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# Germacranolide type sesquiterpene lactones from *Neurolaena macrocephala*

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**Abstract**

Two new neurolenin-type sesquiterpene lactones were found in the leaves of *Neurolaena macrocephala* Sch. Bip. Ex Hemsl. (*Asteraceae*). These compounds were identified by their NMR spectra as the 8$\beta$-isobutyryloxy- and 8$\beta$-(2-methyl)butyryloxy analogs of neurolenin B. Additionally, the known neurolenins B, C and D and the furanoheliangolide 9$\alpha$-acetoxy-8$\beta$-isovaleryloxy-calyculatolide were found to be present in this species. 

*Keywords:* *Neurolaena macrocephala*; Asteraceae; Heliantheae; Sesquiterpene lactones; Germacranolides; Neurolenins

## 1. Introduction

Recently, we reported the isolation and identification of sesquiterpene lactones from the widely distributed *Neurolaena lobata* (Asteraceae) (Passreiter, Wendisch, & Gondol, 1995) and their various biological activities (François, Passreiter, Woerdenbag, & Van Looveren, 1996; Passreiter & Isman, 1997). In contrast to this species, which is frequently used as remedy against malaria, cancer and other important diseases in several Central American countries (Morton, 1981; Girón, Freire, Alonzo, & Cáceres, 1991), the other eleven species of this genus are only locally distributed (Turner, 1982). In some cases their leaves of these species are used as substitute for *N. lobata*, e.g. the leaves of *N. cobanensis* are used in the same field of indications, provided that *N. lobata* leaves are temporarily not available (Passreiter, Medinilla, Velasquez, & Moreno, 1998). Turner divided the genus into two sections named *Neurolaena* and *Brevipalea,* based on morphological differences of the species (Turner, 1982). Nevertheless the results of the investigations on flavonoids including three *Neurolaena* species (Ulubelen, Kerr, & Mabry, 1980; Kerr, Mabry, & Yoser, 1981), led Turner to propose that *N. lobata* of the section *Neurolaena* is probably more closely related to *N. oaxacana* of the section *Brevipalea*, than it is to *N. macrocephala* of the section *Neurolaena* (Turner, 1982).

Our recent findings with *N. cobanensis* (Passreiter et al., 1998), which is equally to *N. oaxacana* placed in the series *Radiata* of the section *Brevipalea* (Turner, 1982), would seem to support the last assumption relying on the similarities of the sesquiterpene lactone patterns of *N. cobanensis* and *N. lobata*, which mainly differs quantitatively, except for the absence of lobatin B, which could not been isolated, although other furanoheliangolides were present in this plant (Passreiter et al., 1998). Thus, besides its medicinal interest, these taxonomic problems have made the genus *Neurolaena* interesting for chemotaxonomical studies. Therefore, in order to establish if the sesquiterpene lactone patterns confirm the differences suggested by the flavonoids found in *N. lobata* and *N. macrocephala*, both in the section *Neurolaena* (Turner, 1982), we have now investigated the leaves of *N. macrocephala* Sch. Bip. Ex Hemsl., which is endemic to the Gulf of Mexico coast.

## 2. Results and discussion

Purification of the dichloromethane extract of *N. macrocephala* Sch. Bip. Ex Hemsl. by CC on Sephadex LH-20 gave three fractions rich in sesquiterpene lactones. Further purification afforded the neurolenins B (**1**), C (**2**) and D (**3**) as well as the furanoheliangolide 9$\alpha$-acetoxy-

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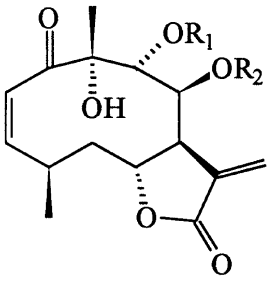
8β-isovaleryloxy-calyculatolide (**6**). Beside these known germacranolides, previously isolated from *N. lobata* (Manchand & Blount, 1978; Passreiter et al., 1995), two further compounds (**4** and **5**) were isolated.

The molecular ion in the mass spectrum of **4** was found at *m/z* 408, together with a fragmentation pattern similar to **1** and other neurolenins (Passreiter et al., 1995). This indicated **4** to be a neurolenin derivative containing a saturated $C_4$-acid ester instead of the $C_5$-acid ester in **1** ($\Delta M^+ = 14$). Accordingly, main fragments were found at *m/z* 71 $[C_3H_7CO]^+$ and 43 $[CH_3CO]^+$, which together with the fragments at *m/z* 366 $[M–CH_2{=}C{=}O]^+$, 338 $[M–C_2H_6C{=}C{=}O]^+$, 296 $[M–(C_2H_6C{=}C{=}O + CH_2{=}C{=}O)]^+$ and 260 $[M–(C_3H_7COOH + CH_3COOH)]^+$ clearly indicated the presence of two ester side chains in **4**, build from isobutyric and acetic acid. The absence of the isovalerianylic acid ester, commonly found in sesquiterpene lactones isolated from *N. lobata* (Passreiter et al., 1995), followed from the lack of the intense fragments at *m/z* 85 $[C_4H_9CO]^+$ and 57 $[85\text{-}CO]^+$. Therefore it is most likely that **4** is only differing from **1** by the ester side chain at C-8.

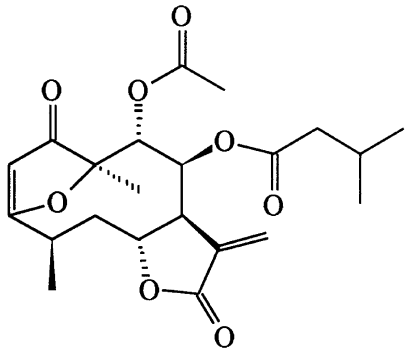
The structure of **4** was elucidated by its $^1H$ and $^{13}C$ NMR spectra. All signals were assigned using the additionally recorded 2-D-COSY and 2-D-HMQC spectra, and by comparison to the data of **1** (Passreiter et al., 1995). From the 21 carbons found in the $^{13}C$ NMR spectra of **4**, 15 were found at shift values very similar to those of **1** (Table 1). These signals were assigned to the 15 carbons of the sesquiterpene lactone skeleton (C-1 to C-15). The signals of the two ester side chains were found at δ 170.21 (C-1″) and 20.54 (C-2″), acetoxy-group, and at δ 175.06 (C=O), 28.23 (CH), 18.72 ($CH_3$) and 18.59 ($CH_3$), isobutyryloxy group.

In the $^1H$ NMR spectrum of **4** all signals were found at shift values similar to those of one **1** (Table 2) except for the signals for two methyl and one methine groups of the isobutyrate moiety, which were registered as a six proton doublet at δ 1.02 (H-3′,4′) and one proton septett at δ 2.36 (H-2′).

Definite proof of the structure of **4** was subsequently obtained from X-ray crystallographic analysis. In the crystal structure of **4** (Fig. 1) the 10-membered ring adopts a distorted boat–boat conformation with a *trans*-orientation for the 8β-isobutyryloxy and 9α-acetoxy groups. The enone moiety is no longer planar, instead of that we observe a dihedral angle of 51.4° between the C=C plane and that of the keto group at C-1 (mean deviations from the least-squares planes; C-1,C-2,C-3, C-4: 0.003 Å, C-2,C-1,O-1,C-10: 0.004 Å). The γ-lactone closed at C-6 and *trans*-annelated to the ten-membered ring adopts a flattened twist conformation (Cremer & Pople (1975); parameters $q^2 = 0.2176$ Å, $\varphi = 198.57°$). The α-hydroxy group at C-10 forms an intramolecular hydro-




| | $R^1$ | $R^2$ |
|---|---|---|
| **1** | Ac | i-Val |
| **2** | i-Val | H |
| **3** | H | i-Val |
| **4** | Ac | i-But |
| **5** | Ac | 2-Me-but |




**6**

Table 1
$^{13}$C NMR data of compounds **4** and **5** (125 MHz, $CDCl_3$)

| | 4 | 5 |
|---|---|---|
| 1 | 204.7 | 204.7 |
| 2 | 125.3 | 125.3 |
| 3 | 148.2 | 148.1 |
| 4 | 28.2 | 28.2 |
| 5 | 40.3 | 40.3 |
| 6 | 76.4 | 76.3 |
| 7 | 41.2 | 41.2 |
| 8 | 73.9 | 73.9[a] |
| 9 | 73.9 | 73.9[a] |
| 10 | 79.3 | 79.3 |
| 11 | 134.8 | 134.9 |
| 12 | 168.9 | 168.8 |
| 13 | 126. 6 | 126.4 |
| 14 | 23.7 | 23.7 |
| 15 | 19.7 | 19.7 |
| 1′ | 175.1 | 174.6 |
| 2′ | 33.9 | 40.8 |
| 3′ | 18.7[a] | 26.1 |
| 4′ | 18.6[a] | 11.6 |
| 5′ | – | 15.7 |
| 1″ | 170.2 | 170.2 |
| 2″ | 20.5 | 20.5 |

[a] Assignment interchangeable.

gen bond with the keto group (H-5...O-1: 2.11, O-5...O-1: 2.609, $\angle_{C5-H5...O1} = 125.3^{\circ}$) and in the crystal the molecules are held together by C–H...O interactions (H-6...O-5 ($-x$, $0.5+y$, $0.5-z$): 2.41 Å) in addition to van der Waals forces. As expected from the NMR spectra, the configuration of **4** was found to be the same as in the other neurolenins **1**–**3**.

Compound **5** was isolated as a white crystalline mixture with **1**. Its structure was therefore elucidated using spectra of the mixture. In contrast to **4**, the GC/MS spectrum of **5** displayed intense signals at $m/z$ 85 $[C_4H_9CO]^+$ and 57 $[85\text{-}CO]^+$, indicating the presence of an saturated $C_5$-acid ester. Likewise to **1** the molecular ion was found at $m/z$ 422, which led to the assumption that both compounds must be isomeric. The assignment of all signals in the $^1$H and $^{13}$C NMR (Tables 1–2) of the mixture was made by 2-D-COSY and 2-D-HMQC experiments and comparison to the spectra of **1**. The signals of the acyl group at C-8 of **5** were found at shift values characteristic for a 2-methylbutyric acid (Lee, Olivier, Urbatsch, & Fischer, 1982; Budesinski & Saman, 1995). Thus, **5** is a structural isomer of **1**, containing an 2-methylbuturyloxy- instead of an 3-methylbutyryloxy substituent at C-8.

The sesquiterpene lactones found in *N. macrocephala* are particularly the same found in *N. lobata* and *N. cobanensis*. As found for these two species, germacranolides of the neurolenin type as well as furanoheliangolides are present, but the neurolenins **4** and **5** have not been found in *N. lobata* and *N. cobanensis* so far (Passreiter & Medinilla Aldana, 1998; Passreiter et al., 1995, 1998; Passreiter, 1998).

From the flavonoid chemistry, due to the lack of flavones and 6-hydroxykaempferol derivatives in *N. macrocephala*, Turner (1982) suggested that *N. lobata* of the section *Neurolaena* seems to be closer related to *N. oaxacana* of the section *Brevipalea* than to *N. macrocephala*, of the same section.

In light of the sesquiterpene lactones isolated from the three *Neurolaena* species that have been investigated so far, all plants seem to be very similar to each other. Since, all three are producing sequiterpene lactones of the neurolenin and furanoheliangolide type, the only difference between *N. macrocephala* and the other species is the occurrence of isobutyric and 2-methylbutyric acid

Table 2
$^1$H NMR data of compounds **4** and **5** (500 MHz, $CDCl_3$)

| | 4 | 5 | | 4 | 5 |
|---|---|---|---|---|---|
| 2 | 6.57 d | 6.56 d | 2′ | 2.36 sept. | 2.15 sept |
| 3 | 5.99 d | 5.97 d | 3′ | 1.03 d | 1.54 dq |
| 4 | 3.09 m | 3.08 m | | | 1.28 dq |
| 5a | 1.81 ddd | 1.80 ddd | 4′ | 1.02 d | 0.82 t |
| 5b | 1.41 ddd | 1.39 ddd | 5′ | – | 1.97 d |
| 6 | 4.55 dd | 4.52 dd | 2″ | 2.06 s | 2.06 s |
| 7 | 2.58 s (br.) | 2.56 s (br.) | OH | 4.07 s | 4.07 s |
| 8 | 5.54 d[a] | 5.53 d[a] | | | |
| 9 | 5.54 d[a] | 5.53 d[a] | | | |
| 13 | 6.30 d | 6.29 d | | | |
| 13b | 5.79 d | 5.78 d | | | |
| 14 | 1.31 s | 1.30 s | | | |
| 15 | 1.11 d | 1.10 d | | | |

[a] Center of an AB-system.*J* (Hz): **4** and **5**: 2,3: 11.98; 3,4: 11.35; 4,15: 6.30; 4,5a: 12.61; 4,5b: 5.05; 5a,5b: 13.87; 5a,6: 5.04; 5b,6: 11.98; 7,13a: 1.26; 7,13b: 1.26; 8,9: 10.1; *ibut*: 2′,3′: 7.57; 2′,4′: 6.94; 2-*Mebut*: 2′,3′a: 13.87; 2′,5′: 6.93; 3′a, 4′: 7.57; 3′b,4′: 7.57.

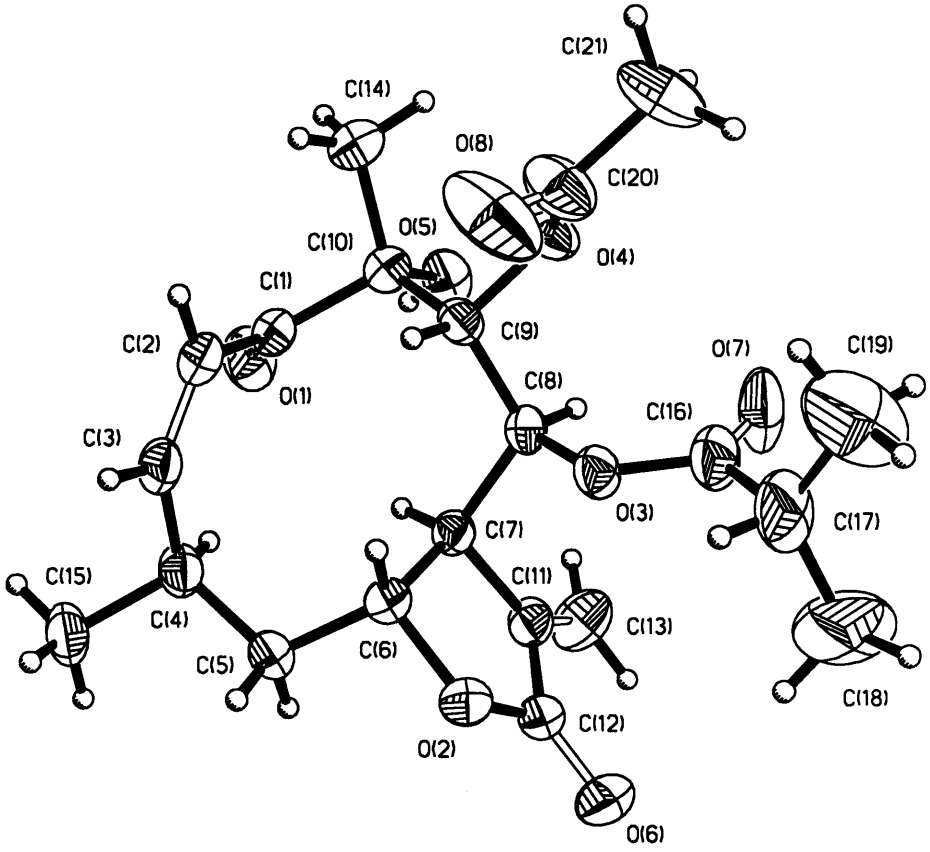



Fig. 1. ORTEP-like draw showing the atom numbering scheme for neurolenin G (**4**) thermal ellipsoid at 30% probability level.

esters. As these two short acids were often found as ester side chain of sesquiterpene lactones in the Asteraceae (Seaman, 1982), it is very questionable, if their occurrence can support the differences between *N. lobata* and *N. macrocephala* of the section *Neurolaena* as strong as the absence of flavones and 6-hydroxykaempferol derivatives in the flavonoid pattern of *N. macrocephala* (Kerr et al., 1981). Since the presented results are the first findings of sesquiterpene lactones in *N. macrocephala,* further and more detailed studies on this species are in progress. They will probably proof the proposed differences between the two sections inside the genus *Neurolaena*.

## 3. Experimental

### 3.1. Plant material

*N. macrocephala* was collected during October 5th and 6th 1996 in Laguna Escondida, 2.5 km NW of the Estacion de Biologia Tropical 'Los Tuxtlas' of the National University of Mexico, 30 km from the town of Catemaco, Veracruz, Mexico. A voucher specimen (MEXU-831848) of the plant was deposited in the herbarium of the Instituto de Biologia, UNAM.

### 3.2. Extraction and isolation

Ground material (416 g) was extracted with $CH_2Cl_2$ in a Soxhlet apparatus. Evaporation of the solvent in vacuum gave 25 g crude extract. A portion of this extract (5.5 g) was separated by CC on Sephadex LH-20 (Pharmacia) with MeOH to give six fractions (TLC monitored, toluene–EtOAc, 3:2). Fraction 4 was chromatographed on a silica gel 60 column with toluene:EtOAc (3:2) to give 12 subfractions. Further purification by prep. HPLC gave pure, crystalline **4** (11 mg), as well as mixtures of **1** and **5** (29 mg) and **2** and **3** (15 mg).

NMR: Bruker ARX 500, 500 MHz ($^1$H NMR) and 125 MHz ($^{13}$C NMR) in $CDCl_3$, calibrated on solvent signal 7.24 ppm ($^1$H) and 77.0 ppm ($^{13}$C).

MS: EI (70 eV) on Hewlett Packard MSD 5972 using GC–MS mode combined with gas chromatograph 5890 plus (Hewlett Packard); column Optima-1 (Macherey & Nagel), 25m × 0.25 mm. Temp. prog. 150 (3 min) to 280°C at 10°C min$^{-1}$. $R_t$ (min): **1**: 17.78: **4**: 17.36; **5**: 17.84.

HPLC: Hewlett Packard 1050 system, equipped with DAD detector. Detector channels set at 225 and 260 nm, RP 18 Hypersil ODS (5 μm) column (12.5 × 5 mm), flow rate 1.8 ml $min^{-1}$ (Passreiter & Medinilla Aldana, 1988; Passreiter, 1998). Solvent system: MeOH–$H_2O$ (9:11). $R_t$ (min.): **4**: 6.8; **5**: 12.0.

TLC: Silica gel 60 $F_{254}$ (Merck) toluene: EtOAc (3:2). Detection with anisaldehyde $H_2SO_4$. $R_f$ **1**: 0.5; **2**: 0.4; **3**: 0.25; **4**: 0.40; **5**: 0.49; **6**: 0.45.

### 3.3. Compound 4 (neurolenin G)

$C_{21}H_{28}O_8$, white crystals, UV $\lambda_{max}$ (MeOH:$H_2O$ (9:11)): 212, 238 (sh) nm. EIMS (*m*/*z* (rel. int.): 408 $[M]^+$ (1); 366 $[M–CH_2{=}C{=}O]^+$ (3); 338 $[M–C_2H_6C{=}C{=}O]^+$ (2); 296 $[M–(C_2H_6C{=}C{=}O + CH_2{=}C{=}O)]^+$ (1); 278 $[296\text{-}H_2O]^+$ (10); 260 $[M–(C_3H_7COOH + CH_3COOH)]^+$ (3); 250 (10); 235 (6); 217 (10); 189 (9); 165 (7); 149 (6); 111 (12); 97 (8); 82 (20); 71 $[C_3H_7CO]^+$ (33); 55 (9); 43 [71-CO and $CH_3CO]^+$ (100).

### 3.4. X-ray data

A crystal of dimensions 0.36 × 0.24 × 0.18 mm crystallized from acetone was used for data collection on a Nicolet P3/F diffractometer equipped with CuKα radiation ($\lambda = 1.54178$ Å) and Ni-filter. Crystal data are $C_{21}H_{28}O_8$, Mr = 408.43, orthorhombic space group $P2_1\,2_1\,2_1$, $a = 10.176(2)$, $b = 12.000(2)$, $c = 17.931(4)$ Å, $V = 2189.6(7)$ $Å^3$, $Z = 4$, $D_x = 1.239$ g $cm^{-3}$, $T = 293$ K. Intensity data were measured by $\omega$–$2\theta$ scans of variable rate. Two octants of data (*hkl*, *-h–k–l*) were collected within the limits $1.50 < \theta < 57.50^\circ$. Data reduction included correction for Lorentz polarization. Absorption corrections ($\mu = 0.793$ $mm^{-1}$) were not applied. 3451 reflections were collected, of which 2986 unique reflections ($R_{int} = 0.0301$) were used in the refinement of 266 parameters. The structure was solved by direct methods (Altomare et al., 1994) and refined by full matrix least squares, treating nonhydrogen atoms anisotropically, using the SHELXTL93 program (Sheldrick, 1993). Hydrogen atoms were included using a riding model, except for H-5 which was located on a difference density Fourier map and its coordinates refined, with a common $U_{iso} = 0.08$ $Å^2$. Convergence was achieved with $R = 0.059$ ($wR2 = 0.1427$). Residuals after the last refining cycle were 0.180 and −0.187 e$Å^3$. Listings of final atomic parameters, bond lengths, bond angles and torsion angles will be given on request.

### 3.5. Compound 5 (neurolenin H)

$C_{22}H_{30}O_8$, white crystals, UV $\lambda_{max}$ (MeOH:$H_2O$ (9:11)): 212, 238 (sh) nm. EIMS: *m*/*z* (rel. int.): 422 $[M]^+$ (1); 380 $[M–CH_2{=}C{=}O]^+$ (1); 362 $[M–CH_3COOH]^+$ (1); 338 $[M–C_4H_8{=}C{=}O]^+$ (2); 320 $[M–C_4H_9COOH]^+$ (1); 296 $[M–(C_4H_8{=}C{=}O + CH_2{=}C{=}O)]^+$ (1); 278 $[296\text{-}H_2O]^+$ (8); 260 $[M–(C_4H_9COOH + CH_3COOH)]^+$ (5); 250 (10); 235 (5); 217 (10); 189 (7); 165 (6); 149 (6); 111 (13); 97 (10); 85 $[C_4H_9CO]^+$ (60); 57 [85-CO]$^+$ (100); 43 (85).

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