CONSTITUENTS OF *HELENIUM AMARUM*. III. ISOLATION AND CHARACTERIZATION OF ISO-HELENIAMARIN, A NEW SESQUITERPENE LACTONE

MAHMOUD A. ELSOHLY, ALPANA S. SHARMA, and CARLTON E. TURNER

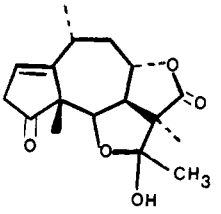
Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi, University, Mississippi 38677

Extracts of *Helenium amarum* (Bitterweed) leaves were shown to have analgesic activity. In addition, cytotoxic properties (KB Cells) and antitumor activities (*in vivo* P388) were observed. In a previous communication (1) we reported the phytochemical investigation of the ethanolic extract of the leaves which resulted in the isolation of the sesquiterpenes tenulin, aromaticin, amaralin, mexicanin I and heleniamarin. In this communication we report a new sesquiterpene lactone, iso-heleniamarin, from the same extract.

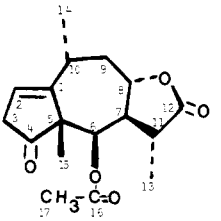
RESULTS AND DISCUSSION

The concentrated ethanol extract of the leaves of *H. amarum* was partitioned between chloroform and water and the chloroform fraction was then partitioned between *n*-hexane and 10% aqueous methanol. The aqueous methanol fraction was chromatographed on a silica gel column and elution was started with chloroform followed by chloroform-methanol mixtures. Elution with 7.5% methanol in chloroform afforded a fraction which upon rechromatography on silica gel G column with 30% ether in benzene as eluant, gave a light yellow oil which showed a single spot on tlc. Crystallization of this yellow oil was extremely difficult but was accomplished once from a hexane-ether mixture of unknown composition as colorless rectangular crystals, mp 124–126°. The ir spectrum showed three carbonyl groups at 1790, 1760, and 1745 cm^{-1}. The mass spectrum showed a molecular ion at *m/z* 306 (4%) for $C_{17}H_{22}O_5$ (HRMS). The presence of a base peak at *m/z* 264 (100%) and the ion at *m/z* 246 (5%) [(M^+-42) and (M^+-60), respectively] indicated the possibility of an acetoxy functional group. This was supported by the peak in the ^{1}H-nmr spectrum at δ 2.23 (3H, s) and the carbonyl absorption in the ir spectrum (1745 cm^{-1}). In addition, the ^{1}H-nmr spectrum showed three aliphatic methyls [δ 1.01 (s, 3H), 1.18 (d, J = 7 Hz, 3H) and 1.37 (d, J = 7Hz, 3H)], a proton on oxygenated carbon [δ 4.53 (m, 1H)], and two downfield protons at δ 5.77 (s, 1H) and δ 6.05 (br; s, 1H). The fact that only one of these downfield protons is olefinic was shown in the ^{13}C-nmr spectrum. In the olefinic/aromatic region there was only a singlet at δ 153.7 and a doublet at δ 119.1 in the off-resonance spectrum. The three carbonyl functions were found in the ^{13}C-nmr at δ 215.8, 177.2, and 170.2.

These data indicated a pseudoguaianolide sesquiterpene lactone with one acetoxy group, a trisubstituted double bond and a cyclopentenone ring. The presence of the double bond β,γ to the keto group was indicated from the ir band (1790 cm^{-1}) and the trisubstituted double bond. These structure features suggested a relationship to heleniamarin (**1**) (2) similar to that of isotenulin to tenulin. This was proven to be the case by chemical correlation. Thus, treatment of **1** with 10% sodium carbonate resulted in the formation of iso-



[1]



[2]

heleniamarin (**2**) which was identical in all respects (ir; ^{1}H-nmr, ms, tlc, and [α]D) to the natural product. This is the first reported isolation and structure of iso-heleniamarin. Iso-heleniamarin was inactive in the analgesic and antitumor screens.

A model of iso-heleniamarin showed that the angle between the protons on carbon 6 and 7 was almost 90° resulting in a singlet in the ^{1}H-nmr at δ 5.77. It is also important to point out that the chemical shift of the methyl group of acetate in the ^{1}H-nmr was at a rather low field (δ 2.23) which was confusing in the initial stages of the structure work.

EXPERIMENTAL[1]

PLANT MATERIAL, EXTRACTION AND FRACTIONATION OF H. AMARUM LEAVES, AND COLUMN CHROMATOGRAPHY OF RESIDUE E (COLUMN A).—These were previously described in an earlier report (1).

ISOLATION OF ISO-HELENIAMARIN (**2**).—Elution of Column A with 7.5% methanol in chloroform mixture resulted in a fraction (872 mg.) which showed one major component by tlc, Rf 0.61 [benzene-ether (1:1)]. This fraction was rechromatographed on a processed silica gel (35 g) column (1.8 x 32 cm) packed in 30% ether in benzene. Elution with the same solvent resulted in a fraction containing pure iso-heleniamarin (386 mg) as a pale yellow oil. When crystallization was accomplished, after several trials, from a hexane-ether mixture rectangular, colorless crystals of iso-heleniamarin (**2**) were obtained; mp 124–126°; $[\alpha]^{24}D$ +1.8° (*c* 0.25, $CHCl_3$); ir max (KBr) 1790, 1760, 1745, 1460, 1390, 1240, and 987 cm^{-1}; ms, M+*m/z* 306 (4%) for $C_{17}H_{22}O_5$ (obs. 306.14714, calc. 306.14673) and other ions at *m/z* 264 (100%), 246 (5%) and 222 (6%); ^{1}H-nmr, (60 MHz, $CDCl_3$) δ 1.01 (s, 3H), 1.18 (d, *J*=7Hz, 3H), 1.37 (d, *J*=7Hz, 3H), 2.23 (s, 3H), 4.53 (m, 1H), 5.77 (s, 1H) and 6.05 (br, s, 1H); ^{13}C-nmr (15.03 MHz, $CDCl_3$) δ 215.8 (s), C-4; 177.2 (s), C-12; 170.1 (s), C-16; 153.7 (s), C-1; 119.1 (d), C-2; 76.9 (d), C-8; 69.4 (d), C-6; 59.3 (s), C-5; 53.0 (d), C-7; 43.6 (t), C-9; 40.1 (t), C-3; 38.5 (d), C-11; 28.2 (d), C-10; 20.5 (q), C-13*; 20.0 (q), C-17*; 19.6 (q), C-14*; and 12.3 (q), C-15.

[1]See reference #1 for equipment used. ^{13}C-nmr spectra were recorded on JEOL 60 FX instrument in $CDCl_3$ with tetramethylsilane as internal standard.

*Assignments may be interchanged. The C-13 nmr assignments were based on multiplicities and chemical shift data as compared to those reported for other related sesquiterpenes (3).

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