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MARGINATIN, A NEW GERMACRANOLIDE FROM *VERNONIA* SPECIES

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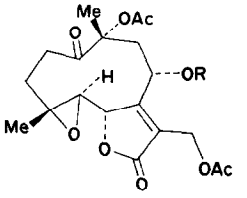
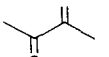
Key Word Index—*Vernonia marginata*; *V. fasciculata*; *V. arkansana*; Compositae; sesquiterpene lactone; germacranolide; marginatin.

Abstract—A new germacranolide, marginatin, has been isolated from three species of *Vernonia* from the central United States, *V. marginata*, *V. fasciculata* and *V. arkansana*, and its structure has been determined.

IN THE course of our biochemical systematic investigations of more than 45 species of *Vernonia* from throughout the Western Hemisphere,[1–3] a new germacranolide, which we have named marginatin (**1**), was found to be the major sesquiterpene lactone component of three species which occur in the central region of the United States: *V. marginata* (Torr.) Raf., range centered in West Texas; *V. fasciculata* Michx. and *V. arkansana*, DC, whose ranges are centered in the north central United States. Other closely related North American species were previously reported[4] to elaborate as their major sesquiterpene lactone constituent either glaucolide-A (**2a**) or B (**2b**); in some instances, marginatin occurs with (**2a**).



(**2a**) R =



(**2b**) R = Ac

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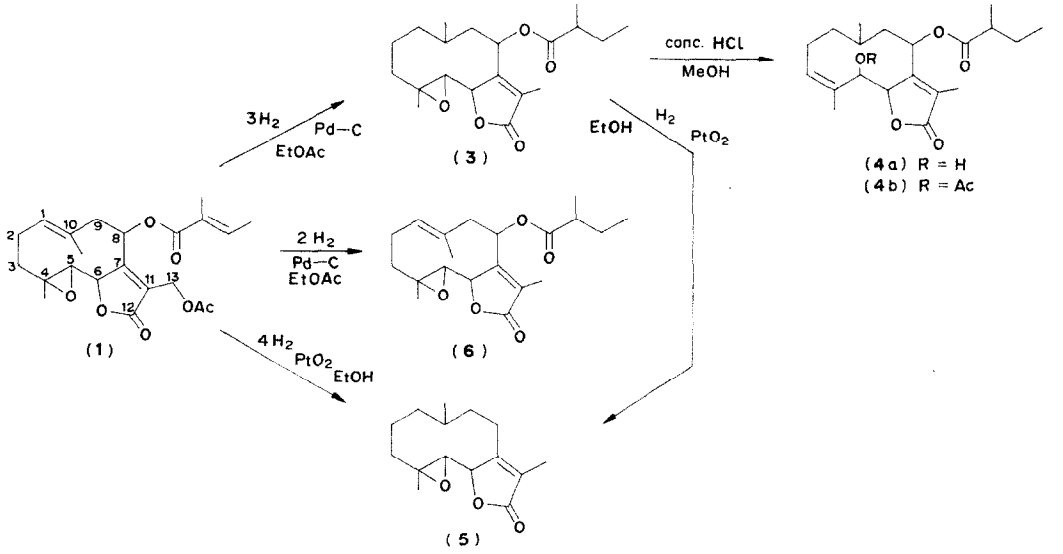
[1] PADOLINA, W. G. (1973) Ph.D. thesis, The University of Texas at Austin.

[2] ABDEL-BASET, Z. H., SOUTHWICK, L., PADOLINA, W. G., YOSHIOKA, H., MABRY, T. J. and JONES, JR., S. B. (1971) *Phytochemistry* **10**, 2201.

[3] MABRY, T. J., ABDEL-BASET, Z. H., PADOLINA, W. G. and JONES, JR., S. B. (1974) *Biochem. Syst. and Ecol.*, prepared.

[4] PADOLINA, W. G., YOSHIOKA, H., NAKATANI, N., MABRY, T. J., MONTI, S. A., DAVIS, R. E., COX, P. J., SIM, G. A., WATSON, W. H. and WU, I. B., (1974) *Tetrahedron* **30**, 1161.

Marginatin (**1**), which was first obtained from the chloroform extract of leaves of *V. marginata*, has the following properties: $C_{22}H_{28}O_7$, m.p. 104–105°, $[\alpha]_D^{25}$ −6·0°, UV absorption at λ_{max} = 215 nm (ε 25600), IR 1760, 1710 and 1236 cm^{-1}. The UV and IR data for marginatin indicated the presence of a conjugated γ-lactone ring. The PMR spectrum of marginatin (in acetone-d_6; Table 1) exhibited a broad singlet at δ 4·92 (2H) and an acetate singlet at δ 2·02 (3H) which, by analogy with the PMR spectrum of glaucolide-A (**2a**) and B (**2b**),[4] is indicative of an allylic acetate group. A triplet at δ 5·20 (1H, *J* 7 Hz) when shifted upfield by the addition of $Pr(fod)_3$ appeared as a double-doublet at δ 4·70 (1H, *J* 10, 4 Hz). Another doublet at δ 4·98 (1H, *J* 9 Hz) was also shifted upfield by $Pr(fod)_3$ and appeared as a broad doublet at δ 4·05 (1H, *J* 10 Hz) (0·02 mmol of $Pr(fod)_3$ was used to 0·19 mmol of **1**). In the upfield region of the PMR spectrum of **1** a singlet appeared at 1·40 (3H) which could be assigned to a tertiary methyl group. In addition, a set of overlapping signals from δ 1·95 to 1·66 (9H) were assigned to three vinyl methyl groups. In the low-field region, a multiplet appeared at δ 7·00 (1H) which could be assigned to a β-proton of an α,β-unsaturated ester.



SCHEME 1. CHEMICAL TRANSFORMATIONS OF MARGINATIN (**1**).

Catalytic hydrogenation of marginatin (**1**) over palladium–carbon in ethyl acetate yielded a mixture of the stereoisomers of tetrahydrodesacetoxymarginatin (**3**) (PMR spectral data in Table 1) in which hydrogenolysis of the C_{13} allylic acetate ester had occurred along with the reduction of two of the three double bonds in the compound. The conjugated γ-lactone moiety and an ester group were still present in (**3**) (IR 1750, 1725 cm^{-1}). Furthermore, a new vinyl methyl signal at δ 2·03 (*d*, 3H, *J* 2 Hz) appeared in the PMR spectrum of (**3**) proving the presence of an allylic acetate moiety in marginatin which, by analogy with similar hydrogenolysis reactions for glaucolide-A and B[1,4] must be associated with the conjugated γ-lactone. The saturation of the other double bonds in (**1**) was indicated by the absence of the low-field vinyl proton signals in the PMR spectrum of (**3**). The functional groups in marginatin as indicated by the spectral and elemental analysis data were in accord with a germacranolide skeleton.

MS and PMR data indicated the presence of the tiglate ester in marginatin. The base peak in the MS of marginatin was at *m/e* 83 followed in intensity by another peak at *m/e* 55 (40%). Similarly, the base peak in the MS of **3** appeared at *m/e* 57 followed in intensity by another peak at *m/e* 85 (50%). These fragmentations are typical of the cleavage of esters of angelic, tiglic and senecioic acids. Both tiglic and angelic acids have a β-proton, *cis* to the carbonyl in the former and *trans* in the latter. Senecioic acid, however, only has an α-proton. In the PMR spectrum of marginatin, a proton signal appeared at δ 7·00 (m, 1H), thus in the region where the β-proton of tiglic acid occurs.[5] The remaining oxygen atom in marginatin could be assigned to an epoxide from consideration of the allylic alcohol (**4**) (and its acetate, **4b**) obtained from the reaction of (**3**) in concd hydrochloric acid and methanol. A doublet at δ 4·52 (*J* 9 Hz) in the PMR spectrum of the alcohol (**4**) (in acetone-d_6; Table 1) could be assigned to H_5 since it was shown to be coupled to H_6 (δ 5·12, *J* 9, 2 Hz); H_6 was also coupled to the C_{11} vinyl methyl group (δ 1·93, *J* 1 Hz). Since upon acetylation of (**4a**) to (**4b**) the δ 4·52 signal shifted to δ5·65 (*d*, *J* 9 Hz), the alcohol function can be assigned to C_5.

Spin decoupling experiments with (**3**) established coupling interactions between H_5 and H_6; and H_6 with the C_{13}-vinyl methyl group thus indicating that marginatin (**1**) is lactonized to C_6.

The dihydrotiglate moiety present in (**3**) was hydrogenolyzed by the catalytis hydrogenation of (**3**) to yield (**5**) which was also obtained from (**1**) directly. Since this reaction with (**3**) must be activated by the C_7–C_{11} double bond, the tiglate ester must be at C_8 in marginatin (**1**). Moreover, the multiplicity of the H_8 signal in (**3**) suggests that C_9 bears at least two protons; therefore, the double bond associated with the C_{10} vinyl methyl group can be placed between C_1 and C_{10}. However, since the multiplicity of H_8 in the PMR spectrum of (**3**) might be due to sterioisomers formed during hydrogenation, (**6**) was prepared by a controlled catalytis hydrogenation. An analysis of the PMR spectrum of (**6**), dihydrodeacetoxymarginatin, provided additional support for the presence of the C_1–C_{10} double bond. The spectrum of (**6**) exhibited a doublet at δ 2·01 (3H, *J* 2 Hz), assigned to the C_{13} vinyl methyl group; another doublet at δ 2·70 (1H, *J* 10), assigned to H_5; a doublet of quartets at δ 4·75 (1H, *J* 10, 2), assigned to H_6; a triplet at δ 5·10 (1H, *J* 6), assigned to H_8; and a broad triplet at δ 5·45 (1H, *J* 7), assigned to H_1. The multiplicity of the latter signal requires that the vinyl proton H_1 be adjacent to a methylene group, in accord with structure (**1**) for marginatin.

Comparison of the ^{13}C-NMR spectrum for marginatin with those obtained for glaucolide-A (and B),* whose total structure was established by X-ray crystallography,[4] indicate that the stereochemistry at the C_4, C_5, C_6 and C_8 positions in marginatin are the same as those in glaucolide-A and B (**2a**, b) respectively. No direct data are available to indicate the stereochemistry about the C_1–C_{10} double bond, although most, but not all, germacranolides containing this function have a *trans* configuration for this double bond.

It is of interest to note that marginatin (**1**) has a simpler structure than does glaucolide-A (**2a**) and B (**2b**) and since (**1**) dominates in what are considered to be recently evolved species,[3,6] it appears that in these taxa and for these compounds loss mutation is an advanced evolutionary character.

* The ^{13}C-NMR data will be described in a later paper: Bhacca, N. S., Wehrli, F., Mabry, T. J. and Padolina, W. G., in preparation.

[5] (a) Geissman, T. A. and Griffin, T. S. (1971) *Revista Lationoamer de Química* **2**, 81; (b) Frazer, R. R. (1960) *Can. J. Chem.* **38**, 549.

[6] Jones, Jr., S. B., private communication.

TABLE 1. PMR DATA FOR

Compound	H_5	H_6	H_8	H_{13}
(**1**)†	2·83 (*d*, 1H, *J* 8)	4·98 (*d*, 1H, *J* 9)	5·20 (*t*, 1H, *J* 7)	4·92 (*brd s*, 2H)
(**3**)†	2·58 (*d*, 1H, *J* 10)	4·88 (*dq*, 1H, *J* 10, 2)	5·40 (*dd*, 1H, *J* 11, 3)	VM 2·03 (*d*, 3H, *J* 2)
(**4a**)‡	4·52 (*d*, 1H, *J* 9)	5·12 (*dq*, 1H, *J* 9, 2)	6·00–5·30 (*c*, 2H)	VM 1·93 (*d*, 6H, *J* 2)
(**4b**)‡	5·65 (*d*, 1H, *J* 9)	5·22 (*brd, d*, 1H, *J* 9)	5·82–5·10 (*c*, 2H)	VM 1·95 (*d*, 6H, *J* 2)
(**5**)†	2·59 (*d*, 1H, *J* 10)	4·57 (*brd, d*, 1H, *J* 10)	–	VM 1·87 (*d*, 3H, *J* 1·5)
(**6**)‡	2·70 (*d*, 1H, *J* 10)	4·75 (*dq*, 1H, *J* 10, 2)	5·10 (*t*, 1H, *J* 6)	VM 2·01 (*d*, 3H, *J* 2)
1† + $Pr(fod)_3$	Shifted upfield	4·05 (*d*, 1H, *J* 10)	4·70 (*dd*, 1H, *J* 10, 4)	3·55 (*brd s*, 2H)

* Spectra were determined on a Varian A60 spectrometer. Chemical shifts are in δ units (ppm) relative to tetramethylsilane as internal standard. Parentheses contain signal multiplicity, number of protons, and coupling constant, *J* in Hz. Signal multiplicity is designated by the following symbols: *s* = singlet, *d* = doublet, *t* = triplet, *dq* = doublet composed of two quartets, *c* = complex. For **3**, **4a**, **4b** and **5** the spectral data are for mixtures of stereoisomers formed during hydrogenation.

EXPERIMENTAL

Spectra were measured on Varian A60 and HA100 (NMR); Beckman IR5A (IR); CEC110 (MS, 70eV, direct insertion); and Perkin–Elmer 141 polarimeter (specific rotation).

Marginatin (**1**). Dried and ground leaves (220 g) of *Vernonia marginata** were extracted with $CHCl_3$ and worked-up in the usual way[7] to yield 3·4 g of crude syrup. Pure marginatin (**1**) was obtained either by chromatography over a column of silica gel (C_6H_6–EtOAc, 3:1) or trituration of the crude syrup with C_6H_6–Et_2O, m.p. 104–105°; $[\alpha]_D^{25}$ −6·0°; UV (EtOH) λ_{max} = 215 nm (ϵ 25600); IR 1760, 1710, 1615, 1236 cm^{-1}; PMR data are in Table 1. (Anal. Calcd for $C_{22}H_{28}O_7$: C, 65·35; H, 6·94; O, 27·72. Found: C, 65·56; H, 6·73; O, 27·40%.)

Hydrogenation of marginatin (**1**) *to tetrahydrodesacetoxymarginatin* (**3**). A solution of 500 mg of **1** in 100 ml of EtOAc was hydrogenated under NTP with 500 mg prereduced 5% Pd–C catalyst. The reaction was stopped after the uptake of 3 equivalents of H_2. The soln was conc *in vacuo* and purified by preparative TLC to yield, after crystallization from MeOH, 300 mg of fine needles of a mixture of isomers of tetrahydrodesacetoxymarginatin (**3**): m.p. 83–85°; $[\alpha]_D^{25}$ −87·8°; UV (EtOH) λ_{max} = 210 nm (ϵ 15800); IR 2950, 1750, 1720, 1205 cm^{-1}; PMR data in Table 1. (Anal. Calcd for $C_{20}H_{32}O_5$: (M$^+$ + 1), 351·2171. Found: (M$^+$ + 1), 351·2180; A weak M$^+$ peak was observed.)

Isomerization of tetrahydrodesacetoxymarginatin (**3**) *to the allylic alcohol* **4**a. 200 mg of (**2**) in 4 ml MeOH was added to 0·7 ml of conc HCl and the mixture reacted overnight. The solvents were removed under vacuum and the products separated by preparative TLC (C_6H_6–Et_2O, 1:2) to yield 80 mg of an oily product (**4**) (IR 3500, 2950, 1770, 1710 cm^{-1}; PMR data in Table 1) characterized as the acetate **4**b which also isolated as an oil: $[\alpha]_D^{25}$ −62·0°; UV (EtOH) λ_{max} = 210 nm (ϵ 12200); IR 2950, 1700, 1710, 1240 cm^{-1}; PMR data are in Table 1. (Anal. Calcd for $C_{22}H_{32}O_6$: MW, 392·2199. Found: mol wt. (MS), 392·2194.)

Hydrogenation of marginatin (**1**) *to the epoxy lactone* (**5**). A soln of 100 mg of **1** in 100 ml of EtOH was hydrogenated NTP with 100 mg of prereduced PtO_2. The reaction was stopped after the uptake of 4 equivalents H_2 and after conc, preparative TLC (hexane: Et_2O, 1:1) and recrystallization from petrol., 70 mg of fine needles of

* Information on the collection sites of *V. marginata*, *V. fasciculata* and *V. arkansana* and the deposition of vouchers are recorded elsewhere.[1,2]

[7] MABRY, T. J., MILLER, H. E., KAGAN, H. B. and RENOLD, W. (1966) *Tetrahedron* **22**, 1139.

MARGINATIN AND ITS DERIVATIVES*

C_4-Me	C_{10}-Me	OAc–Me	Miscellaneous
1·40 (*s*, 3H)	1·95–1·66 (*c*, 9H)	2·02 (*s*, 3H)	Tiglate vinyl methyl 1·95–1·66 (*c*, 9H) tiglate vinyl-H 6·90 (*c*, 1H) C_1-vinyl proton 5·55 (*brd t*, 1H, *J* 7)
1·62 (*s*, 3H)	1·05–0·65 (*c*, 6H)	—	2-*Me*-butyrate 1·16 and 1·13 both (*d*, *J* 7) integrating for 3H butyrate terminal Me 1·05–0.65 (*c*, 6H)
Overlapping w/C_{13}-VM	1·03 (*d*, 3H, *J* 7)	—	2-*Me*-butyrate 1·07 (*d*, 3H, *J* 7) butyrate terminal-Me 0·89 (*dd*, 3H, *J* 6, 2) C_3-vinyl proton 6·00–5·30 (*c*, 2H) C_5-OH 6·00–5·00 (*brd s*, 1H) lost w/D_2O
Overlapping w/C_{13}-VM	1·08 (*d*, 3H, *J* 7)	2·06 (*s*, 3H)	2-*Me*-butyrate 1·08 (*d*, 3H, *J* 7); butyrate terminal-Me 0·89 (*dd*, 3H, *J* 6, 2) C_3-vinyl proton 5·82–5·10 (*c*, 2H)
1·55 (*s*, 3H)	0·92 (*brd s*, 3H)	—	
1·39 (*s*, 3H)	1·76 (*d*, 3H, *J* 1)	—	2-*Me*-butyrate 1·13 and 1·10 both (*d*, *J* 7) integrating for 3H butyrate terminal-Me 0·93 (*dd*, 1H, *J* 6, 3) C_1-vinyl proton 5·45 (*brd t*, 1H, *J* 7)
1·01/1·16 (*s*, 3H)§	1·90–1·40 (*c*, 9H)	1·01/1·16 (*s*, 3H)§	Tiglate vinyl methyl 1·90–1·40 (*c*, 9H) tiglate vinyl-H 6·67 (*q*, 1H, *J* 8) C_1-vinyl proton 5·18 (*t*, 1H, *J* 8)

† In chloroform-d.
‡ In acetone-d_6.
§ Denotes multiplicity and integration for each signal.
^{VM} Vinyl methyl.

the epoxy lactone (**5**) were obtained: m.p. 138–141°; $[\alpha]_D^{25}$ −6·2°; UV (EtOH) λ_{max} = 212 nm (ε 12000); IR 2950, 1750 cm^{-1}; PMR data are in Table 1. (Anal. Calcd for $C_{15}H_{22}O_3$: MW, 250·1569. Found: MW (MS), 250·1570.)

Hydrogenation of tetrahydrodesacetoxymarginatin (**3**) *to the epoxy lactone* (**5**). A soln of 100 mg of (**3**) in 20 ml EtOH was hydrogenated at NTP with 100 mg of prereduced PtO_2. The reaction was stopped after the absorption of 1 equivalent H_2 and worked-up as the catalyst was filtered off. The filtrate was conc under H_2O pump vacuum to yield 40 mg of the epoxy lactone **5** (identical m.p. and IR and PMR spectra).

Hydrogenation of marginatin (**1**) *to dihydrodesacetoxymarginatin* (**6**). A soln of 500 mg of marginatin (**1**) in 500 ml EtOAc was hydrogenated at NTP with 500 mg prereduced 5% Pd–C. The reaction was stopped after the uptake of 2 equivalents of H_2 and worked-up. The catalyst was as before, giving 250 mg of an oily mixture of isomers of **6**: $[\alpha]_D^{25}$ −37·0° UV (EtOH) λ_{max} = 210 nm (ε 13500); IR 2950, 1750, 1710 cm^{-1}; PMR data are in Table 1. (Anal. Calcd for $C_{20}H_{28}O_5$: MW, 348·1937. Found: MW (MS), 348·1936.)

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