PICROHELENIN, A NEW CYTOTOXIC AND BITTER PSEUDOGUAIANOLIDE FROM SENDAI *HELENIUM AUTUMNALE* L.

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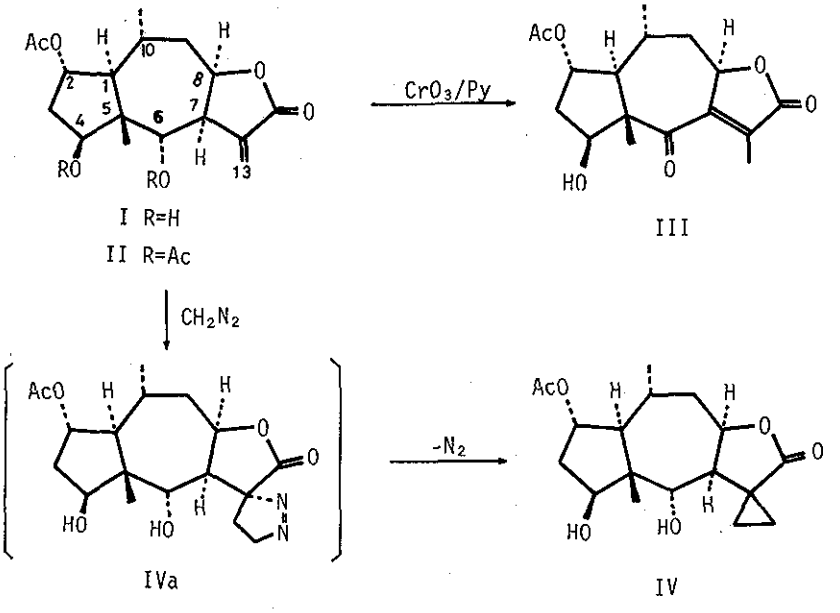
Picrohelenin, a cytotoxic and intensely bitter pseudoguaianolide from the epigeal parts of Sendai *Helenium autumnale* L. (Compositae) has been established to be the structure I. The taste threshold of picrohelenin in man is 1.92×10^{-7}– 3.7×10^{-7} *M*. Picrohelenin proved to be inhibitory to tissue culture HeLa cells and its ID_{50} was 1.5 μg/ml.

Helenium autumnale L. (Compositae) is a bitter herb of North American origin. Recently, several of the constituents of American *H. autumnale* have been reported to exhibit cytotoxic or antitumor activity[1,2]. In this communication we wish to report on the isolation and structural elucidation of a new cytotoxic and bitter pseudoguaianolide named picrohelenin from Sendai *H. autumnale*.

Picrohelenin (I), mp 170°, $C_{17}H_{24}O_6$, was isolated from the methanol extract of the epigeal parts by partition and column chromatography. I displyed IR bands at 3500 (hydroxyl), 1700 (γ-lactone), 1725 (acetyl) and 1655 cm^{-1} (conjugated double bond) and a pair of low field doublets (J≈2 Hz) in the NMR spectrum at 5.88 and 6.36 ppm, characteristic of an α-methylene-



I R=H
II R=Ac

IVa

III

IV

Scheme 1

γ-lactone grouping. In addition, I exhibited each one secondary methyl, tertiary methyl and acetyl methyl signals at 1.08 (doublet, J=7 Hz), 1.10 and 2.07 ppm, respectively. A doublet of doublets at 3.74 ppm (1H, J_1=4 Hz, J_2= 12 Hz) which collapsed to a doublet (J=12 Hz) when D_2O was added, a triplet at 4.37 ppm (1H, J=9 Hz), a multiplet (1H) at 4.74 ppm and a triplet of doublets (1H, J_1<2 Hz, J_2=9 Hz) at 4.94 ppm were unequivocally assigned to the protons on C_6, C_4, C_8 and C_2 by the addition of a shift reagent and/or the aid of double resonance technique.

The presence of two free hydroxyl groups on the C_4 and C_6 carbons was evidenced by the formation of acetate (II), mp 171°, $C_{21}H_{28}O_8$, in which signals attributed to methine protons on the carbons bearing the hydroxyls were shifted downfield, as expected.

When I was treated with chromium trioxide in pyridine, a monoketolactone (III), mp 173-174°, $C_{17}H_{22}O_6$, was formed. The structure of III was deduced

from inspection of its UV maxima [241 nm (ε 10000) and 316 nm (ε 1000)], IR (3550, 1755, 1740(sh), 1720(sh) and 1670 cm^{-1}), NMR (disappearance of the doublet of doublets at 3.74 ppm and synchronous appearance of a singlet methyl signal at 2.18 ppm) and mass (m/e 322) spectra (Scheme 1). Conversion of I to III definitely indicates that one of the hydroxyl groups is situated at C_6 in the pseudoguaianolide nucleus. In the NMR spectrum of III a doublet of sharp doublets at 4.45 ppm with coupling constants of 8.5 Hz and 11.5 Hz, which is the typical X portion of an ABX pattern, was assignable to the proton at C_4 bearing the hydroxyl group. A doublet of doublets (J_1=5 Hz, J_2=9 Hz) at 4.90 ppm was attributable to the proton at C_2 bearing the acetoxyl group.

The CD spectrum of I showed a negative Cotton effect at 259 nm ([θ] -4076) due to the n→π* transition of a *cis*-fused α-methylene-γ-lactone chromophore[3], which is in fair agreement with small allylic coupling of the C_{13}-proton (J≈2 Hz)[4,5].

The configuration of I was determined on the basis of the observation of the nuclear Overhauser effects (NOE) and the coupling constants between the respective protons. When the singlet methyl signal was saturated (added equimolar of $Eu(fod)_3$ in $CDCl_3$ solution), 24% of the NOE at the C_{10}-methine proton but the lack of an observable NOE at the C_4-methine proton were found. These facts lead us to conclude that picrohelenin has the structure I with absolute configuration as shown.

Picrohelenin is responsible for the intensely bitter taste and cytotoxicity of Sendai *H. autumnale*. Of chemotaxonomical interest the Sendai speciments contain no detectable amount of helenalin which is an usual constituent of American *H. autumnale*[6]. Sensory evaluation of the bitterness are given in Table I. The taste threshold of picrohelenin in man is doses of 1.92×10^{-7}–3.7×10^{-7} *M* which are below the threshold of quinine sulfate (3×10^{-6} *M*)[7].

Table I Concentrations of Isobitter Solutions of Picrohelenin and Caffeine

	Molarity	Relative Bitterness
Picrohelenin	7.72×10^{-7}	2670
Caffeine	2.06×10^{-3}	1

The α-methylene-γ-lactone moiety is allowable for retention of the bitter taste[8], because methylenation of I to α-cyclopropyl γ-lactone (IV), mp 255-258°, $C_{18}H_{26}O_6$, having an analogous chromophore, abolished its bitterness (Scheme 1).

Several α-methylene-γ-lactone derivatives have recently gained attention as potential experimental tumor inhibitors[9]. These compounds have been known to display cytotoxicity, which was ascribed to their cysteine scavenging properties. Picrohelenin showed growth inhibitory activity against HeLa cells in culture. The ID_{50} was 1.5 μg/ml[10] which was approximately as twice cytotoxic as bleomycin A_2.

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