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GLAUCOLIDE-A AND -B, NEW GERMACRANOLIDE-TYPE SESQUITERPENE LACTONES FROM *VERNONIA* (COMPOSITAE)

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Abstract—The structures of two new germacranolides, glaucolide-A (**1a**) and -B (**1b**), isolated from more than 25 species of *Vernonia* from the Western Hemisphere, have been established by a series of chemical transformations, and spectral and X-ray crystallographic studies.

Our chemical investigations of species of the genus *Vernonia* (Compositae) from the Western Hemisphere have resulted in the detection of several novel germacranolide sesquiterpene lactones including glaucolide-A (**1a**) as the major sesquiterpene lactone constituent of *V. glauca* (L) Willd. and 25 other species from North and South America,[1,2] and glaucolide-B (**1b**) as the major sesquiterpene lactone component of *V. baldwinii.* Torr. from the Southwestern United States.[1,2] Both of the compounds described here, glaucolide-A (**1a**) and -B (**1b**), were isolated, usually as a mixture with several compounds, by chloroform extraction of dried leaves from plants of species of *Vernonia.*

We describe here the structure elucidation of **1a** and **1b**; the detailed distribution of these and other related sesquiterpene lactones in representative species of *Vernonia* from throughout the Western Hemisphere will be presented later.[3]

Glaucolide-A

Glaucolide-A (**1a**) which was first isolated in 1968,[4] was shown in the present study to have the following properties: $C_{23}H_{28}O_{10}$, m.p. 153–154·5°, $[\alpha]_D^{25} - 29°$, IR 1770, 1730, 1240 cm^{-1} (indicative of a conjugated γ-lactone) UV (EtOH) $\lambda_{max_1} = 211$ nm (ϵ 21300) and a shoulder at 290 nm (ϵ 135). The PMR spectrum of **1a** (in chloroform-d; Table 1) exhibited broadened singlets at 5·76 (1H), 6·22 (1H), and 1·95 ppm (3H), typical proton resonances of a methacrylate ester; also, a broad singlet at 1·59 ppm (3H) and a sharp singlet at 1·67 ppm (3H) indicated the presence of two tertiary Me groups; and two sharp singlets at 2·07 (3H) and 2·09 ppm (3H) showed the presence of two acetate esters. In the same PMR spectrum of **1a** a complex signal at 5·20–4·75 ppm (2H) overlapping with a broad singlet at 4·88 ppm (2H) was resolved by a paramagnetic NMR shift reagent, $Pr(fod)_3$. Addition of gradually increasing amounts of $Pr(fod)_3$ in a benzene solution of **1a** until a 10:1 mole ratio of **1a** to the shift reagent was obtained caused an upfield shift of the broad singlet at 4·88 ppm (2H) to give an AB pattern centered at 3·90 ppm (2H). This AB pattern is assigned to two geminal methylene protons. In the same shift reagent experiment the complex signal at 5·20–4·75 ppm (2H) was resolved into two doublets with centers at 3·65 (1H, $J = 7$ Hz) and 3·20 ppm (1H, $J = 9$ Hz) indicative of two protons, each with at least one neighboring proton.

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Table 1. PMR data for glaucolide-A(1a) and its derivatives[a]

Compound	H_3	H_6	H_8	H_{13}	C_4/C_{10}-Me	OAc-Me	Miscellaneous
1[b]		5·20–4·75 (c, 2H)	5·20–4·75 (c, 2H)	4·88 (brd s, 2H)	1·67/1·59 (s, 3H)#	2·07 (s, 3H) 2·09 (s, 3H)	methacrylate vinyl-H 6·22 (brd s, 1H) and 5·76 (brd s, 1H) methacrylate vinyl-Me 1·95(brd s, 3H)
2[c]		5·00–4·55 (c, 2H)	5·00–4·55 (c, 2H)	VM 1·96 (d, 3H, J = 2)	1·60/1·52 (s, 3H)#	2·05 (s, 3H)	isobutyrate-Me 1·2 (d, 3H, J = 7) and 1·17 (d, 3H, J = 7)
3[c]	3·38 (d, 1H, J = 8)	4·70–4·20 (c, 1H)	5·61 (t, 1H, J = 9)	2°-Me 1·37–0·70 (c, 9H)	1·55 (s, 6H)	1·94 (brd s, 3H)	isobutyrate-Me 1·37–0·70 (c, 9H)
4[c]	3·75 (dd, 1H, J = 9, 4)	4·97 (dq, 1H, J = 9, 1·5)	6·05 (t, 1H, J = 6)	VM 1·85 (d, 3H, J = 1·5)	1·61/1·45 (s, 3H)#	1·90 (s, 3H)	isobutyrate-Me 1·18 (d, 3H, J = 7) and 1·12 (d, 3H, J = 7); C_3-OH 4·55 (d, 1H, J = 4); C_4-OH 5·21 (s, 1H)
5[c]	5·31 (d, 1H, J = 10)	5·05 (dq, 1H, J = 10, 1·5)	6·13 (dd, 1H, J = 9, 6·5)	VM 1·88 (d, 3H, J = 1·5)	1·61/1·52 (s, 3H)#	2·09 (s, 3H) 1·96 (s, 3H)	isobutyrate-Me 1·17 (d, 3H, J = 7) and 1·12 (d, 3H, J = 7); C_4-OH 5·50 (s, 1H)
6[c]	3·97 (dd, 1H, J = 9, 3)	5·12 (d, 1H, J = 9)	6·13 (dd, 1H, J = 10, 7·5)	4·28 (c, 2H)	1·53/1·49 (s, 3H)#	1·82 (s, 3H)	methacrylate vinyl-Me 1·92 (brd s, 3H) methacrylate vinyl-H 6·12 (brd s, 1H) and 5·58 (brd s, 1H); C_3-OH 4·83 (brd d, 1H, J = 3); OMe 3·32 (s, 3H)
7[c]	4·29 (d, 1H, J = 10)	4·61 (dd, 1H, J = 10, 3)	5·20 (c, 1H)	2°-Me 1·01 (d, 3H, J = 7)	1·68/1·42 (s, 3H)#	1·91 (s, 3H)	isobutyrate-Me 1·10 (d, 3H, J = 7) and 1·07 (d, 3H, J = 7); C_3-OH 4·33 (c, 1H); C_4-OH 4·85 (s, 1H)
7[b]	4·25 (d, 1H, J = 10)	4·58 (dd, 1H, J = 10, 3)	5·20 (c, 1H)	2°-Me 1·08 (d, 3H, J = 7)	1·68/1·48 (s, 3H)#	2·02 (s, 3H)	isobutyrate-Me 1·17 (d, 3H, J = 7) and 1·13 (d, 3H, J = 7)
8[b]		4·30 (c, 1H)	5·52 (c, 1H)	2°-Me 1·20 (brd s, 3H)	1·65/1·58 (s, 3H)	2·05 (s, 3H)	isobutyrate-Me 1·20 (brd s, 3H)
9[c]	4·27 (d, 1H, J = 10)	4·60 (d, 1H, J = 10)	5·20 (dd, 1H, J = 11, 7)	2°-Me 1·07 (brd s, 3H)	1·67/1·40 (s, 3H)#	1·90 (s, 3H)	isobutyrate-Me 1·07 (brd s, 3H); C_3-OH 4·50–4·00 (brd s, 1H); C_4-OH 4·89 (s, 1H)
10a[b]	4·40 (d, 1H, J = 9)	4·70 (dd, 1H, J = 9, 4)	5·14 (t, 1H, J = 5)	2°-Me 1·10 (d, 3H, J = 7)	1·35/1·25 (s, 3H)#	—	isobutyrate-Me 1·13 (d, 3H, J = 7) and 1·10 (d, 3H, J = 7)
10b[b]	4·18 (d, 1H, J = 10)	4·56 (dd, 1H, J = 10, 3)	5·12 (c, 1H)	2°-Me 1·08 (d, 3H, J = 7)	1·45/1·38 (s, 3H)#	—	isobutyrate-Me 1·13 (brd d, 6H, J = 7)
10b[c]	4·19 (d, 1H, J = 10)	4·55 (dd, 1H, J = 10, 3)	5·05 (td, 1H, J = 8·5, 2)	2°-Me 0·98 (d, 3H, J = 7)	1·38/1·32 (s, 3H)#	—	isobutyrate-Me 1·09 (d, 3H, J = 7) and 1·07 (d, 3H, J = 7); OH 4·80–4·00, 3·70–2·70 (brd s) lost w/D_2O
11[b]	4·20 (d, 1H, J = 10)	4·57 (dd, 1H, J = 10, 4)	5·00 (c, 1H)	2°-Me 1·06 (d, 3H, J = 7)	1·32 (s, 3H)		isobutyrate-Me 1·15 (d, 3H, J = 7) and 1·13 (d, 3H, J = 7); C_{10}-Me 0·82 (d, 3H, J = 7)

[a]Spectra were determined on a Varian A-60 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 units. Signal multiplicity is designated as follows: s, singlet; d, doublet; dd, doublet of doublets; dq, doublet of quartets; t, triplet; td, triplet of doublets; c, complex. VM = vinyl methyl; 2°-me = secondary methyl.

[b]Spectrum determined on a Varian HA-100 spectrometer.

[c]In chloroform-d.

[d]In acetone-d_6.

#Denotes multiplicity and integration for each signal.

The presence of a ketone, a methacrylate ester and an allylic acetate in glaucolide-A (**1a**) was evident from the data for two products from the hydrogenation of **1a** over Adam's catalyst, di- and tetra- hydrodesacetoxyglaucolide-A (**2** and **3**, respectively). The weak UV absorption at 290 nm, which appeared as a shoulder in the UV spectrum of **1a**, is now a distinct absorption peak for **2** and **3** indicating the presence of a ketone group in all three compounds.

The presence of an allylic acetate in **1a** was evident since in the formation of **2** and **3** hydrogenolysis of an acetate moiety had occurred to form a vinyl Me group (1·96 ppm d, $J = 2$ Hz) in **2**. The IR and UV spectra showed that the *conjugated* γ-lactone was still present in **2** and absent in **3**; thus the double bond which activated hydrogenolysis must be associated with the conjugated γ-lactone.

In addition, it was evident from the PMR of **2** that hydrogenation of **1a** to yield **2** had converted the methcrylate group to an isobutyrate moiety. [Set of doublets centered at 1·20 (3H, $J = 7$ Hz) and 1·17 ppm (3H, $J = 7$ Hz) and the absence of the two vinyl proton singlets in the downfield region.]

Consideration of all the above data for **2** and **3** together with the spectral and elemental analysis results for **1a**, **2** and **3** suggested that glaucolide-A (**1a**) consisted of a germacranolide skeleton with one acetate moiety as part of the conjugated γ-lactone function, a second acetate group, one methacrylate ester and a ketone group.

Evidence that the remaining unassigned O atom is part of an epoxide ring adjacent to the lactone ring was obtained in the following manner.

Solvolysis of **2** in trifluoroacetic acid with a trace of water gave **4** quantitatively. The presence of two new OH groups in **4** was shown by analysis of the PMR spectrum before and after deuterium exchange. That one of the two OH groups in **4** was secondary and the other tertiary was confirmed by the conversion of **4** to its monoacetate **5** (PMR spectrum in Table 1; IR, 3450 cm^{-1}).

Spin decoupling experiments established (Table 2) that the C_6 proton of **4** couples homoallylically with the C_{13} vinyl Me group as well as with the methine proton on the C atom bearing the secondary OH group; these data establish the relative positions of the functional groups as shown in partial structures A for **4** and B for **1a**. Additional support that the conversion of **2** to **4** had opened an epoxide ring without formation of a double bond, was obtained by the isolation of the methyl ether **6** from the treatment of **1a** with a dilute solution of conc hydrochloric acid in methanol. The PMR spectrum of **6** before and after deuterium exchange established that the methyl ether was attached at C_4 and the OH group at C_5; D_2O washed out the OH proton signals and transformed the H_5 signal at 3·97 ppm from a double–doublet (1H, $J = 9$, 3 Hz) to a doublet ($J = 9$ Hz).

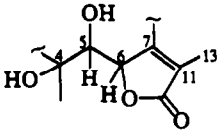
Assignment of the methacrylate group to C_8 and the remaining acetate moiety to C_{10} in glaucolide-A (**1a**) was made as follows. Acid hydrolysis of the tetrahydro derivative **3**, obtained as a mixture of (stereo) isomers by hydrogenation of **1**, yielded diol **7** (this and subsequent products with a saturated lactone ring will be referred to in this discussion as if they were a single isomer unless otherwise noted) whose PMR spectrum showed H_5 as a doublet at 4·29 ppm (1H, $J = 10$ Hz) coupled with H_6 whose signal shows a double–doublet at 4·61 ppm (1H $J =$ 10, 3Hz). Moreover, the lowfield proton triplet at 6·05 ppm in the conjugated-lactone diol **4** appeared as a more complex signal at 5·20 ppm in the saturated lactone diol **7** indicating that the saturation of the lactone ring introduced another coupling in-

Table 2. Summary of results from the spin decoupling experiment on 4[a]

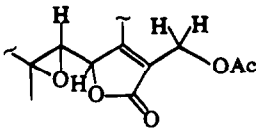
Signal irradiated	Signal observed	Response
1.15	2.60	septet-like pattern collapsed to singlet
	2.80	slightly broadened
2·60	1·15	isobutyrate-Me doublets collapsed to two singlets at 1·20 and 1·17
2·77	6·05 (t, 1H, $J = 6$)	triplet collapsed to a singlet
3·75	4·96 (dq, 1H, $J = 9,1·5$)	doublet of quartets collapsed to a broad singlet
	4·55 (d, 1H, $J = 4$)	doublet collapsed to a singlet
4·55	3·75 (dd, 1H, $J = 9,4$)	double-doublet collapsed to a doublet ($J = 9$)
4·96	3·75 (dd, 1H, $J = 9,4$)	double-doublet collapsed to a doublet ($J = 4$)
	1·85 (d, 1H, $J = 1·5$)	doublet collapsed to a singlet

[a]Signals denoted in the same manner as in Table 1.

teraction with this low-field proton. This was confirmed by the introduction of deuterium at C_7, accomplished by the catalytic deuteration of glaucolide-A (**1a**) in ethanol-O-d in the presence of platinum oxide to give **8** (PMR spectral data, Table 1), and the subsequent conversion of **8** to the diol **9**, the deuterated analogue of diol **7**. Comparison of the PMR spectrum of diol **7** with that of deuterio diol **9** (Table 1) showed the simplification of the complex signal of 5·20 ppm (1H) and the double–doublet at 4·61 ppm (1H, $J = 10$, 3 Hz) in the PMR spectrum of **7** to a double–doublet at 5·20 ppm (1H, $J = 11$, 7 Hz) and a doublet at 4·60 ppm (1H, $J = 10$ Hz), respectively, in the PMR spectrum of **9**. These observations locate one ester function at C_8 and a methylene group at C_9 in glaucolide-A (**1a**).

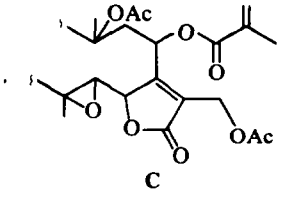


A



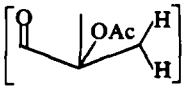
B

Acid catalyzed hydrolysis of **3** furnished the triol isomer **10** whose PMR spectrum revealed the loss of an acetate group and the presence of an isobutyrate moiety, at C_8 since the C_8 proton signal for **10** does not shift upfield (1H, 5·12 and 5·05 ppm in chloroform-d and acetone-d_6, respectively). These observations locate the acetate group at C_{10} (a tertiary position) and lead to partial structure C for glaucolide A (**1a**).



C

The mass spectral fragmentation pattern of dihydrodesacetoxyglaucolide-A (**2**) provided a clue for locating the ketone group at C_1 in glaucolide-A (**1a**). An ion was observed at *m/e* [M-128], corresponding to the loss of $C_6H_8O_3$, which most likely represents the loss of the fragment below:



Positioning of the ketone group at C_1 adjacent to the acetate ester at C_{10} was confirmed by the isolation of **11** from the product mixture of the dissolving metal reduction of tetrahydrodesacetoxyglaucolide-A (**3**) in zinc-acetic acid. The PMR spectrum of **11** (Table 1) showed the loss of an acetoxyl group and the appearance of a high-field doublet at 0·82 (3H, $J = 7$) in accord with the formation of a new secondary Me group.

The spectral and chemical data presented above established structure **1a** (without stereochemistry) for glaucolide-A.

Because this is the first sesquiterpene lactone with the rare C_{13} allylic acetate function[5] to be examined by X-ray crystallography, both the natural product **1a** and its derivative **2** with the C_{13} acetoxy portion were determined in order to establish the relative stereochemistry of the five asymmetric centers in glaucolide-A (**1a**) and the conformation of the 10-membered carbocyclic ring.

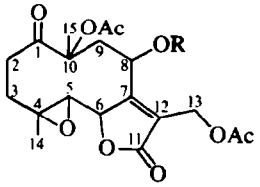
Both glaucolide-A (**1a**) and its derivative **2** crystallize in the orthorhombic space group $P2_12_12_1$ with $z = 4$ and cell dimensions for **1a** of $a = 9·075(6)$ Å, $b = 18·901(6)$ Å, and $c = 13·654(6)$ Å and for **2**, $a = 11·682(6)$, $b = 17·899(6)$ Å and $c = 10·242(6)$ Å. X-ray data for **1a** were measured with Zr-filtered MoK_α ratiation on a Hilger and Watts computer-controlled four-circle diffractometer; X-ray data for **2** were collected on a Philips PAILRED diffractometer using CuK_α radiation and a graphite monochromator. The crystal structures were solved by direct methods and after least-squares refinement the current values of R are 0·085 over 1705 reflections for **1a** and 0·095 over 1478 reflections for **2**. The conformations of the 10-membered carbocyclic ring in **1a** and **2** are similar to that of shiromodiol[6] (**15**), as shown by the endocyclic torsion angles of the rings (Table 3) and the structures below. Although the ten-membered rings in **1a**, **2** and **15** have similar conformations, corresponding bonds have different torsion angles; for example, C_7 in shiromodiol (**15**), the atom carrying the exocyclic substituent C_{11}, plays the same role as C_1 in glaucolide-A (**1a**). The preferred conformation for the 10-membered carbocylic ring in these germacranolides is not greatly influenced by the novel C_{13} allylic acetate function present in **1a**. The two-dimensional structure shown below for glaucolide-A conforms with the latest conventions proposed for germacranolides.[7]

Glaucolide-B

Glaucolide-B (**1b**), $C_{21}H_{26}O_{10}$, m.p. 75–77°, $[\alpha]_D^{25}$ – 50·0°, is, like glaucolide-A (**1a**), a conjugated γ-lactone (IR 1745, 1235 cm^{-1}) with a strong UV absorption at $\lambda_{max_1} = 230$ nm (ε 10,000) and $\lambda_{max_2} = 290$ (ε 65).

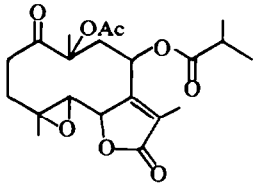
The PMR spectrum of glaucolide-B (**1b**) (in chloroform-d; Table 4) was similar to that observed for **1a** with the exception that no vinyl proton signals appeared in the downfield region and there were three acetate singlets at 2·13, 2·10, and 2·06 ppm.

In addition, the UV and IR spectral data of **1b** were similar to those for **1a**. Thus it appeared from the spectral data (and it was subsequently proven)



1a: R = 

1b: R = Ac



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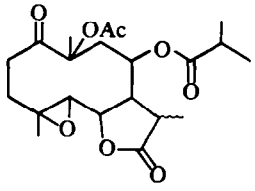

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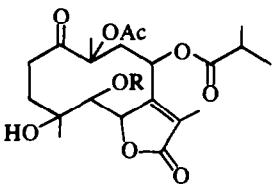
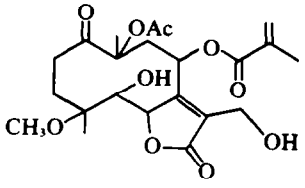


4: R = H
5: R = Ac



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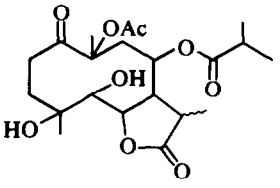

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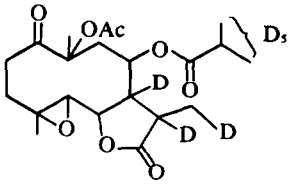

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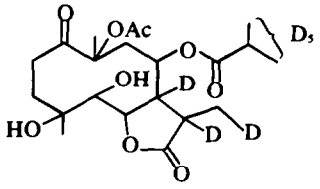


9

Table 3. Table on torsion angles

Bond	Glaucolide-A (1a)	Dihydrodesacetoxy-glaucolide-A (2)	Bond	Shiromodiol (15)
C1–C2	− 143	− 141	C7–C8	− 129
C2–C3	55	54	C8–C9	53
C3–C4	55	60	C9–C10	64
C4–C5	− 149	− 152	C10–C1	− 166
C5–C6	102	99	C1–C2	112
C6–C7	− 57	− 53	C2–C3	− 49
C7–C8	103	103	C3–C4	86
C8–C9	− 148	− 152	C4–C5	− 151
C9–C10	61	59	C5–C6	79
C10–C1	68	68	C6–C7	57

Table 4. PMR data for glaucolide-B(**1b**) and its derivatives[a]

Compound	H_5	H_6	H_8	H_{13}	C_4/C_{10}–Me	OAc–Me	Miscellaneous
1b[b]		5·10–4·60 (c, 2H)	5·10–4·60 (c, 2H)	4·92 (brd s, 2H)	1·63/1·58 (s, 3H)[d]	2·13(s, 3H) 2·10(s, 3H) 2·06(s, 3H)	
12[b]		5·00–4·55 (c, 2H)	5·00–4·55 (c, 2H)	VM 1·98 (d, 3H, J = 2)	1·62 (s, 6H)	2·12(s, 3H) 2·06(s, 3H)	
13[b]		5·80–5·30 (c, 1H)	4·60–4·10 (c, 1H)	2°–Me 1·30 (d, 3H, J = 7)	1·75/1·66/1·52 (brd s, 6H)	2·10(s, 3H) 2·03(s, H)	
14[c]	4·15 (brd d, 1H, J = 9)	5·02 (brd d, 1H, J = 9)	5·28 (t, 1H, J = 6)	VM 1·77 (d, 3H, J = 2)	1·36/1·33 (s, 3H)[d]	—	OH 4·35–3·80 (brd) lost after D_2O exchange

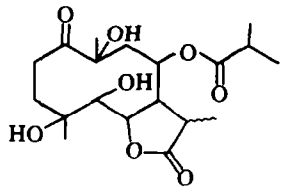
[a] Spectra were determined on a Varian A-60 spectrometer. Chemical shifts are in δ-units (ppm) relative to tetramethylsilane as internal standard. Parentheses contain signal multiplicity, number of protons, and coupling constant, J, in Hz. Signal multiplicity is denoted by same symbols as in Table 1.

[b] In chloroform-d.

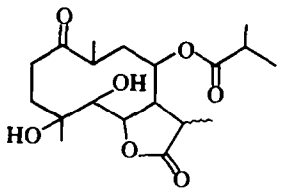
[c] In acetone-d_6.

[d] Denotes multiplicity and integration for each signal.

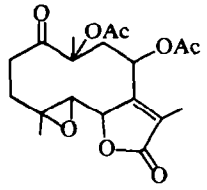
VM = vinyl methyl.



10a,b



11



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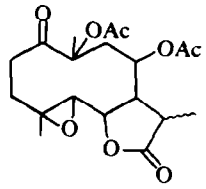
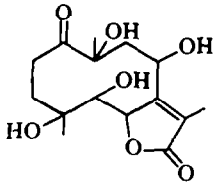
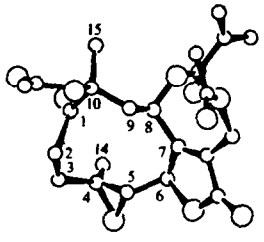


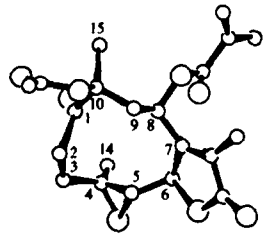
13



14



1a



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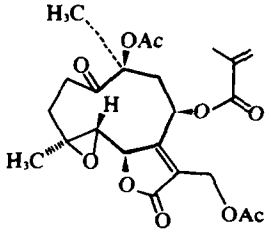
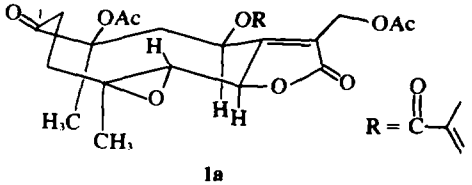
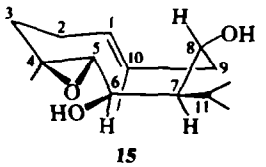


1a



1a



15

that glaucolide-B (**1b**) differed from **1a** only in that the methacrylate ester in the latter is replaced by an acetate function.

Hydrogenation of **1b** yielded desacetoxyglaucolide-B (**12**) and dihydrodesacetoxyglaucolide-B (**13**) (PMR data, Table 3). Both **12** and **13** showed the characteristic UV absorption at 290 nm for a ketone chromophore.

Glaucolide-A (**1a**) and -B (**1b**) were both converted to the same (by m.p., PMR, IR, UV, MS) tetrahydroxy derivative **14** by treatment of **2** and **12** each with conc hydrochloric acid in methanol (PMR data for **14**, Table 2).

The evidence discussed above established that glaucolide-B (**1b**) is the C_8 acetate analogue of glaucolide-A (**1a**). The isolation of identical tetrahydroxy derivatives, an analogous complement of products from the same reactions and ^{13}C NMR spectroscopic studies[8] of **1a** and **1b** indicate that both of the new germacranolides have the same relative stereochemistry.

EXPERIMENTAL

M.ps were determined on a Fischer-Johns m.p. apparatus and are uncorrected. UV absorption spectra were determined on a Beckman DB spectrophotometer. IR spectra were determined in chloroform soln on a Beckman IR5A recording spectrophotometer. Optical rotations (α) were determined on a Perkin-Elmer 141 polarimeter with the compounds dissolved in chloroform at a concentration of 0·5%. PMR spectra were determined on a Varian A-60 nuclear magnetic resonance spectrometer using TMS as an internal standard; spin decoupling experiments were carried out on a Varian HA-100 nuclear magnetic resonance spectrometer at the University of Texas, Department of Chemistry Research Instruments Laboratory under the direction of Dr. Ben Shoulders. High and low resolution mass spectra (MS) were determined at the University of Texas, Department of Chemistry Mass Spectrometry Laboratory under the direction of Dr. Conrad Cone. Microanalyses were performed by Alfred Bernhardt Microanalytical Laboratories, West Germany.

Light petroleum refers to the fraction that boils at 30–60°. Silica gel powder, 60–100 mesh, was used for column chromatography. TLC plates were prepared with silica gel-G at a thickness of 0·3 mm. Preparative thin layer chromatography plates were prepared using silica gel-G at a thickness of 0·75 mm.

Isolation of glaucolide-A (**1a**). Ground, dried leaves (990 g) of *V. noveboracensis*[1,2] were extracted with chloroform and the extract was worked-up in the usual way.[9] To the thick syrup thus obtained was added 1250 ml of 90% EtOH and the resultant mixture was then heated on a steam bath and allowed to cool. To precipitate the more polar components, 1250 ml of 4% aqueous lead (II) acetate soln was added and the soln was filtered and the filtrate concentrated under water pump vacuum until only a mixture of water and an oily residue remained. After extraction several times with chloroform, the extracts were combined and dried under high vacuum to yield 5·7 g (0·57%) of crude extract. The crude syrup was then chromatographed on activated silica gel (200 g) with chloroform–ether (8:1) as eluting solvent. Fractions of 20 ml each were collected and monitored by TLC, after which, fraction numbers 21–34 were combined and concentrated; the material which crystallized was triturated with isopropyl ether to yield 2·14 g (0·2%) of crude **1a** which was recrystallized from MeOH as plates: m.p. 153–154·5°; $[\alpha]_D^{25} - 29·0°$; UV (EtOH) $\lambda_{max_1} = 211$ nm (ϵ 21300), $\lambda_{max_2} = 290$ nm (ϵ 135); IR 1770, 1740, 1240, 830 cm^{-1}; PMR data are in Table 1. (Found: C, 59·82; H, 5·96; O, 34·04. Calcd for $C_{23}H_{28}O_{10}$: C, 59·60; H, 6·03; O, 34·45%).

Hydrogenation of glaucolide-A (**1a**) *to dihydrodesacetoxyglaucolide-A* (**2**) *and tetrahydrodesacetoxyglaucolide-A* (**3**). A soln of **1** (500 mg) in 100 ml EtOH was hydrogenated at atmospheric pressure and room temp in the presence of 500 mg of prereduced PtO_2. The reaction was stopped after the uptake of two equivs of H_2 and the catalyst was filtered off. The filtrate was concentrated under water pump vacuum to yield, after preparative TLC (chloroform:ether, 4:1) and recrystallization from MeOH, 170 mg of plate-like crystals of **2**, m.p. 169–171°; $[\alpha]_D^{25} - 64·8°$; UV (EtOH) $\lambda_{max_1} = 214$ nm (ϵ 13600), $\lambda_{max_2} = 290$ nm (ϵ 98); IR 3000, 1760, 1710, 1242 cm^{-1}; PMR data are in Table 1. (Found: mol wt, 408·1784 (mass spectrum). Caldc for $C_{21}H_{28}O_8$: mol wt, 408·1788).

Extraction of another band (lower R_f-value) from the silica gel-G plate yielded, after recrystallization from MeOH, 210 mg of granular crystals of the mixture of isomers of **3**, m.p.-wide range; $[\alpha]_D^{25}$ 0·0; UV (EtOH) $\lambda_{max_1} = 208$ nm (ϵ 11100), $\lambda_{max_2} = 290$ nm (ϵ 54); IR 3000, 1770, 1710, 1240 cm^{-1}; PMR data are in Table 1. (Found: mot wt, 410·1944 (mass spectrum) Calcd for $C_{21}H_{30}O_8$: mot wt, 410·1941. Found: C, 61·29; H, 7·48; O, 31·06. Calcd for $C_{21}H_{30}O_8$ C, 61·46; H, 7·32; O, 31·22%).

Solvolysis of dihydrodesacetoxyglaucolide-A (**2**) *to diol* **4**. To 2 ml of trifluoracetic acid (with a trace of water) was added 60 mg of **2** and the mixture was allowed to react for 4 min at room temp. Excess trifluoroacetic acid was removed under high vacuum and chloroform was added to the crude product. The chloroform soln was washed with dil $NaHCO_3$aq until the washings were slightly basic, then washed with brine until the washings were neutral. The chloroform extract was dried (Na_2SO_4), concentrated under water pump vacuum, and purified by preparative TLC (chloroform:ether, 4:1) to yield, quantitatively, the diol **4**, recrystallized from ether–light petroleum as fine granular crystals: m.p. 72–74°; $[\alpha]_D^{25} - 7·0°$; UV (EtOH) $\lambda_{max_1} = 220$ nm (ϵ 13900), $\lambda_{max_2} = 290$ nm (very weak); IR 3550, 3450, 3000, 1760, 1720, 1240 cm^{-1}; PMR data in Table 1 and 2. (Found: $(M^+ + 1)$, 427·198 (mass spectrum). Calcd for $C_{21}H_{31}O_9$: $(M^+ + 1)$, 427·197).

Acetylation of diol **4** *to the diol acetate* **5**. The treatment of **4** (50 mg) in 0·5 ml pyridine with 0·1 ml Ac_2O for 12 h at room temp gave, after normal workup, purification by preparative TLC (chloroform:ether, 4:1), and crystallization from ether–n-hexane, 65 mg of granular crystals of **5**, m.p. 60–63°; $[\alpha]_D^{25} - 26·0°$; UV (EtOH) $\lambda_{max_1} = 208$ nm (ϵ 13000), $\lambda_{max_2} = 272$ nm (ϵ 209); IR 3450, 3000, 1760, 1710, 1240 cm^{-1}; PMR data in Table 1; M^+, *m/e* 468.

Acid hydrolysis of glaucolide-A (**1a**) *to methyl ether* **6**. Hydrolysis of **1a** (137 mg) in a soln of 0·1 ml conc HCl in 4 ml of abs MeOH for 15 h at room temp and subsequent evaporation of the excess MeOH and HCl under high vacuum yielded, after preparative TLC (chloroform:ether, 2:1) and crystallization from n-hexane–ether, 30 mg of granular crystals of **6**: m.p. 98–100°; $[\alpha]_D^{25} + 24·8°$; UV (EtOH) $\lambda_{max_1} = 208$ (ϵ 12800), $\lambda_{max_2} = 280$ nm (ϵ 113); IR 3560, 3500, 2970, 1760, 1730, 1240 cm^{-1}; PMR data in

Table 1. (Found: mol wt, 454·1841 (mass spectrum). Calcd for $C_{22}H_{30}O_{10}$; mol wt, 454·1839).

Acid hydrolysis of tetrahydrodesacetoxyglaucolide-A* (**3**) *to the diol* **7** *and triol isomers* **10a** *and* **10b**. To **3** (200 mg) partially dissolved in 2·0 ml MeOH, was added 0·7 ml conc HCl and the mixture was left in a wrist-action shaker overnight at room temp. The excess MeOH and HCl were removed under high vacuum and the products were separated by preparative TLC (chloroform:acetone, 4:1) to yield, after recrystallization from n-hexane–ether, 30 mg of granular crystals of **7**, m.p. 104–106°; $[\alpha]_D^{25}$ + 97·6°; UV (EtOH) λ_{max_1} = 208 nm (ϵ 13000), λ_{max_2} = 284 nm (ϵ 100); IR 3400, 2950, 1765, 1720, 1260 cm^{-1}; PMR data in Table 1. (Found: $(M^+ - 18)$, 410·1939 (Mass spectrum; no M^+ peak was observed). Calcd for $C_{21}H_{30}O_8$: $(M^+ - 18)$, 410·1941).

Two isomers of the triol were separated by preparative TLC (chloroform:acetone, 4:1). After recrystallization from n-hexane–ether, 30 mg of granular crystals of **10a** were obtained: m.p. 205–207°; $[\alpha]_D^{25}$ + 87·2°; UV (EtOH) λ_{max_1} = 208 nm (ϵ 11000), λ_{max_2} = 300 (very weak) IR 3500, 3000, 1770, 1720 cm^{-1}; PMR data in Table 1. (Found: $(M^+ - 18)$, 368·1834 (mass spectrum; no M^+ peak was observed). Calcd for $C_{19}H_{28}O_7$: $(M^+ - 18)$, 368·1835).

The other triol isomer **10b** was isolated in the same manner as described for **10a** to yield, after crystallization from n-hexane–ether, 40 mg of granular crystals of **10b**: m.p. 106–109°; $[\alpha]_D^{25}$ + 79·0°; UV (EtOH) λ_{max_1} = 208 nm (ϵ 10400), λ_{max_2} = 280 nm (ϵ 60); IR 3500, 2950, 1760, 1720 cm^{-1}; PMR data in Table 1. (Found: $(M^+ - 18)$, 368·1828 (mass spectrum; the $(M^+ + 1)$ peak was too weak to be measured). Calcd for $C_{19}H_{28}O_7$: $(M^+ - 18)$, 368·1835).

Octadeuteriodesacetoxyglaucolide-A (**8**). A soln of **1** (500 mg) in 100 ml abs EtOD was catalytically deuterated in the presence of 500 mg of prereduced PtO_2 at atmospheric pressure and room temp. The reaction was stopped after the uptake of two equivs of D and the catalyst was filtered off. The filtrate was concentrated under water pump vacuum to yield, after preparative TLC (chloroform:ether, 4:1) and recrystallization from MeOD, 180 mg of plate-like crystals of tetradeuteriodesacetoxyglaucolide-A whose R_f value is identical with that of **2** and whose key signals in the PMR and IR spectra were similar to those of **2**. In the mass spectrum of tetradeuteriodesacetoxyglaucolide-A the molecular ion pattern showed a series of deuterated products, the most abundant of which (from peak intensities) was the tetradeuterio derivative, M^+, 412. Another reaction product with an R_f-value identical to that of **3** was isolated and recrystallized from MeOD, to yield 210 mg of granular crystals of **8** whose key signals in the IR and PMR spectra are identical with those of **3**.

Acid hydrolysis of deuteriotetrahydrodesacetoxyglaucolide-A (**8**). Using the same conditions (substituting conc DCl and MeOD) leading to **7**, **10a** and **10b** from **3**, 200 mg of **8** yielded 40 mg of the deuterio-diol **9** which was recrystallized as granular crystals from n-hexane–ether: m.p. 108–110°; R_f-value, UV, and IR spectra are similar with those of **7**; PMR data in Table 1 and mass spectrum shows a mixture of deuterated products, the most abundant of which (by peak height comparison) was the octadeuterio derivative, M^+, 436.

Zinc-acetic acid reduction[10] *of tetrahydrodesacetoxyglaucolide-A* (**3**). To **3** (80 mg) dissolved in 40 ml glacial AcOH was added 8·0 g of Zn dust, and the mixture was refluxed for 24 h, after which the Zn was filtered off. The filtrate was concentrated under water pump vacuum, diluted with chloroform, and washed with 5% NaOH aq followed with brine until the washings were neutral. The chloroform soln was dried (Na_2SO_4) and concentrated to yield, after preparative TLC (chloroform:acetone, 4:1) and recrystallization from acetone, 20 mg of fine needle-like crystals of **11**, m.p. 207–209°; $[\alpha]_D^{25}$ + 13·4°; UV (EtOH) λ_{max_1} = 208 nm (ϵ 11000), λ_{max_2} = 270 nm (ϵ 90); IR 3450, 2950, 1765, 1710 cm^{-1}; PMR data in Table 1. (Found: $[(M^+ + 1) - 17]$, 354·2045 (mass spectrum; no M^+ peak was observed). Calcd for $C_{19}H_{30}O_6$: $[(M^+ + 1) - 17]$, 354·2041).

Isolation of glaucolide-B (**1b**). Dried and ground leaves (302 g) of *V. baldwinii* Torr[1,2] were extracted with chloroform and worked-up in the same manner as in the isolation of **1a** to yield 7·2 g of crude syrup which was chromatographed over a column of silica gel (70 g) and eluted with 300 ml of chloroform followed by 420 ml of chloroform–acetone (6:1). Fractions of 20 ml each were collected and monitored by TLC, after which fractions 16–24 were combined, concentrated, and triturated with light petroleum to yield 1·86 g of crude **1b** which could be recrystallized from ether–light petroleum as fine granular crystals: m.p. 75–77°; $[\alpha]_D^{25}$ − 50·0°; UV (MeOH) λ_{max_1} = 213 nm (ϵ 10000), λ_{max_2} = 290 nm (ϵ 65); IR 1745, 1235 cm^{-1}; PMR data in Table 4. (Found: C, 57·53; H, 6·10. Calcd for $C_{21}H_{26}O_{10}$: C, 57·53; H, 5·98%).

Hydrogenation of glaucolide-B (**1b**) *to desacetoxyglaucolide-B* (**12**) *and dihydrodesacetoxyglaucolide-B* (**13**). A soln of **1b** (500 mg) in 100 ml abs EtOH was hydrogenated at atmospheric pressure and room temp in the presence of 500 mg prereduced PtO_2. The reaction was stopped after the uptake of 2 equivs of H_2. After filtering off the catalyst, the soln was concentrated under water pump vacuum to yield, after preparative TLC (chloroform:ether, 4:1) and recrystallization from MeOH, 200 mg of plate-like crystals of m.p. 146–148°; $[\alpha]_D^{25}$ − 76·2°; UV (EtOH) λ_{max_1} = 212 nm (ϵ 11000), λ_{max_2} = 284 nm (ϵ 100); IR 1750, 1710, 1240 cm^{-1}; PMR data in Table 4. (Found: mol wt, 380, 1469 (mass spectrum). Calcd for $C_{19}H_{24}O_8$: mol wt, 380·1471).

Also isolated after preparative TLC and recrystallization from n-hexane–acetone were 190 mg of fine granular crystals of **13**, m.p. 195–200° $[\alpha]_D^{25}$ 0·0°; UV (EtOH) λ_{max_1} = 206 nm (ϵ 11200), λ_{max_2} = 284 nm (ϵ 120); IR 1710, 1240 cm^{-1}; PMR data in Table 4. (Found: mol wt, 382, 1618 (mass spectrum). Calcd for $C_{19}H_{26}O_6$: mol wt, 382·1628).

Acid hydrolysis of dihydrodesacetoxyglaucolide-A (**2**). To **2** (200 mg) partially dissolved in 1·5 ml of abs MeOH was added 0·7 ml conc HCl and the mixture was left in a wrist-action shaker overnight at room temp. The excess MeOH and HCl was removed under high vacuum and the reaction products were separated by preparative TLC (chloroform:acetone, 4:1) to yield 50 mg of a diol which had identical m.p. and UV, IR, and PMR spectra with **4** from the trifluoroacetolysis reaction; and 60 mg of **14**, crystallized from ether–light petroleum as granular crystals: m.p. 41–43°; $[\alpha]_D^{25}$ − 0·6°; UV (EtOH) λ_{max} = 216 nm (ϵ 13000); IR 3450 (broad) 1750, 1720 cm^{-1}; PMR data in Table 4. (Found: $(M^+ - 18)$, 296·1255 (mass spectrum; no M^+ peak was observed). Calcd for $C_{15}H_{20}O_6$: $(M^+ - 18)$, 296·1260).

Acid hydrolysis of desacetoxyglaucolide-B (**12**). The hydrolysis of **12** (200 mg) was accomplished in the same

*Conditions for this acid hydrolysis were provided by Dr. R. Toubiana.

manner as the hydrolysis of **2** yielding, after preparative TLC (chloroform : acetone, 4 : 1) a tetrahydroxy derivative identical by IR, UV and PMR with **14**. A diol and a triol were also isolated from the product mixture.

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