Sesquiterpene Lactones from *Neurolaena oaxacana*[⊥]

Claus M. Passreiter,*[,†] Jesus Sandoval-Ramirez,[‡] and Colin W. Wright[§]

Institut für Pharmazeutische Biologie, Heinrich-Heine-Universität Düsseldorf, Universitätsstrasse 1, D-40225 Düsseldorf, Germany, Facultad de Ciencias Quimicas, Benemerita Universidad Autonoma de Puebla, Puebla, Mexico, Postgraduate Studies in Pharmaceutical Technology, The School of Pharmacy, University of Bradford, West Yorkshire, BD7 1DP, U.K.

Received February 3, 1999

Twelve sesquiterpene lactones, two new (**1** and **2**) and 10 known neurolenin-type germacranolides and furanoheliangolides (**3**−**12**) were isolated from *Neurolaena oaxacana,* and their structures were elucidated by NMR and GC−MS analysis. The chemotaxonomic importance of these findings is discussed. As *N. lobata* is used against dysenteries, neurolenin B (**4**) and a mixture of the neurolenins C (**5**) and D (**6**) were tested against *Entamoeba histolytica* and *Giardia intestinalis.*

The genus *Neurolaena* (Asteraceae) occurs in tropical areas of southern Mexico, Guatemala, and other Central American states.[1] Except for the widely distributed *N. lobata,* which is frequently used in the traditional medicine of most countries in this region,[2,3] all other species are less popular due to their limited distribution. Nevertheless, some species are used in their particular area of distribution as alternatives when *N. lobata* leaves are unavailable.[4]

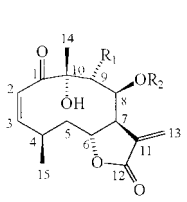
Taxonomic considerations have also made the genus *Neurolaena* interesting, and its placement within the Asteraceae was a subject of considerable discussion.[5−7] Relationships within the genus, as seen by the flavonoid chemistry of some species, are more complex than would be expected from their morphology. Turner has divided the genus *Neurolaena* into two sections, *Brevipalea* and *Neurolaena,* based on differences in length of the receptacular bracts of the species.[1] For these botanical reasons, *N. macrocephala* is more closely related to *N. lobata,* both members of the section *Neurolaena,* than it is to *N. oaxacana* of the section *Brevipalea,* although the lack of flavones and 6-hydroxykaempferol derivatives seems to differentiate *N. macrocephala* from the other two.[8,9]

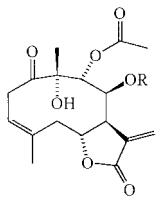
Recently, we reported the occurrence of sesquiterpene lactones in *N. lobata, N. cobanensis,* and *N. macrocephala,*[4,10,11] and their biological activities.[12,13] Our findings could not support the chemical differences within the section *Neurolaena* between *N. macrocephala* and *N. lobata* as found for the flavonoids.[8,9] Although *N. macrocephala* is the only species in which ester derivatives built from isobutyric acid have been found,[4,10] it generally contains the same type of sesquiterpene lactones as *N. lobata* and *N. cobanensis.* In continuation of our studies on sesquiterpene lactones, we now report the occurrence of sesquiterpene lactones from *N. oaxacana,* a member placed together with *N. cobanensis* in the series *Radiata* of the section *Brevipalea.*[1]

Additionally, we have tested the activities of the main sesquiterpene lactones against *Entamoeba histolytica* and *Giardia intestinalis,* both well known to cause gastrointestinal infections, as *N. lobata* is also used against dysentery in Guatemala and other Meso-American states.

Results and Discussion

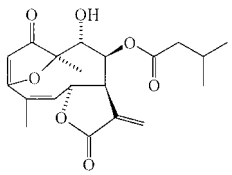
The dichloromethane extract of *N. oaxacana* B.L. Turner (Asteraceae) leaves afforded two new neurolenins **1** and **2** in addition to the known neurolenin-type germacranolides neurolenins A (**3**), B (**4**), C (**5**), D (**6**), G (**7**), and H (**8**), lobatin A (**9**), lobatin B (**10**), and the two calyculatolides (**11** and **12**), previously isolated from *N. lobata*[10] and *N. cobanensis,*[4] respectively. Both new compounds (**1** and **2**) contained an isobutyryloxy substituent, which was obvious from an intense fragment ion at *m/z* 71 in the MS of each compound. Whereas the molecular ion (*m/z* 366) indicated **1** to be a neurolenin derivative containing only one ester moiety, it could be assumed that **2** (M^+ 408) was a diester derivative containing an additional acetyl group. Because the MS of **2** contained an intense fragment at *m/z* 250, only found for **9** at that intensity, compound **2** could be a derivative of **9**, differing in the ester moiety at C-8.

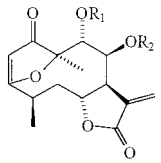




	R^1	R^2
1	OH	iBut
3	H	iVal
4	OAc	iVal
5	OiVal	H
6	OH	iVal
7	OAc	iBut
8	OAc	Mebu

	R
2	iBut
9	iVal





10

	R^1	R^2
11	H	iVal
12	Ac	iVal

The structures of **1** and **2** were further elucidated from their NMR spectra. The ^{1}H NMR of **1** displayed signals for 24 protons. The signals of 17 protons, representing the

* To whom correspondence should be addressed. Tel.: +49 211 8114172. Fax: +49 211 8111923. E-mail: passreit@uni-duesseldorf.de.
† Heinrich-Heine-Universität Düsseldorf.
‡ Benemerita Universidad Autonoma de Puebla.
§ University of Bradford.
⊥ Dedicated to Prof. Dr. B. C. Lippold on the occasion of his 60th birthday.

10.1021/np990038t CCC: $18.00
Published on Web 07/14/1999

neurolenin moiety, were found at shift values similar to those of **6**, clearly suggesting that **1** was a neurolenin derivative containing two free hydroxy groups. The remaining signals, representing an isobutyryloxy substituent, were found as a one-proton multiplet at δ 2.33 and two doublets for three protons each at δ 0.96 and 0.97. As all coupling constants were found in the same range as previously found for **6**, compound **1** possesses the same relative configuration as **6**. Placement of the ester moiety at C-8 clearly followed from the lowfield position of the signal for H-8 (δ 5.26), found at δ 3.99 in the case of a free alcohol at this position.[10]

The ^{1}H NMR of **2** showed the signals for the sesquiterpene lactone moiety found in **9**,[10] containing two ester groups attached to the 8β- and 9α-hydroxy groups. The spectrum of **2** also displayed a three-proton singlet at δ 2.15, indicating the presence of an acetate at C-9. Signals for the other acid moiety were found at shift values again representing an isobutyryloxy substituent attached to C-8. The structure of **2**, the 8β-isobutyryloxy derivative of 8-desisovaleryloxy-lobatin A, was further confirmed by its ^{13}C NMR spectrum. In accordance with the proposed structure, there were signals for 15 carbons at similar shift values to those found for **9**.[10] Two of the remaining six carbons were assigned to C-1″ (δ 170.9) and C-2″ (δ 20.5) of the acetic acid attached to C-9. The other four carbons were found at shift values characteristic for an isobutyric acid ester. Because all carbon and proton signals of the sesquiterpene lactone moiety in **2** were found at nearly the same shift values as for **9**, and all coupling constants were in the same range, it can be assumed that the relative configurations are the same in **9** and **2**. Compounds **1** and **2** are reported here for the first time in nature.

In a previous paper we reported the isolation of an inseparable mixture of **5** and **6** from *N. lobata*.[10] Thus, assignments of all partly overlapping signals in the ^{1}H and ^{13}C NMR in the mixture of **5** and **6** were made only on the basis of their concentrations in the mixture.[10] After repeated MPLC we were able to separate **5** and **6** from *N. oaxacana*. In the spectra of pure **5** and **6**, we found that the ^{13}C NMR signals of C-8 and C-9 were assigned incorrectly.[10] The correct assignments are δ 74.7 (**5**) and 76.4 (**6**) for C-8 and 73.9 (**5**) and 74.2 (**6**) for C-9.

The leaves of *N. oaxacana* contain the same types of sesquiterpene lactones as previously found in *N. cobanensis*,[4] which is placed together with this species in the series *Radiata* of the section *Brevipalea*. Differences between *N. oaxacana* and *N. cobanensis* include the absence of lobatin B (**10**) and different isobutyryloxy derivatives in the latter species. The sesquiterpene lactone pattern of *N. lobata* is very similar to that of *N. cobanensis* and *N. oaxacana*, except for the isobutyric acid esters, which have not yet been found in *N. lobata*. Because such compounds are also found in *N. macrocephala*,[11] these slight differences cannot support the assumptions made from the flavonoid chemistry.

As *N. lobata* is also used as remedy against dysenteries in Guatemala and other countries of Central America,[2,3] the main sesquiterpene lactones of the genus *Neurolaena* were tested against *E. histolytica* and *G. intestinalis*, both often causing gastrointestinal infections.[16,17] The activities obtained are given in Table 1. Neurolenin B (**4**) was found to be active against *E. histolytica* with a minimum inhibitory concentration (MIC) of 7.6 μM, which is only 2.6 times less active than the standard compound emetine. The mixture of **5** and **6** (larger amounts were only available as 1:1 mixture from *N. lobata* leaves) was found to be less active. Compound **4** showed a slightly higher activity against *G. intestinalis*. The MIC of **4** was found at 3.8 μM. The mixture of **5** and **6** was again two-fold less active. Although the neurolenins are less active than the standard compounds, emetine and metronidazole, they are probably involved in the antidysenteric activity of *N. lobata*, as they are found in the leaves in high concentrations.[18]

Table 1. Minimum Inhibitory Concentrations (in μM) of the Major Neurolenins (**4**−**6**) against *Entamoeba Histolytica* and *Giardia Intestinalis* in Vitro

compound	*E. histolytica*	*G. intestinalis*
neurolenin B (**4**)	7.60	3.80
neurolenin C/D (**5** and **6**) (1:1)	8.40	8.40
emetine	2.90	
metronidazole		1.17

Experimental Section

General Experimental Procedures. NMR: Bruker ARX 500, 500 MHz (^{1}H NMR) and 125 MHz (^{13}C NMR) in $CDCl_3$, calibrated on solvent signals at 7.24 ppm (^{1}H) and 77.0 ppm (^{13}C). GC−MS: EI (70 eV) using the GC−MS mode on a MSD 5972 combined with a 5890 plus gas chromatograph (Hewlett−Packard); column 25 m × 0.25 mm (Optima-1, Macherey & Nagel). Temperature progression: 150°(3 min) to 280° at 10° min^{-1}. HPLC: HP 1050 system, equipped with DAD detector. Detector channels set at 225 and 260 nm, with a RP_{18} Hypersil ODS (5 μm) column (12.5 × 5 mm). Mobile phase: MeOH−H_2O (9:11) at 1.8 mL min^{-1}. TLC: Si gel 60 F_{254} (Merck) toluene−EtOAc (3:2). Detection with anisaldehyde H_2SO_4.

Plant Material. Leaves of *N. oaxacana* Turner were collected 1.4 km north of La Esperanza, Oaxaca, Mexico, and identified by R. Torres, Instituto de Biologia, UNAM, Mexico City. Vouchers (no. 14381) are on deposit at the Institut für Pharmazeutische Biologie, Heinrich-Heine-Universität Düsseldorf.

Isolation. Dried and powdered leaves (300 g) were extracted with CH_2Cl_2 in a Soxhlet apparatus. A portion (20 g) of the resulting dried extract (30.2 g) was purified by column chromatography on Sephadex LH-20 with MeOH as eluent (4 columns filled with 500 g each). Fraction 4 (160 mL, residue 3.1 g) was found to contain sesquiterpene lactones (TLC and HPLC). Further purification by column chromatography on Si gel 60 (300 g) with toluene−EtOAc (3:2) as eluent, followed by preparative TLC, using the same solvent system, gave pure **1** (4 mg), **2** (2.9 mg), **3** (2 mg), **4** (32 mg), **5** (2 mg), **6** (4.5 mg), **7** (4 mg), **8** (1 mg), **9** (5.4 mg), **10** (1 mg), **11** (2 mg), and **12** (1 mg).

Desacetyl-neurolenin G (1): UV [MeOH−H_2O (9:11)] λ_{max} 215 nm; $[\alpha]^{20}_D = -28°$ (*c* 1%, MeOH); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.49 (1H, d, J = 12.0 Hz, H-2), 6.27 [1H, s (br), H-13a], 5.91 (1H, t, J = 11.4, 12.0 Hz, H-3), 5.74 (1H, d, J = 1.26 Hz, H-13b), 5.26 (1H, dd, J = 1.9, 9.5 Hz, H-8), 4.42 (1H, dd, J = 5.1, 11.4 Hz, H-6), 4.05 (1H, d, J = 9.5 Hz, H-9), 3.13 (1H, m, H-4), 2.59 [1H, s (br), H-7], 2.33 (2H, m, H-2′), 1.80 (1H, ddd, J = 4.4, 5.1, 13.2 Hz, H-5a), 1.39 (1H, m, H-5b), 1.31 (3H, s, H-14), 1.10 (3H, d, J = 6.3 Hz, H-15), 0.97 (3H, d, J = 6.3 Hz, H-3′), 0.96 (3H, d, J = 6.3 Hz, H-4′); EIMS *m/z* 366 $[M]^+$ (1), 348 (5), 296 (1), 278 (5), 260 (3), 250 (5), 217 (4), 189 (3), 165 (3), 149 (5), 111 (5), 71 (35), 43 (100).

Lobatin D (2): UV [MeOH−H_2O (9:11)] λ_{max} 210 nm; $[\alpha]^{20}_D = -53°$ (*c* 1%, MeOH); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.29 (1H, d, J = 3.3 Hz, H-13a), 5.88 (1H, t, J = 8.0, 9.6 Hz, H-3), 5.84 (1H, dd, J = 1.38, 10.5 Hz, H-8), 5.67 (1H,

d, J = 2.9 Hz, H-13b), 5.64 (1H, d, J = 10.5 Hz, H-9), 4.92 (1H, dd, J = 3.6, 4.2 Hz, H-6), 3.56 (1H, dd, J = 9.6, 15.9 Hz, H-2a), 3.06 (1H, dd, J = 8.0, 15.9 Hz, H-2b), 2.80 (1H, dd, J = 3.6, 15.2 Hz, H-5a), 2.72 (1H, dd, J = 4.2, 15.2 Hz, H-5b), 2.59 (1H, m, H-7), 2.29 (1H, m, H-2′), 2.15 (3H, s, H-2″), 1.83 (3H, s, H-15), 1.31 (3H, s, H-14), 1.03 (3H, d, J = 6.3 Hz, H-3′), 1.02 (3H, d, J = 6.3 Hz, H-4′); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 210.7 (s, C-1), 175.1 (s, C-1′), 170.9 (s, C-1″), 168.0 (s, C-12), 136.7 (s, C-4), 134.4 (s, C-11), 124.4 (t, C-13), 121.4 (d, C-3), 80.5 (s, C-10), 76.5 (d, C-8), 76.5 (d, C-9), 72.5 (d, C-6), 42.9 (t, C-5), 41.7 (d, C-7), 34.4 (d, C-2′), 36.1 (t, C-2), 25.4 (q, C-14), 22.2 (q, C-15), 20.5 (q, C-2″), 18.8 (q, C-3′), 18.5 (q, C-4′); EIMS m/z 408 $[M]^+$ (1), 366 (1), 296 (1), 292 (1), 278 (2), 250 (20), 217 (5) 149 (5), 125 (7), 109 (6), 71 (37), 43 (100).

Bioassays. Compound **4** and a mixture of **5** and **6** (1:1), previously isolated from *N. lobata*, were tested for their activity against *E. histolytica* and *G. intestinalis* in vitro. The antiamoebic and antigiardial assays were carried out using previously described methods,[14,15] except that assessment of parasite growth was carried out by visual inspection of the microtiter plate under an inverted microscope.

Acknowledgment. We are grateful to Mrs Heike Fürtges for technical assistance. C.M.P. gratefully acknowledges a travel grant to Mexico given by the Deutscher Akademischer Austauschdienst (DAAD).

References and Notes

(1) Turner, B. L. *Plant Syst. Evol.* **1982**, *140*, 119−139.
(2) Girón, L. M.; Freire, V.; Alonzo, A.; Cáceres, A. *J. Ethnopharmacol.* **1991**, *34*, 173−187.
(3) Morton, J. F. *Atlas of Medicinal Plants of Middle America: Bahamas to Yucatan*; Charles C. Thomas: Springfield, IL, 1981; p 949.
(4) Passreiter, C. M.; Medinilla, B.; Velasquez, R.; Moreno, P. *Pharm. Pharmacol. Lett.* **1998**, *8*, 119−121.
(5) Robinson, H.; Bretell, R. D. *Phytologia* **1973**, *25*, 439−445.
(6) Stuessy, T. F. In *The Biology and Chemistry of the Compositae;* Heywood, V. H., Harborne, J. B., Turner, B. L., Eds.; Academic: London, 1977; Vol. 2, pp 621−671.
(7) Turner, B. L.; Powell, A. M. In *The Biology and Chemistry of the Compositae;* Heywood, V. H., Harborne, J. B., Turner, B. L., Eds.; Academic: London, 1977; Vol. 2, 699−737.
(8) Kerr, K. M.; Mabry, T. J.; Yoser, S. *Phytochemistry* **1981**, *20*, 791−794.
(9) Ulubelen, A.; Kerr, K. M.; Mabry, T. J. *Phytochemistry* **1980**, *19*, 1761−1766.
(10) Passreiter, C. M.; Wendisch, D.; Gondol, D. *Phytochemistry* **1995**, *39*, 133−137.
(11) Passreiter, C. M.; Stoeber, S. B.; Ortega, A.; Maldonado, E.; Toscano, R. A. *Phytochemistry*, in press.
(12) François, G.; Passreiter, C. M.; Woerdenbag, H. J.; Van Looveren, M. *Planta Med.* **1996**, *62*, 126−129.
(13) Passreiter, C. M.; Isman; M. B. *Biochem. Syst. Ecol.* **1997**, *25*, 371−377.
(14) Wright, C. W.; O'Neill, M. J.; Phillipson, J. D.; Warhurst, D. C. *Antimicrob. Agents Chemother.* **1988**, *32*, 242−248.
(15) Wright, C. W.; Melwani, S. I.; Phillipson, J. D.; Warhurst, D. C. *Trans. Royal Soc. Trop. Med. Hyg.* **1992**, *86*, 517−519.
(16) Guerrant, R. L. *Rev. Infect. Dis.* **1986**, *8*, 218−227.
(17) Boreham, P. F. L. *Pharm. J.* **1991**, 271−274.
(18) Passreiter, C. M.; Medinilla Aldana, B. *Planta Med.* **1998**, *64*, 427−430.

NP990038T