2-METHOXYDIHYDROHELENALIN FROM THE RHIZOMA OF SENDAI *HELENIUM AUTUMNALE* L.

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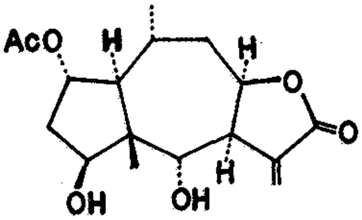
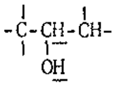
Helenalin and 2-methoxydihydrohelenalin II, a first naturally occurring pseudoguaianolide, were isolated from the rhizoma of Sendai *Helenium autumnale* (Compositae). The structure and stereochemistry of II were confirmed on the basis of chemical and physical means.

We previously reported on the isolation and structural elucidation of three new sesquiterpene lactones from the epigeal parts of Sendai *Helenium autumnale* (Compositae), in which the pseudoguaianolide I showed a significant inhibitory activity against HeLa cells[1]. Of chemotaxonomical interest the Sendai specimens showed marked differences in chemical constitution between American populations[2]. Helenalin, a usual constituent of American *H. autumnale*, could not be found in the epigeal parts of Sendai plants.

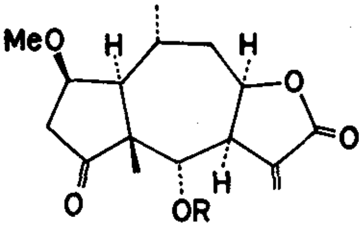
During the course of the study of Sendai *H. autumnale*, we found that extracts of the rhizoma showed cytotoxic activity. In this communication we wish to report on the fractionation of the extracts, from which 2-methoxydihydrohelenalin, a first naturally occurring pseudoguaianolide, was isolated.



Two major sesquiterpenes II and IV were isolated from the acetone extract of the fresh rhizoma in 0.001% and 0.006% yields, respectively. I and helenium lactone were not detected in the rhizoma. IV, $C_{15}H_{18}O_4$, mp 169-172°, $[\alpha]_D^{25}$ -89.4° ($CHCl_3$), was identical with helenalin[3]. Helenalin was reported to have highly cytotoxic[4] as well as antineoplastic activities[5,6] *in vivo* and *in vitro*. II, mp 182-183°, $[\alpha]_D^{25}$ +108.9° ($CHCl_3$), has the molecular formula $C_{16}H_{22}O_5$ (m/e 294). The IR spectrum displayed bands at 3530 cm^{-1} (hydroxyl), 1765 cm^{-1} (γ-lactone), 1730 cm^{-1} (five membered ketone) and 1660 cm^{-1} (conjugated double bond). The NMR spectrum (in $CDCl_3$, 100 MHz) of II revealed the presence of the α-methylene-γ-lactone group appearing as the typical low field doublets (J=2.6 Hz) at 5.82 and 6.32 ppm. Each one tertiary methyl, secondary methyl and methoxy groups appeared at 0.96, 1.18 (doublet, J=6.0 Hz) and 3.25 ppm, respectively. A doublet (J=4.8 Hz) at 3.10 ppm and a double doublet (J_1=2.8 Hz, J_2=4.8 Hz) at 4.28 ppm were assigned to the  $-\overset{|}{\underset{|}{C}}-\underset{\underset{\underline{OH}}{|}}{\underline{H}\!\!CH}-\overset{|}{C}H-$ grouping, because the former signal disappeared and the latter double doublet converted to a doublet (J=2.6 Hz) on addition of D_2O. Extensive double resonance experiments led to the following assignment of the pseudoguaianolide ring system: δ 3.96 (t, J=4.5 Hz, H-2), 4.28 (dd, J_1=2.8 Hz, J_2=4.8 Hz, H-6), 3.52 (m, H-7), 4.90 (m, H-8), 5.82 (d, J=2.6 Hz, H-13a), 6.32 (d, J=2.6 Hz, H-13b), 1.18 (d, J=6.0 Hz, H-14), 0.96 (s, H-15), 3.10 (d, J=4.8 Hz, -OH), 3.25 (s, -OMe). II gave a monoacetate III, $C_{18}H_{24}O_6$, mp 220-221°. NMR ($CDCl_3$) δ: 2.00 (s, -COMe), 5.24 (d, J=2 Hz, H-6).

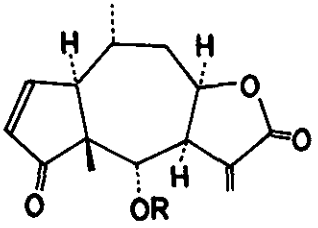
The CD spectrum of II showed negative Cotton effect at 260 nm ([θ] -4241) due to the $n\rightarrow\pi^*$ transition of a *cis*-fused α-methylene-γ-lactone chromophore[7], which was in accordance with small coupling constant of 13-proton to 7-proton (J=2.6 Hz)[8,9].

These spectral data allowed assignment as 2-methoxydihydrohelenalin II. II was synthesized from IV according to the literature[10], and identified by comparing with the natural material. II was first isolated from natural source.



II: R=H

III: R=Ac

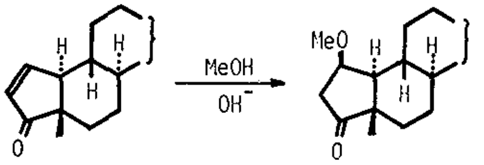


IV: R=H

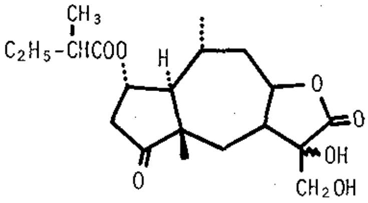
V: R=Ac

Tentative assignment of the configuration at C-2 was based upon the following evidence. Androst-15-en-17-ones VI, which have an analogous structural environment, gave the 15β-substituted derivatives VII[11], stereospecifically. The proton of the corresponding methine (15α-H; 2β-H) in VII and florigradin VIII was reported to exhibit triplet (J=5 Hz)[11] and triplets of doublet (J=8 and 7 Hz)[12], respectively. The C-2 proton of II appeared triplet (J=4.5 Hz) at 3.96 ppm, which suggested the presence of the β-methoxy group. The acetate III gave helenaline acetate V, $C_{17}H_{20}O_5$, mp 185-187°, on treatment with hydrochloric acid.

The structures of other minor components are under the investigation.



VI VII



VIII

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