

## Isolation of (S)-(-)-2,3-Dihydro-2,6-dimethyl-4H-benzopyran-4-one from Roots of *Leontopodium alpinum*

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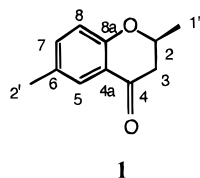
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The isolation of (S)-(-)-2,3-dihydro-2,6-dimethyl-4H-benzopyran-4-one (**1**) from the essential oil produced by natural roots and also from genetically transformed ("hairy") root cultures of *Leontopodium alpinum* is described. This is the first reported natural occurrence of **1**. Structural assignments were made on the basis of spectroscopic data and by comparison with a synthetic sample.

*Leontopodium alpinum* Cass. (Compositae, Inuleae), commonly known as Edelweiss, is a protected plant indigenous to the alpine regions of Europe. The plant has some suggested medicinal properties.<sup>1,2</sup> Chemical analyses have led to the identification of hydrocarbons<sup>3</sup> and flavanoids<sup>4</sup> in the aerial parts of *L. alpinum*. More recently, this group has identified sterols and two hydroxycinnamic acids in the roots and aerial parts of *L. alpinum*.<sup>5</sup>

Because of the protected status of Edelweiss, plant material cannot be collected from the wild. We therefore developed plants from commercially available seeds and grew these to maturity under greenhouse conditions in a peat-based compost. Natural roots were obtained by separation from aerial parts. However, this source only generated a small quantity of plant material, and genetically transformed ("hairy") roots were used as an additional source of biomass. Powdered root material from both sources, when subjected to steam distillation, yielded an essential oil: natural roots ca. 2.0% and hairy roots ca. 0.6%. Gas chromatographic analyses of both oils showed them to be a complex mixture of more than 30 compounds, with the hairy root oil qualitatively similar to that isolated from natural roots, although quantitative differences are apparent.<sup>6</sup> Chemical analysis of the essential oil produced by the roots has resulted in the isolation of a minor constituent (0.3%), which has been identified as (S)-(-)-2,3-dihydro-2,6-dimethyl-4H-benzopyran-4-one (**1**). This is the first reported isolation of **1** as a natural product.



The isolate analyzed for C<sub>11</sub>H<sub>12</sub>O<sub>2</sub>, suggesting a bicyclic aromatic molecule. A carbonyl function was evident by a band in the IR spectrum at  $\nu_{\max}$  1680 cm<sup>-1</sup>. The <sup>1</sup>H NMR spectrum showed a signal for an aromatic methyl group ( $\delta$  1.60, Me-6) together with a secondary methyl group ( $\delta$  1.45, d,  $J$  = 7.3 Hz, Me-2). The

secondary methyl group showed a coupling to a deshielded one-proton multiplet at  $\delta$  4.50 ( $J$  = 7.3, 6 Hz, H-2). A deshielded doublet at  $\delta$  2.70 ( $J$  = 6 Hz, H-3) integrated for two protons.

A series of signals also appeared in the aromatic region of the spectrum. Further unambiguous assignments for structure **1** were based on two-dimensional <sup>1</sup>H-<sup>1</sup>H COSY NMR studies. In the COSY spectrum, coupling was clearly observed between the deshielded methine at  $\delta$  4.50, the methyl at  $\delta$  1.45, and the methylene at  $\delta$  2.70. On the basis of these data and the molecular requirement for two oxygen atoms, we established the partial structure -OCH(CH<sub>3</sub>)CH<sub>2</sub>C=O. The coupling pattern in the aromatic region of the molecule indicated two *ortho*-coupled protons ( $J$  = 9 Hz), one of which was further coupled in a *meta* relationship (2 Hz). A third proton resonated as a fine *meta*-coupled doublet downfield at  $\delta$  7.70 (H-5), and the strongly deshielded nature of this proton placed it in close proximity to the carbonyl. The aromatic methyl substituent was placed beside this proton to accommodate the observed coupling. On the basis of these conclusions, we determined the total structure to be 2,3-dihydro-2,6-dimethyl-4H-benzopyran-4-one (**1**). This is the first reported natural occurrence of **1**. Final proof of the structure came from the synthesis of **1** using methods reported in the literature.<sup>7,8</sup> The (S)-(-)-configuration of the C-2 methyl was assigned by correlation of the optical activity of the isolate with literature values of simple chromanones with established stereochemistry.<sup>9</sup>

### Experimental Section

**General Experimental Procedures.** NMR spectra are for CDCl<sub>3</sub> solutions measured on a Bruker instrument operating at 300 Mz (<sup>1</sup>H) and 100.6 MHz (<sup>13</sup>C), with TMS as internal standard. FTIR spectra were obtained on a Pye Gamma 6 spectrometer. Mass spectra were recorded at 70 eV. GC analyses were recorded using a Perkin-Elmer Sigma 3 instrument fitted with a Carbowax 20M column operated with a temperature program (100–200 °C, ramp rate 2° min.).

**Plant Material.** *L. alpinum* plants were developed from commercially available seed (W. J. Unwin Ltd.,

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Cambridge, U.K.). Hairy roots were developed by infecting sterile plants with *Agrobacterium rhizogenes*.<sup>10</sup>

**Extraction and Isolation.** Powdered root material from both natural roots and hairy roots was subjected to steam distillation using an Apparatus for the Determination of Essential Oils in Vegetable Drugs.<sup>11</sup> This powdered material yielded an essential oil: natural roots ca. 2.0% and hairy roots ca. 0.6%.<sup>12</sup> The volatile oil was chromatographed on Si gel impregnated with AgNO<sub>3</sub> (15%) gradient eluted with petroleum ether:ethyl acetate and CHCl<sub>3</sub>:MeOH mixtures. Samples were collected in 10 mL fractions. One of the CHCl<sub>3</sub>-MeOH fractions yielded homogeneous (*S*)-(-)-2,3-dihydro-2,6-dimethyl-4*H*-benzopyran-4-one (**1**) (0.3%).

**(*S*)-(-)-2,3-Dihydro-2,6-dimethyl-4*H*-benzopyran-4-one (**1**).** This compound was isolated as an oil:  $[\alpha]_D^{25} -28^\circ$  (*c* 0.1, MeOH); IR (film)  $\nu_{\max}$  1680 (C=O, ketone), 1452, 1258 cm<sup>-1</sup>; EIMS (70 eV)  $m/z$  [M<sup>+</sup>] 176 (65), 161 (20), 134 (100), 106 (24), 78 (18), 51 (15), 39 (12), 28 (6); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  1.45 (3H, d, *J* = 7.3 Hz, Me-1'), 2.30 (3H, s, Me-6), 2.70 (2H, d, *J* = 6 Hz, CH<sub>2</sub>-3), 4.50 (1H, dt, *J* = 6, 7.3 Hz, H-2), 6.80 (1H, d, *J* = 9 Hz, H-8), 7.30 (1H, d, *J* = 2, 9 Hz, H-7), 7.70 (1H, d, *J* = 2 Hz, H-5); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75.4 MHz) 18.32 (Ar-CH<sub>3</sub>), 19.80 (CH<sub>3</sub>), 42.6 (C-3), 72.3 (C-2), 114.5 (ArCH), 118.6 (C-4a), 124.4 (ArCH), 128.8 (ArC), 134.8 (ArCH),

155.7 (C-8a), 190.9 (C-4). Anal. Calcd for C<sub>11</sub>H<sub>12</sub>O<sub>2</sub>: C, 75.00; H, 6.81. Found: C, 74.25; H, 6.92.

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## References and Notes

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