Stereostructures of Neurolenins A and B, Novel Germacranolide Sesquiterpenes from *Neurolaena lobata* (L.) R.Br.[1]

Percy S. Manchand* and John F. Blount

Chemical Research Department, Hoffmann-La Roche Inc., Nutley, New Jersey 07110

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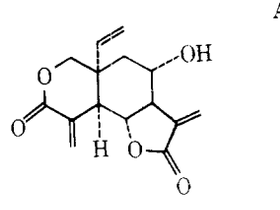
Two novel germacranolide sesquiterpenoids, neurolenins A (**4**) and B (**5**), have been isolated from *Neurolaena lobata* (L.) R.Br. (Compositae) and their stereostructures determined from spectral and X-ray crystallographic analyses; **4** and **5** possessed the α-methylene-γ-butyrolactone moiety, but were inactive against sarcoma-180 in rats.

A number of sesquiterpenoids possessing the α-methylene-γ-butyrolactone moiety are known to exhibit significant cytotoxic and, if a second α,β-unsaturated group is also present, in vivo antitumor activities.[2] Examples of these compounds are vernolepin (**1**),[3] euparotin acetate (**2**),[4] and elephantopin (**3**).[5] Based on in vitro experiments, particularly



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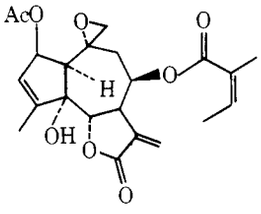

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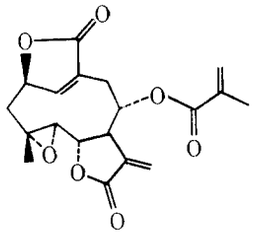

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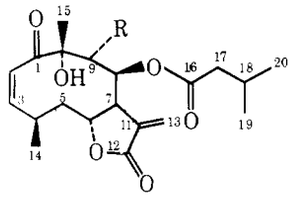
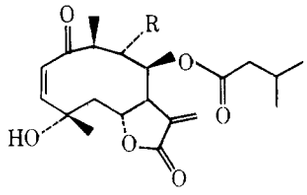


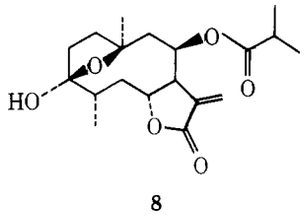
4, R = H

5, R = O—C(=O)—C(CH$_3$)$_3$



6, R = H

7, R = OAc



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those in which the α-methylene lactone group has been shown to react rapidly and preferentially with the sulfhydryl group, Kupchan has suggested that these sesquiterpenoids probably act by selective alkylation of growth-regulatory biological macromolecules, via a Michael-type reaction of the α-methylene lactone group.[6] In addition, the report by Loeb[7] that there are present in certain DNA polymerases sulfhydryl groups which are susceptible to inhibition by thiol reagents (e.g., *p*-mercurichlorobenzoate) lends some credence to Kupchan's suggestion and also to the speculation that these sesquiterpenoids probably inhibit DNA replication.[8] Despite extensive isolation[2] and synthetic[9] studies in this area, to the best of our knowledge no therapeutically acceptable compound has yet emerged.

In this article we report the isolation and structural elucidation of two novel sesquiterpenoids, neurolenins A (**4**) and B (**5**), both of which possess the aforementioned structural requirements for cytotoxic and antitumor activities, but were inactive against sarcoma-180 in rats.[10]

The neurolenins, extremely bitter substances, were isolated from a methylene chloride extract of the West Indian medicinal plant *Neurolaena lobata* ("zeb-a-pique", "herbe-a-pique", "cow-gall bitter", Compositae),[11] a plant apparently used in the Antilles for the treatment of cancer,[12] but which had not been studied previously. A curious feature of this plant is that its fresh leaves and stems impart a yellow stain to the skin when handled.

Neurolenin A (**4**), $C_{20}H_{28}O_6$, mp 127–128 °C, had IR ($CHCl_3$, cm^{-1}) absorptions indicative of the following functional groups: hydroxyl (3500), γ-lactone (1763), ester (1737), α,β-unsaturated ketone (1685), and terminal methylene (1630). Because absorption in the 235-nm region appeared as a barely discernible shoulder on the main peak at 208 nm, the UV spectrum of **4** was not definitive about the presence of an α,β-unsaturated ketone; however, cogent evidence for the presence of this functionality was readily adduced from inspection of the ^1H and ^{13}C NMR spectra. Thus, absorptions due to an AB quartet (J = 11 Hz) at δ 5.80 and 6.55 in the ^1H NMR spectrum are attributed to protons α (on C-2) and β (on C-3), respectively, to a carbonyl group; corresponding absorptions in the ^{13}C NMR spectrum (see Table I) appeared as doublets at 125.3 (C-2) and 146.6 (C-3) ppm, with absorptions due to the ketone carbonyl (C-1) as a singlet at 205.7 ppm. Other significant absorptions in the ^1H NMR spectrum of neurolenin A include those assigned to an isopropyl group (6 H doublet at δ 0.89, J = 7 Hz), a secondary methyl group (3 H doublet at δ 1.31, J = 7 Hz), a methyl group on a fully substituted carbon atom bearing an oxygen function (3 H singlet at δ 1.44), a one-proton multiplet at δ 3.09 due to H-18, and a one-proton doublet of doublets at δ 4.50 (J = 11 and 2 Hz) ascribed to H-6. As there was only one D_2O exchangeable proton (at δ 4.15) in neurolenin A, it was inferred that a single hydroxyl group was present, and since it was resistant to acetylation (acetic anhydride–pyridine), it was considered tertiary.

Further scrutiny of the extract led to the isolation of a second, closely related sesquiterpenoid, neurolenin B (**5**), $C_{22}H_{30}O_8$, whose IR spectrum showed hydroxyl (3500 cm^{-1}) but only two carbonyl absorptions (1760 and 1690 cm^{-1}). The Raman spectrum, however, disclosed absorptions due to four carbonyl groups (1780, 1745, 1710, and 1690 cm^{-1}), whose presence was fully substantiated by inspection of the ^{13}C NMR spectrum (singlets at 204.3, 170.8, 170.0, and 168.6 ppm; see Table I). The ^1H NMR spectrum of neurolenin B was very similar to that of neurolenin A, and additionally indicated that the extra carbonyl in the former was part of a secondary acetyl group (3 H singlet at δ 2.09 and 1 H singlet at δ 5.50).

The foregoing spectral evidence is compatible with either **4** or **6** for neurolenin A and either **5** or **7** for neurolenin B. Formulas **6** and **7** both contain an oxygen function at C-4, a

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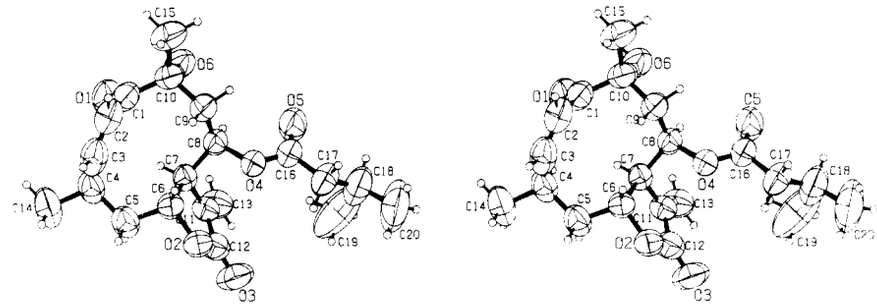

Figure 1. A stereoscopic drawing of neurolenin A (**4**).

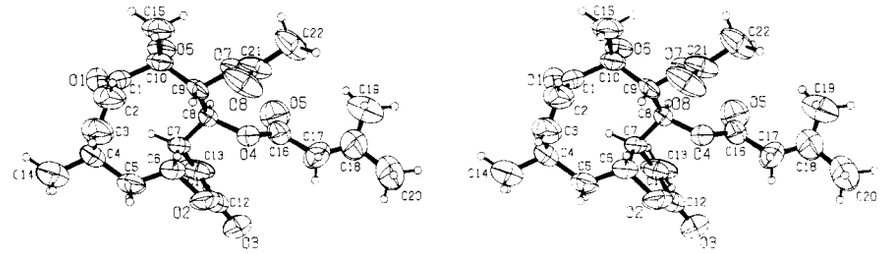

Figure 2. A stereoscopic drawing of neurolenin B (**5**).

Table I. ^{13}C NMR Data[a]

carbon atom[b]	4	5
1	205.7 (s)	204.3 (s)
2	125.3 (d)	125.3 (d)
3	146.6 (d)	147.9 (d)
4	25.4 (d)	24.9 (d)
5	40.2 (t)	40.2 (t)
6	75.9 (d)	76.3 (d)
7	42.4 (d)	41.2 (d)
8	73.9 (d)	73.9 (d)
9	40.4 (t)	73.8 (d)
10	76.5 (s)	79.3 (s)
11	136.1 (s)	134.8 (s)
12	171.3 (s)	170.8 (s)
13	124.5 (t)	126.2 (t)
14	19.9 (q)	19.6 (q)
15	28.1 (q)	23.6 (q)
16	169.0 (s)	170.0 (s)
17	43.0 (t)	42.5 (t)
18	28.3 (d)	28.2 (d)
19	22.2 (q)	22.3 (q)
20	22.2 (q)	22.3 (q)
21		168.6 (s)
22		20.5 (q)

[a] Determined at 25.2 MHz in $CDCl_3$. Chemical shifts are in parts per million with Me_4Si as an internal standard. [b] Assignments are based on chemical shifts and off-resonance decoupled spectra, and are tentative.

Table II. X-Ray Crystallographic Data and Experimental Details

	4	5
formula	$C_{20}H_{28}O_6$ (364.44)	$C_{22}H_{30}O_8$ (422.47)
space group	$P2_1$	$P2_1$
a, Å	12.826 (2)	13.984 (2)
b, Å	7.238 (1)	6.862 (1)
c, Å	12.148 (1)	12.706 (2)
β, deg	116.26 (1)	106.07 (1)
Z	2	2
d_{calcd}, g cm^{-3}	1.196	1.197
μ (Cu Kα), cm^{-1}	7.3	7.6
crystal size, mm	0.10 × 0.15 × 0.6	0.05 × 0.08 × 0.5
max θ, deg	57	57
no. of reflections	1489	1744
no. of obsd reflections	1158	1485
absorption correction	none	none
least-squares refinement	full matrix	full matrix
heavier atoms	anisotropic	anisotropic
hydrogen atoms	iso (fixed)	iso (fixed)
final R	0.048	0.056
final R_w	0.048	0.066
final difference map largest peak, e Å^{-3}	0.1	0.2

feature (or its equivalent such as an epoxide or double bond) that is common to most known germacranolide sesquiterpenoids,[13] and were therefore considered likely structures for neurolenins A and B, respectively. Definitive proof of the structures for the neurolenins was subsequently obtained from X-ray crystallographic analyses, which established structure **4** for neurolenin A and **5** for neurolenin B. Pertinent X-ray crystallographic data are listed in Table II, and stereoscopic drawings for **4** and **5** are displayed in Figures 1 and 2, respectively. As can be seen from the drawings, **4** and **5** contain an α-methylene-γ-butyrolactone trans-fused at C-6 and C-7 to a ten-membered ring, an isopentanoate ester at C-8, and an α,β-unsaturated ketone between C-1 and C-3; the double bond normally present at C-4 has presumably migrated into conjugation with the ketone (vide infra). The acetate group in **5** was located at C-9.

Perusal of the literature[2] indicates that those germacranolide sesquiterpenoids with confirmed antitumor and cytotoxic activities possess, in addition to the α-methylene-γ-butyrolactone moiety, an oxygen function or double bond at C-4. The significance of this additional structural feature in determining the biological activities of these sesquiterpenoids is not immediately apparent, but it is of some interest to note that tirotundin (**8**), recently isolated from *Tithonia rotundifolia* by Herz, had no confirmed activity in the P388 lymphocytic leukemia screen and was inactive in the B 16 melanocarcinoma and Lewis lung screens.[14] As in the case of the neurolenins, tirotundin lacked oxygenation at C-4.

Experimental Section

General. Melting points were determined in capillaries on a Thomas-Hoover melting point apparatus and are uncorrected. Unless otherwise indicated, infrared (IR) and nuclear magnetic resonance spectra (NMR) were determined in $CHCl_3$ and $CDCl_3$, respectively. ^1H and ^{13}C NMR spectra were recorded at 100 and 25.2 MHz, respectively. Chemical shifts are expressed in parts per million (ppm) with tetramethylsilane as an internal standard and coupling constants (J) in hertz (s = singlet, d = doublet, t = triplet, m = multiplet). Mass spectra (MS) were determined using a direct inlet system with an ionization energy of 70 eV; m/e values are given with relative intensities (%) in parentheses. Thin-layer chromatograms (TLC) were made from Merck (Darmstadt) silica gel G, and spots were made visible by spraying with 10% ceric sulfate in 10% H_2SO_4 and heating the plates to 110 °C.

Extraction of *Neurolaena lobata* (L.) R.Br. (syn. *Conyza lobata*, Compositae). Finely ground, dried leaves (2.0 kg) of *N. lobata*, collected in Trinidad (July 1977), were steeped in 12 L of CH_2Cl_2 for 6 days. The mixture was filtered, and the filtrate was evaporated to give 48 g of a green gum, which was dissolved in 1 L of ethyl acetate and stirred 5 times with 20 g of neutral charcoal (4 h each time). Removal of the charcoal and solvent gave 15 g of a light brown gum, which was chromatographed on 300 g of neutral alumina (Woelm, Grade II, dry pack) with 50% ethyl acetate in hexane as eluent. Fractions containing **4** and **5** (ascertained by TLC using 50% ethyl acetate in hexane as eluent) were combined and the solvents removed to give 3.3 g of a gum. The latter was separated by preparative-scale TLC (5 mm thick PF_{254} silica gel plates with 55% ethyl acetate in hexane as eluent, short wavelength UV light) into crude neurolenin A (**4**; R_f 0.66, 800 mg) and crude neurolenin B (**5**; R_f 0.60, 264 mg).

Neurolenin A (4). The preceding crude sample of **4** was crystallized first from a mixture of ethyl acetate (1 mL) and hexane (6 mL) at 0 °C overnight and then from ethyl acetate (0.5 mL) in hexane (3.0 mL) at 0 °C to give 320 mg of **4** as colorless crystals: mp 127–128 °C; $[\alpha]^{25}_D$ −257.7° ($CHCl_3$, c 1.00); UV 208 nm (ϵ 14 050), 235 sh (~6000), 305 (76); ORD (MeOH) $[\Phi]_{238}$ −47 813, $[\Phi]_{200}$ +60 000; CD (MeOH) $[\theta]_{345}$ −88, $[\theta]_{321}$ +124, $[\theta]_{296}$ −269, $[\theta]_{262}$ +3400, $[\theta]_{219}$ −77 000; IR 3500, 1763, 1737, 1685, 1630 cm^{-1}; Raman (neat) 1780, 1760, 1685, 1640 cm^{-1}; ^1H NMR δ 0.89 (6 H, d, J = 7 Hz), 1.12 (3 H, d, J = 7 Hz), 1.44 (3 H, s), 2.26 (2 H, d, J = 7 Hz), 3.09 (1 H, m), 3.70 (1 H, br s, exchangeable with D_2O), 4.50 (1 H, d of d, J = 12 and 5 Hz), 5.32 (1 H, d of t, J = 7 and 2 Hz), 5.77 (1 H, d, J = 1 Hz), 5.96 (1 H, d, J = 11 Hz), 6.27 (1 H, d, J = 1 Hz), 6.52 (1 H, d, J = 11 Hz); MS m/e 364 (M^+, 0.01).

Anal. Calcd for $C_{20}H_{28}O_6$: C, 65.92; H, 7.74. Found: C, 66.25; H, 7.85.

Neurolenin B (5). Crude **5** isolated above was crystallized from ethyl acetate–hexane (1:5) at 0 °C to give colorless crystals: mp 165–166 °C; $[\alpha]^{25}_D$ −350.0° ($CHCl_3$, c 0.76); UV 207 nm (ϵ 15 650), 235 sh (~6200), 305 (75); ORD (MeOH) $[\Phi]_{327}$ −9000, $[\Phi]_{240}$ −59 531, $[\Phi]_{208}$ +112 500; CD (MeOH) $[\theta]_{310}$ −4000, $[\theta]_{264}$ +2000, $[\theta]_{215}$ −100 000; IR 3500, 1760, 1690, 1625 cm^{-1}; Raman (neat) 3500, 1780, 1745, 1710, 1690, 1640 cm^{-1}; ^1H NMR δ 0.86 (6 H, d, J = 7 Hz), 1.13 (3 H, d, J = 7 Hz), 1.34 (3 H, s), 2.09 (3 H, s), 2.63 (1 H, s), 3.12 (1 H, m), 4.19 (1 H, exchangeable with D_2O), 4.57 (1 H, d of d, J = 11 and 5 Hz), 5.57 (2 H, s), 5.82 (1 H, d, J = 2 Hz), 6.02 (1 H, d, J = 11 Hz), 6.31 (1 H, d, J = 2 Hz), 6.61 (1 H, d, J = 11 Hz); MS m/e 422 (M^+, 0.01).

Anal. Calcd for $C_{22}H_{30}O_8$: C, 62.55; H, 7.16. Found: C, 62.47; H, 7.12.

X-Ray Crystallography. The crystallographic data for **4** and **5**, which were collected on a fully-automated Hilger-Watts diffractomer (Cu Kα radiation, θ–2θ scans, pulse height discrimination), are summarized in Table II. Listings of final atomic parameters, final anisotropic thermal parameters, bond lengths, bond angles, and torsion angles are given in Tables III–XII as supplementary material. The structure and relative stereochemistry of **4** and **5** were solved by a multiple solution procedure.[15]

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Registry No.—**4**, 67506-31-4; **5**, 67506-30-3.

Supplementary Material Available: Listings of final atomic parameters, final anisotropic thermal parameters, bond lengths, bond angles, and torsion angles are given in Tables III, IV, V, VI, and VII, respectively, for **4** and Tables VIII, IX, X, XI, and XII for **5** (10 pages). Ordering information is given on any current masthead page.

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