, H-4), 2.96 (1H, dd, J_{gem} 16.5 J_{cis}=11.1, H-4), 4.48 (dddd, *J*=10.5, 7.6, 5.4, 4.6, H-3), 6.14 (2H, s, OCH_2O), 6.74 (1H, s, H-5), 7.59 (1H, s, H-8) ppm; ^{13}C NMR ($CDCl_3$) δ: 162.4 (C1), 76.7 (C3), 36.0 (C4), 114.9 (4a), 106.0 (C5), 147.7 (C6), 154.2 (C7), 107.3 (C8), 134.9 (C8a), 32.7 (C1'), 18.1 (C2'), 13.8 (C3'); EIMS m/z (%): 234 [M$^+$] (36), 232 (41), 163 (31) 162 (100), 191 (29), 134 (65).

Anal. Calcd. For $C_{13}H_{14}O_4$: C 66.66, H 6.02. Found: C 66.59, H 6.09.

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