

# Crystal Structure of a Novel Furo-Furan Lactone from *Heliotropium eichwaldi*

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A novel furo-furan lactone **1** has been isolated as fine needles from the ethyl acetate-soluble fraction of *Heliotropium eichwaldi*. This is the first report of any furo-furan from the genus *Heliotropium*.  $\beta$ -Sitosterol 3-*O*- $\beta$ -D-glucoside **2** has also been obtained for the first time from this source. The structure of **1** was elucidated by X-ray diffraction studies.

**Key words:** *Heliotropium eichwaldi*, Boraginaceae, Furo-Furan Lactone, X-Ray Diffraction Studies

## Introduction

*Heliotropium eichwaldi* Steud (Boraginaceae) is an annual herb which grows in cultivated fields, gardens and shallow lands of the province of Sindh, Pakistan [1]. The plant is emetic, antidote and antiseptic. It is well known to be used for the treatment of scorpion stings, bee stings, mad dog bite, snake bite, earache, and cleaning and healing of wounds, warts and ulcers [1, 2]. Previous investigations on *H. eichwaldi* have yielded pyrrolizidine alkaloids and terpenoids [2]. The ethnopharmacological and chemotaxonomic importance of the genus *Heliotropium* prompted us to carry out further studies on *H. eichwaldi*. As a result, we now report the isolation and structural elucidation of a novel furo-furan lactone **1** along with  $\beta$ -sitosterol 3-*O*- $\beta$ -D-glucoside (**2**), reported for the first time from this species.

## Results and Discussion

The methanolic extract of the whole plant of *H. eichwaldi* was divided into *n*-hexane-, EtOAc-, *n*-BuOH- and water-soluble fractions. A series of column chromatography separations applied to the EtOAc fraction resulted the isolation of compounds **1** and **2**.

Compound **1** was obtained as fine colorless needles, m.p. 172 °C. The (+)-FAB HRMS showed a quasi

[M+H]<sup>+</sup> peak at  $m/z = 275.1131$  (calcd. 275.11308 for C<sub>12</sub>H<sub>19</sub>O<sub>7</sub>). The IR spectrum showed the presence of an intense peak at 1799 cm<sup>-1</sup> corresponding to C=O vibrations and a broad peak in the region 3400–3200 cm<sup>-1</sup> suggesting the presence of OH functionalities. The molecular formula was confirmed by broadband (BB) and DEPT <sup>13</sup>C NMR spectroscopy which showed twelve well resolved signals comprising of three methyl, one methylene, four methine and four quaternary carbon atoms. The most downfield signal at  $\delta = 176.2$  ppm could be assigned to the lactone carbonyl carbon atom. It further showed oxygen-containing quaternary carbons at  $\delta = 80.1, 92.2$  and  $96.5$ , oxymethine carbons at  $\delta = 83.6, 88.9$  and  $113.0$ , as well as an oxymethylene carbon at  $\delta = 62.5$  ppm. The signals of three methyl groups were observed at  $\delta = 17.4, 17.9$  and  $18.1$  ppm, respectively. In the <sup>1</sup>H NMR spectrum three oxymethine protons appeared at  $\delta = 4.05$  (dd,  $J = 8.0, 2.5$  Hz),  $4.47$  (q,  $J = 6.0$  Hz) and  $5.27$  ppm (1H, s). The oxymethylene protons resonated at  $\delta = 3.60$  (1H, dd,  $J = 12.0, 2.5$  Hz) and  $\delta = 3.93$  ppm (1H, dd,  $J = 12.0, 8.0$  Hz). It further exhibited signals of three secondary methyls as doublets at  $\delta = 0.96$  ( $J = 7.0$  Hz),  $1.0$  ( $J = 7.0$  Hz) and  $1.28$  ppm ( $J = 6$  Hz). The presence of a one proton septet at  $\delta = 2.2$  ppm indicated the presence of an isopropyl group.

Table 1.  $^1\text{H}$  (500 MHz) and  $^{13}\text{C}$  (125 MHz) NMR spectroscopic data of compound **1** recorded in  $\text{CD}_3\text{OD}$ .

| C  | $\delta_{\text{H}}$ (mult., J in Hz) | $\delta_{\text{C}}$ | HMBC ( $^1\text{H}$ - $^{13}\text{C}$ ) |
|----|--------------------------------------|---------------------|---|
| 2  | 4.47 (q, $J = 6.0$ )                 | 83.6, CH            | C-2a, C-2', C-4, C-6a, C-6b             |
| 2a | –                                    | 96.5, C             | –                                       |
| 4  | –                                    | 176.2, C            | –                                       |
| 4a | –                                    | 80.1, C             | –                                       |
| 5  | 4.05 (dd, $J = 2.5, 8.0$ )           | 88.9, CH            | C-4, C-4a, C-5', C-6b                   |
| 6a | 5.27 (s)                             | 113.0, CH           | C-2, C-2a, C-4a, C-5, C-6b              |
| 6b | –                                    | 92.2, C             | –                                       |
| 1' | 1.28 (d, $J = 6.0$ )                 | 18.1, $\text{CH}_3$ | –                                       |
| 2' | 2.16 (sept, $J = 7.0$ )              | 32.2, CH            | C-2, C-2a, C-3', C-4', C-6b             |
| 3' | 0.96 (d, $J = 7.0$ )                 | 17.9, $\text{CH}_3$ | –                                       |
| 4' | 1.01 (d, $J = 7.0$ )                 | 17.4, $\text{CH}_3$ | –                                       |
| 5' | 3.60 (dd, $J = 2.5, 12.0$ )          | 62.5, $\text{CH}_2$ | C-5                                     |
|    | 3.93 (dd, $J = 8.0, 12.0$ )          |                     |   |

The assignments of the signals in the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra (Table 1) were made with the help of  $^1\text{H}$ - $^1\text{H}$  COSY, HMQC and HMBC correlations while the stereochemistry was fully authenticated by NOESY experiments (Fig. 1).

The structure and relative configuration of compound **1** are established by single-crystal X-ray diffraction studies as illustrated in Fig. 2. Although natural products with two fused furan rings have previously been reported in the literature the present work describes the first ever isolation of a new natural product with three fused furan rings [3–5].

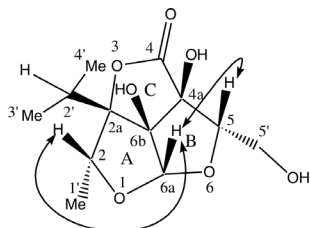


Fig. 1. Structure and important NOESY correlations of compound **1**.

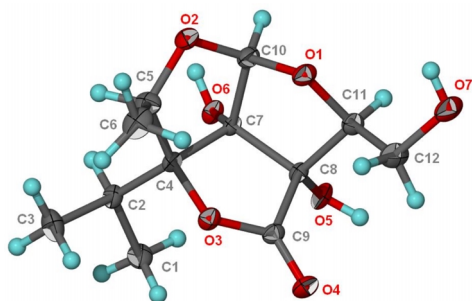


Fig. 2. The molecular structure of **1** in the crystal (displacement ellipsoids drawn at the 30% probability level; H atoms as spheres with arbitrary radii).

The substance was crystallized from  $\text{CHCl}_3/\text{MeOH}$ . The asymmetric unit comprised one molecule of **1** ( $Z = 4$ ) in the orthorhombic space group  $P2_12_12_1$ . Compound **1** is composed of three fused five-membered rings A (O1/C7-C8/C10-C11), B (O2/C4-C5/C7/C10) and C (O3/C4/C7/C8-C9). All three rings were found to be *cis*-fused to each other and adopt envelope conformations. The two hydroxy substituents were found to be pseudo-axially oriented at C-7 and C-8 with *cis* conformation to each other with angles of  $109.5^\circ$ . The hydroxymethylene moiety is attached pseudo-axially to ring A whereas the isopropyl group is found to be equatorially oriented at C-4.

## Experimental Section

### General experimental procedures

Column chromatography (CC) was performed on silica gel (250–400 mesh; E. Merck, Darmstadt, Germany). The purity of the compounds was checked on TLC:  $\text{SiO}_2$  60 F<sub>254</sub> plates (E. Merck, Darmstadt, Germany), detection was done in iodine vapors and by spraying with ceric sulfate in 10%  $\text{H}_2\text{SO}_4$ . Melting points were recorded in glass capillary tubes using a Büchi 535 melting point apparatus. Optical rotations were measured on a Jasco DIP-360 (Japan Spectroscopic Co. Ltd., Tokyo, Japan). The UV spectra were recorded on a Hitachi UV-3200 spectrophotometer while the IR spectrum was recorded on a Jasco 302-A spectrophotometer in KBr. The EI, FAB mass and HRMS ((+)-FAB) spectra were recorded on Jeol JMS-HX-110 and JMS-DA-500 mass spectrometers using glycerol as matrix. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded on a Bruker AMX-500 MHz instrument in  $\text{CD}_3\text{OD}$ . Chemical shifts ( $\delta$ ) are given in ppm, and scalar coupling constant ( $J$ ) are reported in Hertz.

### Plant material

The plant material of *H. eichwaldi* Steud was collected in Karachi in November 2008 and identified by Prof. Dr. Surrayya Khatoun, Plant Taxonomist, Department of Botany, University of Karachi, Pakistan. A voucher specimen (no. 71489) has been deposited in the herbarium of the same Department.

### Extraction and isolation

The shade-dried and chopped whole plant of *H. eichwaldi* (10 kg) was extracted with MeOH ( $3 \times 25$  L) at r. t. for 8 d each. The methanol extract was concentrated *in vacuo*. The greenish-brown residue (240 g) was divided into *n*-hexane (110 g), EtOAc- (18 g), *n*-BuOH- (35 g), and  $\text{H}_2\text{O}$ -soluble (24 g) sub-fractions by solvent extraction. The EtOAc sub-fraction was subjected to column chromatography on silica gel using a mixture of  $\text{CHCl}_3$ -MeOH in order of in-

Table 2. Crystallographic data and numbers pertinent to data collection and structure refinement of **1**.

|   |   |
|---|---|
| Formula   | C <sub>12</sub> H <sub>18</sub> O <sub>7</sub>        |
| <i>M<sub>r</sub></i>  | 274.26  |
| Crystal size, mm <sup>3</sup>   | 0.40 × 0.14 × 0.01                                    |
| Crystal system  | orthorhombic  |
| Space group   | <i>P</i> 2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub> |
| <i>a</i> , Å  | 7.5102(9)   |
| <i>b</i> , Å  | 8.1278(10)  |
| <i>c</i> , Å  | 20.879(2)   |
| <i>V</i> , Å <sup>3</sup>   | 1274.5(3)   |
| <i>Z</i>  | 4   |
| <i>D</i> <sub>calcd</sub> , g cm <sup>-3</sup>  | 1.43  |
| $\mu$ (MoK $\alpha$ ), mm <sup>-1</sup>   | 0.1   |
| <i>F</i> (000), e   | 584   |
| Wavelength; $\lambda$ , Å   | MoK $\alpha$ ; 0.71073                                |
| <i>T</i> , K  | 293(2)  |
| <i>hkl</i> range  | ±9, -9 → +10, -25 → +27                               |
| $\theta$ <sub>max</sub> , deg   | 27.5  |
| Refl. measured / unique / <i>R</i> <sub>int</sub>   | 8839 / 1707 / 0.0305                                  |
| Param. refined / restraints   | 183 / 16  |
| <i>R</i> ( <i>F</i> ) / <i>wR</i> ( <i>F</i> <sup>2</sup> ) <sup>a,b</sup> ( <i>I</i> ≥ 2σ( <i>I</i> )) | 0.0428 / 0.1082                                       |
| <i>R</i> ( <i>F</i> ) / <i>wR</i> ( <i>F</i> <sup>2</sup> ) <sup>a,b</sup> (all refls.)                 | 0.0492 / 0.1140                                       |
| GoF ( <i>F</i> <sup>2</sup> ) <sup>c</sup>  | 1.148   |
| $\Delta\rho$ <sub>fin</sub> (max / min), e Å <sup>-3</sup>  | 0.32 / -0.17  |

<sup>a</sup>  $R1 = \frac{\sum |F_o| - |F_c|}{\sum |F_o|}$ ; <sup>b</sup>  $wR2 = \frac{[\sum w(F_o^2 - F_c^2)^2 / \sum w(F_o^2)^2]^{1/2}}{w}$ ,  $w = [\sigma^2(F_o^2) + (AP)^2 + BP]^{-1}$ , where  $P = (\text{Max}(F_o^2, 0) + 2F_c^2) / 3$ ; <sup>c</sup>  $GoF = [\sum w(F_o^2 - F_c^2)^2 / (n_{\text{obs}} - n_{\text{param}})]^{1/2}$ .

creasing polarity to yield fractions A–D. Fraction B (9 g) which eluted with CHCl<sub>3</sub>-MeOH (9.2:0.8) was again chromatographed using a mixture of CHCl<sub>3</sub>-MeOH in increasing order of polarity to obtain fractions B<sub>1</sub>–B<sub>9</sub>. Fraction B<sub>4</sub> (4 g) which was obtained from CHCl<sub>3</sub>-MeOH (9.2:0.8) was further chromatographed eluting with CHCl<sub>3</sub>-MeOH (9:1) to afford **1** (20 mg). Fraction B<sub>6</sub> (1.4 g) which was obtained with CHCl<sub>3</sub>-MeOH (8.8:1.2) was again chromatographed and eluted with CHCl<sub>3</sub>-MeOH (8.5:15) to afford the compound **2** (1.2 g). Compound **2** was identified through comparison of physical and spectral data with those reported in literature [6].

#### 4a,6b-Dihydroxy-5-(hydroxymethyl)-2a-isopropyl-2-methyl-hexahydro-4H-1,3,6-trioxo-cyclopenta[cd]pentalen-4-one (**1**)

Crystalline solid; m. p. = 172 °C. –  $[\alpha]_D^{25} = +18.5$  (*c* = 0.31, MeOH). – IR (KBR):  $\nu_{\text{max}} = 3200$  (OH), 1799 (C=O), 1438, 1190, 1109, 995 cm<sup>-1</sup>. – <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD) and <sup>13</sup>C NMR (125 MHz, MeOH): see Table 1. – MS (EI, 70 eV): *m/z* (%) = 243 (43) [M-CH<sub>2</sub>OH]<sup>+</sup>, 214 (4), 155 (40), 141 (82), 131 (78), 43 (100). – MS ((+)-FAB): *m/z* = 275, [M+H]<sup>+</sup>. – MS ((-)-FAB): *m/z* = 273 [M-H]<sup>+</sup>. – HRMS ((+)-FAB): *m/z* = 275.1131 (calcd. 275.11308 for C<sub>12</sub>H<sub>19</sub>O<sub>7</sub>, [M+H]<sup>+</sup>).

#### Single-crystal X-ray structure determination of compound **1**

Crystal data and numbers pertinent to data collection and structure refinement of **1** are summarized in Table 2. The structure was solved by Direct Methods and expanded by Fourier techniques using SIR92 [7]. Refinement was done by full-matrix least-squares calculations on *F*<sup>2</sup> with the program SHELXL-97 [8]. In the absence of heavy atoms, the Friedel pairs were merged and no Flack refinement was undertaken. Carbon-bound hydrogen atoms were placed in calculated positions (C–H 0.93 to 0.98, O–H 0.82 Å) and were included in the refinement in the riding model approximation, with *U*<sub>iso</sub> (H) set to 1.2 to 1.5 *U*<sub>eq</sub> (C,O).

Two ether oxygen atoms were disordered over two positions in a ratio of 0.625(5)/0.375. Their anisotropic displacement factors were restrained to be nearly isotropic and set to be similar. Fig. 2 shows the molecular structure.

CCDC 843754 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via [www.ccdc.cam.ac.uk/data\\_request/cif](http://www.ccdc.cam.ac.uk/data_request/cif).

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