CONSTITUENTS OF *HELENIUM AMARUM*. II. ISOLATION AND CHARACTERIZATION OF HELENIAMARIN AND OTHER CONSTITUENTS

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ABSTRACT.—Investigation of the ethanolic extract of the leaves of *H. amarum* resulted in the isolation of the sesquiterpenes aromaticin (**1**), mexicanin I (**2**), tenulin (**4**) and amaralin (**5**), and the flavone hispidulin (**6**). In addition, a new sesquiterpene lactone [heleniamarin, (**3**)] was isolated and its structure determined by spectral and chemical means and proven by x-ray crystallography.

Helenium amarum (Rafin.) H. Rock., commonly known as bitterweed or sneezeweed, is a widely distributed plant in the southern and eastern parts of the United States. The plant is known for its toxicity to livestock (1, 2) and for causing bitterness in milk obtained from dairy animals grazing on it. The bitterness was found to be mainly due to the sesquiterpene lactone, tenulin (**4**), of which only about 0.1% is secreted unchanged in the milk (3). Practitioners of folk medicine have used the plant for different purposes including induction of sneezing to clear nasal passages and making tea to break fevers and to reduce pain during childbirth. Phytochemical investigation of *H. amarum* resulted in the isolation of a number of sesquiterpene lactones of which amaralin (**5**) was found to have analgesic properties (4).

In an effort to screen Mississippian plants for biological activity, extracts from different plant parts of *H. amarum* were prepared for testing. The ethanolic extracts of the leaves of the plant were shown to possess analgesic, cytotoxic (KB cells), and antitumor (*in vivo* P 388) properties. Because of the apparent activity of the crude extracts of the leaves, fractionation and phytochemical investigation of these extracts was warranted.

Dried powdered leaves of *H. amarum* were extracted with ethanol, and the concentrated extract was partitioned between chloroform and water. The chloroform fraction was then partitioned between n-hexane and 10% aqueous methanol. The residue obtained from the aqueous methanol fraction was chromatographed on a silica gel column. Elution with chloroform gave a fraction which, upon crystallization from chloroform-benzene, afforded a crystalline compound, mp 223–225°, identified as aromaticin (**1**). Aromaticin has been previously ioslated from *H. amarum* (4).

Elution with 5% methanol in chloroform followed by evaporation of the solvent and crystallization from chloroform-benzene resulted in the isolation of mexicanin I (**2**). Mexicanin I was previously isolated from *H. mexicanum* (5).

Column chromatography of the mother liquor of Mexicanin I on silica gel G using benzene-ether (1:2) gave a new sesquiterpene lactone named helenimarin (**3**), mp 151–153° (diethyl ether), $C_{17}H_{22}O_5$ (hrms). The ^{1}Hnmr spectrum indicated the presence of four methyl groups [δ 1.22(S), 1.23 (d, $J=7$Hz), 1.28(S) and

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1.50(S)], one olefinic proton (δ 5.78) and two protons on oxygenated carbons [δ 4.07 (d, J=6.5Hz) and 5.03 (br.t, J=10Hz)]. The presence of a tertiary hydroxy group was indicated by the presence of one exchangeable proton at δ 3.60 and the failure of the compound to acetylate with acetic anhydride and pyridine. The similarity of the spectral data of heleniamarin with those of tenulin (**4**) coupled with the presence of a trisubstituted double bond indicated that the only difference could be in the presence of a β-γ unsaturated ketone in heleniamarin. This was further substantiated by the presence of two triplets in the ^{1}Hnmr of heleniamarin at δ 2.82 and 2.97 (J=2Hz) assigned to the methylene protons α to the keto group. Thus tenulin was converted to heleniamarin by passing HCl gas in a chloroform solution of the tenulin (6) followed by preparative tlc. Finally, the structure and stereochemistry of heleniamarin was proven by x-ray crystallography (7). Other examples of sesquiterpenes with β-γ unsaturated ketones include mexicanin A, isolated from *H. mexicanum* (8) and linifolin B, isolated from *H. linifolium* (9).

The residue obtained from the 7.5% methanol in the chloroform fraction was crystallized from chloroform-benzene to give tenulin as the major sesquiterpene lactone (4% of the dry weight of the leaves). Tenulin was previously isolated

TABLE 1. ED_{50} of certain constituents of *H. amarum* (*in vitro* KB cells).

Compound		ED_{50} (μg/ml)
Aromaticin	(**1**)	2.0
Mexicanin I	(**2**)	1.9
Heleniamarin	(**3**)	M100
Tenulin	(**4**)	26.0
Amaralin	(**5**)	4.9
Hispidulin	(**6**)	96

from *H. amarum* and was shown by Herz *et al.* (10) and Herz and Sharma (11) to be an epimeric mixture sesquiterpene lactone of the pseudoguianolide group and was found recently to be the main component in bitterweed responsible for the bitter taste of the milk obtained from cows grazing on the plant (2). Column chromatography of the mother liquor of tenulin on silver nitrate impregnated silica gel G (5%) using benzene-ether (1:2) afforded two compounds, an unidentified compound mp 101–105° and amaralin (**5**). Amaralin was once isolated from *H. amarum* and found to be analgesic properties; but attempts to reisolate the compound from other batches of the plant material were unsuccessful (4).

Continuous elution with 7.5% methanol in chloroform followed by evaporation of the solvent and crystallization of the residue from methanol afforded the flavonoid hispidulin (**6**). Hispidulin was previously isolated from *Ambrosia hispida* (12).

Table 1 shows the ED_{50} of some of the compounds isolated from *H. amarum* leaves using the KB *in vitro* assay. It is clear from the data in table 1 that only the sesquiterpenes with exocyclic methylene groups, α,β to the lactone ring or α,β unsaturated cyclopentenones show cytotoxic activity. The cytotoxicity of sesquiterpene lactones and their structure activity relationships were investigated by Kupchan *et al.* (13). A mechanism for inhibition of cancer growth by the sesquiterpenes tenulin, helenalin, and other related compounds was proposed by Hall *et al.* (14).

EXPERIMENTAL[2]

PLANT MATERIAL.—*Helenium amarum* (Rafin.) H. Rock. was collected in August 1975 in the University, Mississippi area. The plant material was authenticated by Professor Maynard W. Quimby, Department of Pharmacognosy, School of Pharmacy, University of Mississippi. A herbarium specimen is deposited in the herbarium, Department of Pharmacognosy, School of Pharmacy, University of Mississippi. The different parts of the plant were separated, dried, and powdered.

EXTRACTION AND FRACTIONATION OF *H. amarum* LEAVES.—The dried powdered leaves (2 Kg) of *H. amarum* were extracted by percolation with 95% ethanol (70 liters). Removal of the solvent by evaporation under reduced pressure at 40° left a thick residue A (451 g). Residue A was partitioned between 1 liter of water and 4 X 2 liters chloroform. The aqueous layer gave 150 g of residue B upon evaporation while the combined chloroform extracts afforded 325 g of residue C. Residue C was then partitioned between 10% aqueous methanol (1 liter) and hexane (4 X 2 liters). The aqueous methanol fraction afforded 203 g upon evaporation of the solvent (residue E) while the hexane fraction gave 58 g residue D.

COLUMN CHROMATOGRAPHY OF RESIDUE E (Column A).—Residue E (100 g) was dissolved in 125 ml of chloroform, and the solution was then applied on top of a silica gel 60[3] column (8 X 123 cm) (2.27 Kg) packed in chloroform. Elution was started with chloroform, and the polarity of the solvent was gradually increased by the addition of methanol. Fractions of 100 ml were collected and combined according to the their tlc similarities.

ISOLATION OF AROMATICIN (**1**).—A chloroform fraction of column A (3.5 g) was crystallized from chloroform-benzene to give 2.4 g of colorless rhomboid crystals of aromaticin, mp 223–225°; $[\alpha]^{25}D+21.2$ (*c* 0.25, $CHCl_3$); ir νmax (KBr) 1765 and 1700 cm^{-1}; ms, M+ *m/e* 246, for $C_{15}H_{18}O_3$; ^{1}Hnmr δ ($CDCl_3$) 1.23 (d, J=6.5Hz, 3H), 1.33 (s, 3H), 4.55 (m, 1H), 5.55 (d, J=3Hz, 1H), 6.20 (d, J=3Hz, 1H), 6.15 (d,d J=6 Hz, 1H) and 7.55 (d,d J=6 Hz and 2 Hz, 1H).

ISOLATION OF MEXICANIN I (**2**).—Crystallization of a 5% methanol in chloroform fraction of column A from benzene-chloroform gave 130 mg of colorless crystals of Mexicanin I, mp 241–246° (Lit. 257–260) (5); ir νmax (KBr) 3500, 1750 and 1700 cm^{-1}; ms M+ *m/e* 262 for $C_{15}H_{18}O_4$; ^{1}Hnmr δ(DMSO-d_6) 1.07 (s, 3H), 1.13 (d, J=8 Hz, 3H), 4.40 (m, 1H) 4.72 (m, 1H), 5.00 (d, J=6 Hz, 1H exchangeable), 5.64 (d, J=3 Hz, 1H), 6.15 (d,d J=6 Hz and 2 Hz, 1H), 6.17 (d, J=3 Hz, 1H) and 7.77 (dd, J=6 Hz and 2 Hz).

ISOLATION OF HELENIAMARIN (**3**).—The mother liquor of Mexicanin I was chromatographed on a silica gel G Column (500 g) (column B) using benzene-ether (1:2) as the solvent system. Fractions of 20 ml were collected. Fractions 15–27 were combined, and the solvent was evaporated to give 3.1 g of yellowish colored oily residue which, upon repeated crystallization from diethyl ether, gave prismatic crystals of heleniamarin (1.93 g), mp 151–153°; $[\alpha]^{25}D+58.5$ (*c* 0.25, $CHCl_3$); ir νmax (KBr) 3450 (br) 1755 (sh) and 1750 cm^{-1}; uv –max (MeOH) 278 nm (ϵ=66), 226 (ϵ=335); ms M+ *m/e* 306 (obs. 306.14708, Calc. 306.14673) for $C_{17}H_{22}O_5$ (5%) and other ions at 283 (3), 264 (30), 246 (3) and 235 (100); ^{1}Hnmr δ ($CDCl_3$) 1.22 (s, 3H), 1.23 (d, J=7 Hz), 1.28 (s, 3H), 1.50 (s, 3H), 2.82 (t, J=2 Hz, 1H), 2.97 (t, J=2 Hz, 1H), 3.60 (s, 1H, exchangeable with D_2O), 4.07 (d, J=6.5 Hz, 1H), 5.03 (br.t, J=Hz, 1H) and 5.78 (br s, 1H).

CONVERSION OF TENULIN (**4**) TO HELENIAMARIN (**3**).—A solution of 100 mg of **4** in 10 ml dry chloroform was saturated with dry HCl gas at 0° and then left overnight. Removal of the solvent gave an oil which showed two major spots on silica gel tlc plates developed by a benzene-ether (1:1) system. Preparative tlc of the reaction mixture was done, and the band corresponding to **3** scraped and extracted with chloroform-methanol. Further purification was carried out on silver nitrate impregnated silica gel (5%) column with a benzene-ether (1:1) solvent system. The conversion product was found to be identical with the isolated **3** (ir, ms and ^{1}Hnmr).

X-RAY CRYSTALLOGRAPHY OF **3**.—The structure of **3** was finally proven by x-ray crystallography using 1900 reflections above background level collected at −165°. Heleniamarin crystallizes in the space group $P2_1$, with cell dimensions (t=−165°): a=8.843 (8) A°, b=7.991 (7) A°, c=10.938(10) A°, β=96.41 (7). For details of the x-ray crystallography analysis of **3**, see reference 7.

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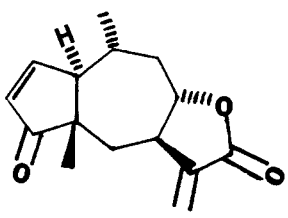
[2]Melting points were determined on Koffler's hot stage apparatus and are uncorrected. The uv spectra were obtained on a Beckman Acta-III spectrophotometer and the ir spectra were determined on a Beckman IR-33 recording spectrophotometer in KBr pellets. ^{1}Hnmr spectra were recorded in $CDCl_3$ on a Jeol-C60-HL instrument with tetramethylsilane as internal standard mass spectra were taken with a high resolution DuPont 21-492 mass spectrometer. Optical rotations were measured on a Perkin-Elmer 141 polarimeter.

[3]Brinkman.

ISOLATION OF TENULIN (4).—Continuous elution of column A with 5% to 7.5% methanol in chloroform followed by evaporation of the solvent afforded a yellowish brown residue (65.7 g). Repeated crystallization from chloroform-benzene gave 23.8 g of colorless prisms of tenulin, mp 188–189°; $[\alpha]^{25}D-14.4$ (*c* 0.25, $CHCl_3$); ir νmax (KBr) 3425, 1775 and 1720 cm^{-1}; ms M+ *m/e* 306 for $C_{17}H_{22}O_5$; ^{1}Hnmr δ($CDCl_3$) 1.23 (s, 3H), 1.30 (d, *J*=6 Hz, 3H), 1.36 (s, 3H), 1.56 (s, 3H), 4.10 (brs, 1H, exch.), 4.49 (d, *J*=6 Hz, 1H), 5.34 (dt, *J*=10 Hz and 3 Hz, 1H), 6.15 (dd, *J*=6 Hz and 2 Hz, 1H) and 7.68 (dd, *J*=6 Hz and 2 Hz, 1H).

ISOLATION OF AMARALIN (5).—The mother liquor of tenulin was chromatographed on a silver nitrate impregnated silica gel G (5%) column (400 g) (column C) with benzene-ether (1:2) as the solvent system; 20 ml fractions were collected. Fractions 27–37 were combined, evaporated, and the residue obtained crystallized from benzene to give colorless needles of amaralin (35 mg), mp 195–198°; ir νmax (KBr) 3425 and 1760 cm^{-1}; ms M+ *m/e* 264 for $C_{15}H_{20}O_4$; ^{1}Hnmr δ ($CDCl_3$) 1.13 (s, 3H), 1.23 (d, *J*=6 Hz, 3H), δ 3.38 (s, 2H), 3.75 (d, *J*=5 Hz, 1H), 4.30 (dt, *J*=10 Hz and 2 Hz, 1H), 5.43 (d, *J*=3 Hz, 1H) and 6.15 (d, *J*=3 Hz, 1H).



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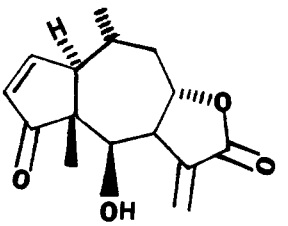

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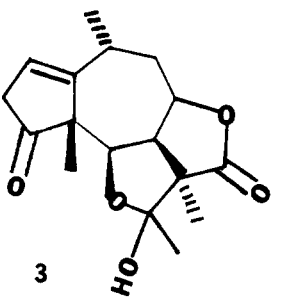

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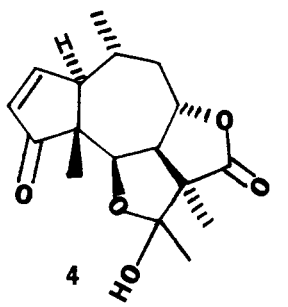

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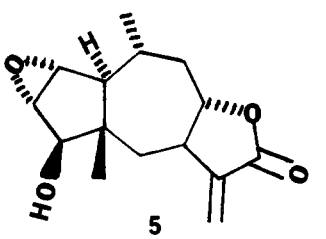

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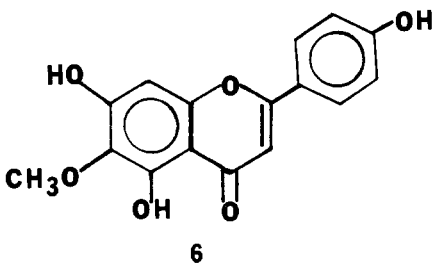

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ISOLATION OF HISPIDULIN (6).—Continuous elution of column A with 7.5% methanol in chloroform afforded a fraction (7.5 g) which was washed with chloroform. The residue crystallized from methanol to give yellow needles of hispidulin (504 mg), mp 285–287° dec.; ir νmax (KBr) 3350, 1660, 1620, 1590 and 1565 cm^{-1}; uv –max (MeOH) 220 nm (log ε 4.14), 274 (4.09), 333 (sh, 4.18) and 337 (sh, 4.18); λmax (0.1N KOH in MeOH) 228 nm (log ε 4.17), 274 (4.08), 325 (3.97), 385 (sh, 4.24) and 390 (sh, 4.25); ^{1}Hnmr δ (DMSO-D_6) 3.81 (s, 3H), 6.14 (s, 1H), 6.30 (s, 1H), 6.95 (d, *J*=9 Hz), 7.93 (d, *J*=9 Hz, 1H) and 13.00 (s, 1H, exchangeable with D_2O).

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LITERATURE CITED

1. J. M. Kingsbury, *Poisonous Plants of United States and Canada*, Prentice Hall, Inc., Englewood, N.J. 1964, p. 412.
2. J. W. Dollahite, L. D. Rowe, H. L. Kim and B. J. Camp, *Southwest. Vet.*, **26**, 135 (1972).
3. G. W. Ivie, D. A. Witzel and D. D. Rushing, *J. Agric. Food Chem.*, **23**, 845 (1975).
4. R. A. Lucas, S. Rovinski, R. J. Kiesel, L. Dorfman and H. B. MacPhillamy, *J. Org. Chem.*, **29**, 1549 (1964).
5. E. Dominguez and J. Romo, *Tetrahedron*, **19**, 1421 (1963).
6. W. Herz, M. V. Laksminathan and R. N. Mirrington, *Tetrahedron*, **22**, 1709 (1966).
7. T. Ottersen, B. Sorensen, M. A. Elsohly and C. E. Turner, *Acta Chem. Scand*, **B**, **32**, 79 (1978).
8. W. Herz, A. Romo de Vivar, J. Romo and N. Wiswanathan, *J. Am. Chem. Soc.*, **85**, 19 (1963).
9. W. Herz, *J. Org. Chem.*, **27**, 4043 (1962).
10. W. Herz, W. A. Rodhe, K. Rabindran, P. Jayaraman and N. Wiswanathan, *J. Am. Chem. Soc.*, **84**, 3857 (1962).
11. W. Herz, and R. P. Sharma, *J. Org. Chem.*, **40**, 2557 (1975).
12. W. Herz and Y. Sumi, *J. Org. Chem.*, **29**, 3438 (1964).
13. S. M. Kupchan, M. A. Eakin and A. M. Thomas, *J. Med. Chem.*, **14**, 1147 (1971).
14. I. H. Hall, K. H. Lee, E. C. Mar, C. O. Starnes and T. G. Waddell, *J. Med. Chem.*, **20**, 333 (1977).