Preparation of 7-Alkoxylated Furanopyrones: Semisynthesis of (−)-Etharvensin, a New Styryl-Lactone from *Goniothalamus arvensis*

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A new furanopyrone derivative, (−)-etharvensin (**1**), was isolated from the stem bark of *Goniothalamus arvensis*. Semisynthesis of the 7-ethoxyfuranopyrone **1** was achieved by addition of EtOH in concentrated acid medium to the unsaturated α-pyrone (+)-altholactone (**2**). This protocol constitutes a novel, direct (single-step), and efficient method to prepare this class of bioactive compounds.

Styryl-lactones are an interesting group of cytotoxic and antitumor agents, many of which have been isolated from *Goniothalamus* species (Annonaceae).[1] As part of our investigation on the isolation, semisynthesis, and bioactivity of acetogenins[2,3] and styryl-lactones,[4,5] we report herein the isolation and structure elucidation of (−)-etharvensin (**1**) from the stem bark of *Goniothalamus arvensis* and the semisynthesis of **1** by alkoxylation of the unsaturated α-pyrone (+)-altholactone (**2**).

Purification of the crude MeOH extract of *G. arvensis* Scheff. stem bark and chromatographic fractionation led to the isolation of etharvensin (**1**). The molecular formula, $C_{15}H_{18}O_5$, was indicated by a small peak at 278 $[M]^+$ in the EIMS, and confirmed by high-resolution mass measurement (HREIMS). The presence of a hydroxyl group was suggested by an IR band at 3416 cm^{-1}, and corroborated by the preparation of a monoacetate derivative (**1a**). Inspection of the ^1H- and ^{13}C-NMR spectra revealed that **1** was closely related to goniotharvensin (**3**), a saturated α-pyrone also isolated from *G. arvensis*.[4] The 2-phenyl-tetrahydrofurano-5-pyrone skeleton of **1** was established by careful examination of the HREIMS fragmentations, 2D homonuclear (COSY 45), and 2D heteronuclear correlation (HMQC) NMR spectra (Table 1).

The presence of an ethoxyl group was suggested in HREIMS by a fragment peak at *m/z* 232.0740 $[M - 46]^+$ ($C_{13}H_{12}O_4$), and confirmed by 1D and 2D NMR experiments, which showed resonances at δ 3.65 (q, 2H, 14-CH_2) and 1.22 (t, 3H, 15-CH_3) in the ^1H, and at δ 60.28 (CH_2) and 15.30 (CH_3) in the ^{13}C NMR, respectively. The 400 MHz COSY 45 spectra of etharvensin (**1**) revealed the correlation between the saturated δ-lactone protons. The H-6a/H-6b (at δ 2.70/ 2.84) and the H-7a (at δ 4.38) were correlated with a methine proton signal at δ 4.02, corresponding to H-7. Indeed, selective irradiation in ^1H NMR of the signal at δ 4.02 simplified the resonances corresponding to the 6 and 7a protons. Moreover, a NOEDIFF (homonuclear Overhauser enhancement) interaction was observed between H-7 (δ 4.02) and H-6a (δ 2.70) and between the methylene of the ethoxy moiety (OCH_2-14, at δ 3.65) and H-7 (δ 4.02). This finding is consistent with an ethoxy group placed at the 7 position,

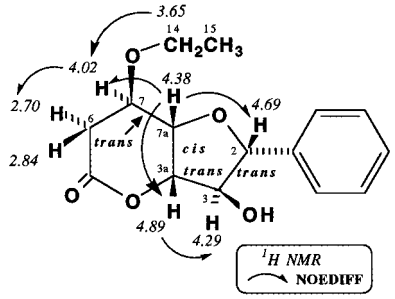

Figure 1. ^1H NMR and NOEDIFF of **1**.

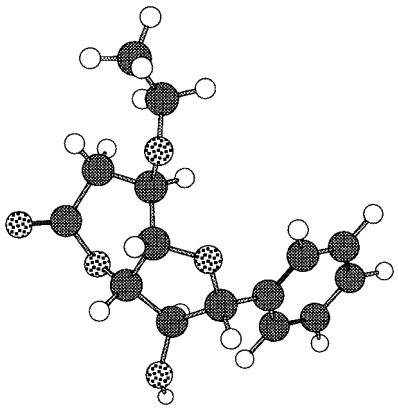

Figure 2. Perspective 3D of the fully energy-minimized structure of etharvensin (**1**) by MM2 calculations.

and it also excludes the location of the ethoxy group at the 3 position (Figure 1).

The relative stereochemistry of the four stereogenic centers in the tetrahydrofuran ring was evidenced by the $^1H-^1H$ coupling constant data and NOEDIFF experiments (Figure 1 and Table 1). The relative configuration was trans (H-3/H-2), trans (H-3a/H-3), and cis (H-7a/H-3a), similar to that of altholactone (**2**), whose configuration was established by X-ray crystallographic analysis,[6] and goniotharvensin (**3**).[4] A trans relationship was established between H-7/H-7a in agreement with a pseudo-chair α-pyrone conformation. Significant enhancement of H-6a (pseudoequatorial) was observed on irradiation of H-7 in a NOEDIFF experiment. To reproduce this configuration, a 3D representation of the fully energy-minimized structure of **1** was calculated using a modeling program with the MM2-derived force field (Figure 2).

The absolute stereochemistry of the chiral secondary hydroxy group at C-3 was determined by preparing the

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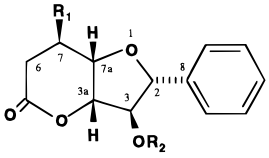
Table 1. 1D and 2D NMR Experiments (400 MHz, $CDCl_3$) of Etharvensin (**1**)

H	δ (*J*, Hz)	coupling in COSY 45	coupling in HMQC (multiplicity by DEPT ^{13}C)
2	4.69 d (6.1)	H-3 (4.29)	85.99 (CH)
3	4.29 dd (2.0, 6.1)	H-3a (4.89), H-2 (4.69)	83.50 (CH)
3a	4.89 dd (2.0, 4.7)	H-7a (4.38), H-3 (4.29)	86.98 (CH)
5			169.48 (C)
6a	2.70 dd (5.8, 16.4)	H-7 (4.02), H-6b (2.84)	33.10 (CH_2)
6b	2.84 dd (3.7, 16.4)	H-7 (4.02), H-6a (2.70)	
7	4.02 ddd (3.6, 3.7, 5.8)	H-7a (4.38), H-6b (2.84), H-6a (2.70)	72.78 (CH)
7a	4.38 br t (3.6, 4.7)	H-7 (4.02), H-3a (4.89)	75.72 (CH)
8			138.15 (C)
9−13	7.33−7.38 m		128.64, 128.31, 126.05 (CH)
14	3.65 q (7.0)	CH_3-15 (1.22)	65.28 (CH_2)
15	1.22 t (7.0)	CH_2-14 (3.65)	15.30 (CH_3)

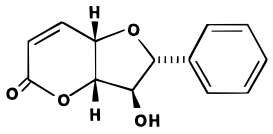
Table 2. ^1H NMR (400 MHz, $CDCl_3$) Data of Mosher Esters **1b** and **1c**

	H-9 to H-13	H-2	H-3	H-3a	H-7a	H-7	H-6a	H-6b
(*S*)-MTPA (**1b**)	7.38−7.30	4.915	5.478	4.878	4.281	4.046	2.713	2.894
(*R*)-MTPA (**1c**)	7.35−7.28	4.782	5.499	4.992	4.294	4.050	2.740	2.904
$\Delta\delta_{S-R}$	+(0.03−0.02)	+0.133	−0.021	−0.114	−0.013	−0.004	−0.027	−0.010

(*R*)- and (*S*)-α-(methoxy)-α-(trifluoromethyl) phenylacetic acid (MTPA) derivatives by the Mosher's ester method.[7,8] Thus, **1** was converted to the (*S*)-MTPA (**1b**) and (*R*)-MTPA (**1c**) esters. The negative [H-3a, $\Delta\delta_{S-R}$ = −0.114] and positive [H-2, $\Delta\delta_{S-R}$ = +0.133] $\Delta\delta_H$ values observed for the signals of the protons on the left and on the right segments, respectively, indicated a 3*R* stereochemistry for **1** (Table 2). Consequently, the absolute configuration for **1** is {2*R*,3*R*,3a*S*,7*R*,7a*S*}, in agreement with the revised stereochemistry for goniofupyrone (**4**) as recently established by synthesis.[9]

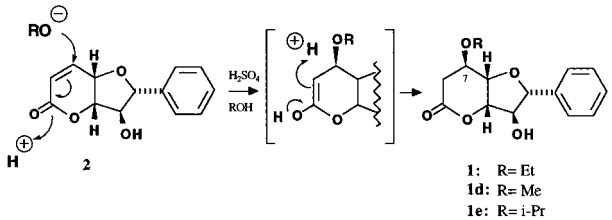


1: R_1= OCH_2CH_3; R_2= H
1a: R_1= OCH_2CH_3; R_2= $COCH_3$
1b: R_1= OCH_2CH_3; R_2= (*S*)-MTPA ester
1c: R_1= OCH_2CH_3; R_2= (*R*)-MTPA ester
3: R_1= R_2= H
4: R_1= OH; R_2=H



2

To confirm the structure of this ethoxylated natural product, etharvensin (**1**) was semisynthesized from the optically active (+)-altholactone (**2**) by a high-yielding single-step method. We first tried to prepare **1** from **2** by epoxidation with *m*-CPBA of the α,β-unsaturated δ-lactone of **2**, followed by lithium aluminum hydride reduction, and *O*-ethylation, but **1** could not be obtained by this method. However, **1** was successfully prepared from **2** by ethoxylation of the unsaturated pyrone by a Michael-type addition of EtOH in concentrated H_2SO_4 (Figure 3). A stereoselective alkoxylation at the C-7 position was achieved because only one diasteroisomer (**1**, 90%) was obtained when **2** was refluxed 1.5 h. To corroborate this stereoselectivity, experiments were



1: R= Et
1d: R= Me
1e: R= i-Pr

Figure 3. Preparation of (−)-etharvensin (**1**) and 7-alkoxylated furanopyrones (**1d**, **1e**) from (+)-altholactone (**2**).

carried out under the same conditions but with MeOH or *i*PrOH in H_2SO_4 acid medium. The 7-methoxy (**1d**) and 7-isopropoxy (**1e**) furanopyrone derivatives were obtained stereoselectively in high yield (Figure 3).

Because **1** was prepared readily from **2** under the conditions outlined above, the possibility that it is an artifact of the isolation process must be considered. This possibility can be discounted, however, inasmuch as no EtOH was used in the extraction or purification of **1**. On the other hand, because altholactone (**2**) is available in large quantity from *G. arvensis*, it represents a good natural starting material for preparation of other styryllactones. This is the first report of a direct semisynthesis of 7-alkoxylated furanopyrones in good yield using simplified substrates.

Etharvensin (**1**) was less active than altholactone (**2**) and goniotharvensin (**3**) in reducing the contractile response induced by noradrenaline on rat aorta. In Ca^{2+}-containing medium, the contractile response was not abolished by **1**−**3** at 100 μM concentrations, while in Ca^{2+}-free solution, **2** and **3** were active, with an IC_{50} of 78 and 80 μM, respectively, suggesting an intracellular mechanism for relaxation of the vascular smooth muscle.[10]

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. IR spectra were run in film on a Perkin-Elmer 843 spectrometer. UV spectra were obtained on a Perkin-Elmer lambda computer 15 UV/vis spectrophotometer. Mass spectra were performed with a VG Auto Spec Fisons spectrometer. ^1H NMR (250 or 400 MHz), ^{13}C NMR, DEPT, and HMQC (62.5 or 100 MHz) spectra were recorded on a Bruker AC-250 or a Varian Unity-

400 instruments. Si gel TLC were detected by UV light (254 nm) and spraying anisaldehyde sulfuric acid.

Plant Material. *G. arvensis* Scheff. (Annonaceae) was collected in the National Park of Varirata, located in the Central Province of Papua, New Guinea. A voucher specimen was deposited in the herbarium of the University of Papua, New Guinea.

Extraction and Isolation. Dried and powdered stem bark of *G. arvensis* (368 g) was macerated with MeOH at room temperature. The crude MeOH extract was partitioned between hexane and aqueous MeOH. The aqueous MeOH solution was again fractionated between CH_2Cl_2 and H_2O to obtain 7 g of CH_2Cl_2 extract. Etharvensin (**1**, 25 mg) was isolated and purified by Si gel 60 H column chromatography (hexane−EtOAc, 4:6 and CH_2Cl_2−Me_2CO, 9:1).

(−)-Etharvensin (**1**): oil; $C_{15}H_{18}O_5$; $[\alpha]_D$ −6.5° (*c* 2.0, EtOH); UV (EtOH) λ_{max} (log ϵ) 214 (2.44), 255 (2.30) and 280 (2.27) nm; IR (dry film) ν_{max} 3416, 3060, 1740, 1636, 1451, 1380, 1237, 1099, 917, 862, 841, 822, 760, 700 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz), ^{13}C NMR ($CDCl_3$, 100 MHz), COSY 45, and HMQC data, see Table 1 and Figure 1; HREIMS *m/z* (%) $[M]^+$ 278.1148 (calcd for $C_{15}H_{18}O_5$, 278.1154) (8), $[M - HOEt]^+$ 232.0740 (calcd for $C_{13}H_{12}O_4$, 232.0735) (12), 162 (38), 133.0653 (calcd for C_9H_9O, 133.0653) (100), 107 (C_7H_7O, 33), 97 (58), 91 (39), 77 (C_6H_5, 15).

3-Acetyletharvensin (**1a**): Treatment of **1** (6 mg) by Ac_2O (1 mL) and pyridine (0.5 mL) overnight at room temperature yielded 6.9 mg of **1a**. $C_{17}H_{20}O_6$; $[\alpha]_D$ −10.7 ° (*c* 1.8, EtOH); UV (EtOH) λ_{max} (log ϵ) 216 (2.44), 236 (2.95) and 280 (2.27) nm; IR (film) ν_{max} 2970, 1749, 1492, 1449, 1369, 1224, 1095, 1044, 917, 761, 699 cm^{-1}; CIMS *m/z* (%) $[MH]^+$ 321 (100), 261 $[MH - HOCOCH_3]^+$ (4); ^1H NMR ($CDCl_3$, 250 MHz) δ 7.37−7.29 (m, H-9 to H-13), 5.29 (dd, H-3, J = 4.0 and < 1.0 Hz), 4.92 (dd, H-3a, J = 3.5 and <1.0 Hz), 4.89 (d, H-2, J = 4.0 Hz), 4.36 (t, H-7a, J = 3.7, 3.5 Hz), 4.06 (ddd, H-7, J = 5.6, 4.0, 3.7 Hz), 3.65 (q, 2H, OCH_2CH_3), 2.89 (dd, H-6b, J = 16.5, 4.0 Hz), 2.70 (dd, H-6a, J = 16.5, 5.6 Hz), 2.14 (s, 3H, $OCOCH_3$), 1.23 (t, 3H, OCH_2CH_3); ^{13}C NMR ($CDCl_3$, 62.5 MHz) 169.90 (C-5), 160.66 ($OCOCH_3$), 138.00 (C-8), 128.71 (C-10, 12), 128.53 (C-11), 126.35 (C-9, 13), 85.86 (C-3a), 83.43 (C-2), 82.91 (C-3), 75.90 (C-7a), 72.80 (C-7), 65.23 (OCH_2CH_3), 33.07 (C-6), 20.82 ($OCOCH_3$), 15.30 (OCH_2CH_3).

Preparation of the C(3)−(*S*)- and (*R*)-MTPA esters of (−)-Etharvensin (**1**). To a stirred solution of **1** (2.5 mg) in CH_2Cl_2 at room temperature was added pyridine, 4-(dimethylamino) pyridine, and (*R*)-MTPA−Cl [to give (*S*)-MTPA ester] or (*S*)-MTPA-Cl [to give (*R*)-MTPA−ester].[8] Each reaction mixture was allowed to sit for 2 h at room temperature, saturated with $NaHCO_3$, and extracted with CH_2Cl_2. Normal workup gave the (*S*)- and (*R*)-MTPA esters of etharvensin (4 mg of **1b** and 4.5 mg of **1c**, respectively).

(*S*)-MTPA−Etharvensin (**1b**): ^1H NMR ($CDCl_3$, 400 MHz) H-2 to H-13, see Table 2; δ 7.48−7.35 (m, 5 H, Ph of MTPA), 3.610 (q, 2H, OCH_2CH_3), 3.530 (s, 3H, CH_3 of MTPA), 1.205 (t, 3H, OCH_2CH_3).

(*R*)-MTPA−Etharvensin (**1c**): ^1H NMR ($CDCl_3$, 400 MHz) H-2 to H-13, see Table 2; δ 7.47−7.35 (m, 5 H, Ph of MTPA), 3.624 (q, 2H, OCH_2CH_3), 3.533 (s, 3H, CH_3 of MTPA), 1.213 (t, 3H, OCH_2CH_3).

General Procedure for Alkoxylation of (+)-Altholactone (**2**). To an EtOH solution (10 mL) of altholactone (**2**, 60 mg, 0.258 mmol) was added dropwise at 0 °C concentrated H_2SO_4 (96%, 1.5 mL). After stirring and refluxing for 1.5 h, H_2O was added to the solution, followed by extraction with CH_2Cl_2. The organic solution obtained was subjected to column chromatography on Si gel 60 H (eluted with $CHCl_3$-EtOAc, 7:3) to afford 65 mg (0.233 mmol, 90%) of a compound that was found to be identical with the previously isolated (−)-etharvensin (**1**).

Biological Assays. Noradrenaline (NA_1, 1μM) was added in Ca^{2+}-containing solution at 37 °C, and afterward the tissue was loaded in Ca^{2+}-free, EDTA-containing solution for 20 min. After this time, agonist (NA_2) was applied until no contraction was induced, indicating complete depletion of internal Ca^{2+} stores sensitive to the agonist. The tissue was incubated for 20 min in Krebs to refill the intracellular Ca^{2+} stores, and a spontaneous increase in the resting tone of the aorta was observed. After washing and 5 min of loading in Ca^{2+}-free solution, styryl-lactone (**1**−**3**) was applied, and after 15 min a new addition of agonist (NA_3) was made.

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