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ANTITUMOR SESQUITERPENE LACTONES FROM *HELENIUM MICROCEPHALUM*: ISOLATION OF MEXICANIN-E AND STRUCTURAL CHARACTERIZATION OF MICROHELENIN-B AND -C*

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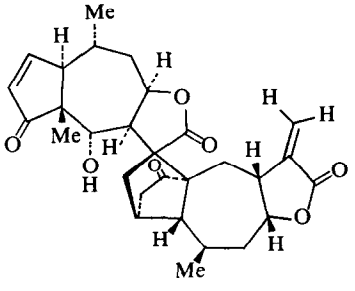
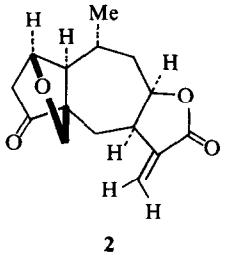
Key Word Index—*Helenium microcephalum*; mexicanin-E; microhelenin-B; microhelenin-C; X-ray; NMR; antitumor sesquiterpene lactones.

We reported recently [1, 2] on the structure determination of two new antitumor sesquiterpene lactones, microlenin (**1**) and microhelenin-A (**2**), which were isolated in addition to helenalin (**3**) from Texas *Helenium microcephalum*. Further investigations on the active fraction from the chloroform extract of this same plant have now led to the isolation of the previously known mexicanin-E (**4**) [3–5]‡ and characterization of another



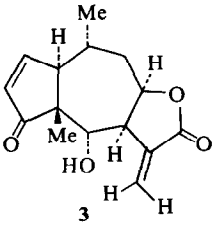
1

two new antitumor principles microhelenin-B (**5**) and -C (**6**). Mexicanin-E, microhelenin-B and -C showed significant (T/C ≥ 125%) inhibitory activity against Walker 256 carcinosarcoma in rats. *In vivo* activity was assayed by Dr. I. H. Hall, Department of Medicinal Chemistry, School of Pharmacy, University of North Carolina at Chapel Hill, by a literature method [6].



2

Mexicanin-E (**4**), $C_{14}H_{16}O_3$, mp 95–100° (from Et_2O–CCl_4), $[\alpha]_D^{26}$ −55.0° (c = 1.0, $CHCl_3$) (lit value [5] $[\alpha]_D^{22}$ −47°) shows IR bands (CCl_4) at 1777, 1667 (α-methylene-γ-lactone), 1712 and 1592 cm^{-1} (cyclopentenone). Although the signals in the ^1H-NMR spectrum (100 MHz, $CDCl_3$) accord well with those previously reported for **4**, the values found here for the chemical shifts and coupling constants do differ slightly. The pair of doublets occur at δ 5.70 (J = 1.5 Hz, H_a-13) and 6.23 (J = 1.5 Hz, H_b-13), the one-proton multiplet is at δ 4.64 (H-8), the two doublets of doublets are at δ 6.28 (J = 2.25, 6.0 Hz, H-3) and 7.83 (J = 2.25, 6.0 Hz, H-2),



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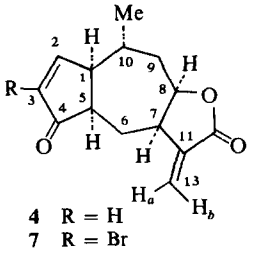

4 R = H
7 R = Br

and the three-proton doublet for the C-10 methyl group is at δ 1.19 (J = 6.0 Hz). The complete structure and relative stereochemistry were confirmed by a single-crystal X-ray analysis of **4** which crystallizes in the monoclinic system, space group $P2_1$, with a = 6.06(1), b = 15.72(1), c = 6.76(1) Å, β = 107.7(1)°, Z = 2. Solution of the crystal structure was effected by direct phase-determining procedures using MULTAN [7], and atomic positional and thermal parameters were refined by full-matrix least-squares calculations to R = 0.055 over 919 statistically significant [$I > 2.0\sigma(I)$] reflections from diffractometer measurements (Ni-filtered Cu-K_α radiation,

*Antitumor Agents. 22.

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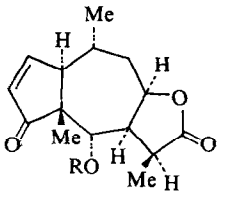
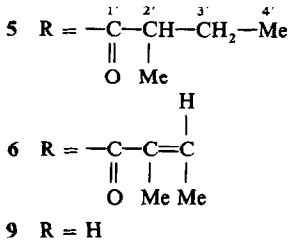
‡The X-ray co-ordinates listed in [3] correspond to an α-oriented C-10 methyl group as shown in **4** but the structural formulae in [3] and [4] represent the configuration as β, an error which has been perpetuated in much of the subsequent literature.

θ–2θ scans). Bond lengths and valency angles all lie close to accepted values and to those found for bromomexicanin-E (**7**) [3, 4]. The seven-membered ring in **4**, defined by endocyclic torsion angles $\omega_{1,5}$ 18.8, $\omega_{5,6}$ −80.7, $\omega_{6,7}$ 67.6, $\omega_{7,8}$ −37.6, $\omega_{8,9}$ 55.6, $\omega_{9,10}$ −87.9, $\omega_{1,10}$ 57.6° has a conformation approximately midway between C_s chair and C_2 twist-chair forms [8] and not significantly different from that in **7** where the corresponding torsion angles are 19, −82, 68, −36, 51, −86, and 60°. The co-occurrence of **4** with **1** and **3** suggests that **4** is the norpseudoguaianolide precursor, with the cyclopentenone ring in its enolic form, involved in a Diels–Alder type condensation with **3** which approaches from the α-face* of **4** to produce **1**.

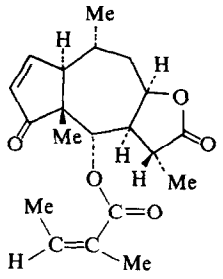




5 R = —C(=O)—CH(Me)—CH_2—Me

6 R = —C(H)(=O)—C(Me)=C(Me)

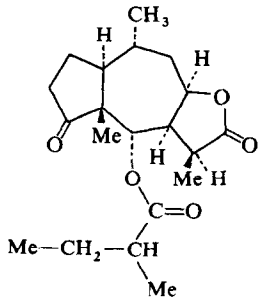
9 R = H



8

Microhelenin-B (**5**), mp 111–113° (from $CHCl_3$), *m/e* 348.1937(M^+), $[\alpha]_D^{28}$ −84.91 (*c* = 1.75, MeOH), has molecular formula $C_{20}H_{28}O_5$. The presence in **5** of a cyclopentenone ring system of the type found in brevilin-A (**8**) [9] was indicated by the appearance of IR bands at 1726 and 1556 cm^{-1} and was substantiated by the presence in the ^{1}H-NMR spectrum ($CDCl_3$) of the characteristic doublets of doublets at δ 7.79 (*J* = 2.3, 6.0 Hz, H-2) and 6.14 (*J* = 3.0, 6.0 Hz, H-3) as found in plenolin (**9**) [10]. Microhelenin-B lacks the lactone α-methylene group but has the corresponding α-methyl-γ-lactone ring [1783 cm^{-1}; δ 1.55 (*d*, *J* = 6.0 Hz, Me-11)]. Three-proton methyl group signals are seen at δ 1.08 (*s*, Me-5) 1.28 (*d*, *J* = 6.0 Hz, Me-10) and 1.08 (*d*, *J* = 7.5 Hz). The last of these is due to an Me group at C-2′ of a 2-methylbutyrate ester the presence of which is also evident from IR bands at 1741, 1230, and 1190 cm^{-1}, and the characteristic mass peak at *m/e* 85.0655 [$MeCH_2CH(Me)C{=}O^+$]. The similarities of the ^{1}H-NMR spectral patterns for the protons at C-6 (*br s*, $W_{\frac{1}{2}}$ = 3.0 Hz, at δ 5.50) and C-8 (*br t*, *J* = 6.0 Hz, at δ 4.80) of **5** and **8** as well as plenolin tiglate, prepared from plenolin (**9**) and tigloyl chloride in C_5H_5N–C_6H_6, suggested that the ester side chain is attached at C-6 and the lactone ring is fused at C-8. The foregoing evidence leads to formulation of structure **5** for microhelenin-B exclusive of stereochemistry.

Microhelenin-C (**6**), $C_{20}H_{26}O_5$, *m/e* 346.1782 (M^+), $[\alpha]_D^{23}$ −85.0° (*c* = 1.30, MeOH), was isolated in only a small quantity as a gum by Si gel column chromatography and preparative TLC (Si gel impregnated with $AgNO_3$). Compound **6** gives IR (CCl_4), ^{1}H-NMR ($CDCl_3$), and MS data very similar to those of microhelenin-B (**5**), indicating the presence of a cyclopentenone ring [1725 and 1583 cm^{-1}; δ 7.79 (*dd*, *J* = 2.0, 6.0 Hz, H-2) and 6.06 (*dd*, *J* = 3.0, 6.0 Hz, H-3)], an α-methyl-γ-lactone ring fused at C-8 [1782 cm^{-1}; δ 1.50 (*d*, *J* = 6.0 Hz, Me-11), 4.78 (*m*, H-8)], a tigloyl group attached at C-6 [1725, 1651, 1260, and 1182 cm^{-1}; δ 6.64 (*brm*, H-3′), 1.73 (*br s*, Me-2′), 1.74 (*br d*, *J* = 7.0 Hz, Me-3′) and 5.50 (*br s*, H-6); *m/e* 246.1252 [M-MeCH=C(Me)-COOH], 83.0494 [$MeCH{=}C(Me)CO^+$] and additional methyl group signals at δ 1.05 (*s*, *Me*-5) and 1.25 (*d*, *J* = 6.0 Hz, Me-10). To confirm further the structure of **6**, its identity with synthetic plenolin tiglate was established by direct comparison (TLC, superimposable IR, NMR and MS).



10

Catalytic hydrogenation of **5** and **6** with 10% Pd–C in EtOH afforded the same amorphous compound **10**, $C_{20}H_{30}O_5$, *m/e* 350.2091 (M^+), IR 1782 (γ-lactone), 1749 1740(sh), and 1193 cm^{-1} (ester and cyclopentanone); the ^{1}H-NMR spectrum lacked the characteristic olefinic and vinylic proton signals seen for **6**. Compound **10** also revealed ^{1}H-NMR signals at δ 0.84 (3H, *s*, Me-5), 1.06 (6H, *d*, *J* = 7.0 Hz, Me-10 and Me-2′), 1.47 (3H, *d*, *J* = 7.5 Hz, Me-11), 2.74 (1H, *dd*, *J* = 6.0, 10.0 Hz, H-7), 3.03 (1H, *dq*, *J* = 7.5, 10.0 Hz, H-11), 4.71 (1H, *br t*, *J* = 6.0 Hz, H-8) and 5.38 (1H, *s*-like, $W_{\frac{1}{2}}$ = 2.5 Hz, H-6) which are strikingly similar to those of tetrahydrobrevilin-A [9] which differs only in the stereochemistry of the methyl at C-11. The assignment of a β-configuration to the C-11 methyl group in **10** is based upon direct comparison of its TLC, IR and NMR spectra with those of the synthetic hydrogenation product from plenolin tiglate. Further support for this assignment is obtained from the observation that hydrolysis of both **5** and **6** with 5% KOH–MeOH at room temp. for 3 hr led to mixtures which showed at least four spots on TLC one

* In accordance with accepted convention the C-7 reference substituent is defined to be β-oriented.

of which corresponds to plenolin (**9**). The preceding evidence leads definitively to **5** and **6**, respectively, for the structures and stereochemistries of microhelenin-B and -C.

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A NEW PHORBOL TRIESTER FROM THE LATICES OF *EUPHORBIA FRANKIANA* AND *E. COERULESCENS*

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Key Word Index—*Euphorbia frankiana*; *E. coerulescens*; Euphorbiaceae; diterpene; 12-*O*-iso-butyl-phorbol-13-acetate 20-angelate.

Euphorbia frankiana Berg. and *E. coerulescens* Haw. are both succulent African species of the subdivision *Polygonae* (subsection *Diacanthium*) of the genus *Euphorbia* [1]. A phytochemical investigation of species of this subdivision [2] indicated that the triterpene profile was uniform; however the diterpene profile was more complex [3]. The subdivision contains species which produce both tigliane, ingenane and daphnane diterpenes [3] but only the species *E. frankiana* and *E. coerulescens* have yielded phorbol to date [3], [4]. The latices of these plants are irritant to skin [5] and the major toxins which are esters of 12-deoxyphorbol have been described [6]. This communication describes the identification of a new cryptic irritant based upon phorbol, which was isolated from the methanol preserved fresh latex.

Water and methanol were removed from the preserved latices by reduced pressure distillation below 40°. The residue was exhaustively extracted with acetone, the acetone removed as before to yield a cream solid residue (40% w/w of dried latex). The crude acetone extract of *E. frankiana* had an irritant dose 50% (ID_{50}) [7] on mice ears of 3 μg/5 μl/ear and of *E. coerulescens* of 7 μg/5 μl/ear. The solid residues from acetone were partitioned between MeOH–H_2O and hexane followed by MeOH–H_2O and ether as previously described [8]. Residues from the ether phase were separated by column chromatography [8] on florosil into two fractions. The less polar fraction consisted of diesters of 12-deoxy-phorbol [9] together with smaller amounts of phorbol ester (**1**), and the polar fraction consisted of 12-deoxy-phorbol monoesters [8].

12-O-isobutyl-phorbol-13-acetate-20-angelate (**1**). *E. frankiana* contained 0.14% w/w of acetone-soluble residue: *E. coerulescens* contained 0.01% w/w of acetone-soluble residue. The non-polar fraction from column chromatography was subjected to PLC on silica gel G (500 μm layers) buffered at pH 7.0, using first $CHCl_3$-ether-C_6H_6 (1:3:3) (H R_f 60) and then after recovery $CHCl_3$–acetone–C_6H_6 (95:6:50) (h R_f20). Compound (**1**) which was still contaminated with esters of 12-deoxy-phorbol was further purified by partition chromatography using digol as stationary phase [10] and finally by repeated elution PLC on silica gel as before using C_6H_6–C_6H_{12}–ether–EtOAc (4:8:3:6). After elution a resin was obtained which produced one spot by analytical TLC (h R_f 67) in the above system. This substance exhibited a yellow colour in UV light after spraying with 60% aqueous H_2SO_4, and a pink colour in daylight after spraying with MeOH–H_2SO_4 (1:1) and heating. In the MS (**1**) exhibited an $M^{+\cdot}$ at *m/e* 558 ($C_{31}H_{42}O_9$) and fragment ions at *me* 498 (10%); 471 (14%); 470 (8%); 458 (10%); 452 (2%); 410 (35%); 398 (16%); 370 (40%); 310 (100%) and 292 (38%). Below the ion at *m/e* 292 the spectrum was similar to that of phorbol triacetate [3].