Furanoheliangolides from Leaves of *Neurolaena macrocephala*[§]

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Six sesquiterpene lactones, two new (**3** and **6**) and four known (**1**, **2**, **4** and **5**) were isolated from the leaves of the Mexican *Neurolaena macrocephala* (Asteraceae). The furanoheliangolide **6**, containing 4β,5-dihydro-9α-hydroxy-atripliciolide as basic structure, was found for the first time in nature. The chemotaxonomic importance of this phytochemical work is discussed.

Introduction

Neurolaena macrocephala SCH. BIP. EX HEMSL. is a member of the genus *Neurolaena*, which includes the widespread, frequently used medicinal plant *N. lobata* (Arnason *et al.*, 1980; Morton, 1981; Nash and Williams, 1976). In contrast to this widely distributed species, *N. macrocephala* only occurs in tropical areas of Veracruz, in the southeast of Mexico (Turner, 1982). Turner (1982) placed *N. macrocephala* into the Section Neurolaena, and therein into the Series Macrocephala. Therefore this species should be closely related to *N. lobata*, which is placed in the Series Neurolaena of the same section (Turner, 1982). As *N. macrocephala* is lacking flavones and 6-hydroxykaempferol derivatives, which were found in both *N. lobata* and *N. oaxacana* (Kerr *et al.*, 1981; Ulubelen *et al.*, 1980), Turner (1982) concluded that the infrageneric placement of those species is not as easy as expected from their morphology.

Since sesquiterpene lactones are also used as taxonomic markers (Seaman, 1982) they may help to clarify the infrageneric relationships inside the genus. In continuation of our studies of the sesquiterpene lactones in plants of this genus (Passreiter *et al.*, 1999a; 1998; 1995), we recently reported the occurrence of mainly neurolenin type sesquiterpene lactones from *N. macrocephala* (Passreiter *et al.*, 1999b). This paper deals with the isolation and identification of further sesquiterpene lactones of the furanoheliangolide type.

Results and Discussion

Three fractions of the purified dichloromethane extract of *N. macrocephala* obtained by CC on Sephadex LH-20 were found to contain sesquiterpene lactones. Further purification of two fractions by CC on silica gel and preparative HPLC afforded the new compounds **3** and **6**, as well as the known lactones **1, 2,** and **5**, previously isolated from *Neurolaena* species (Passreiter *et al.*, 1999a; 1995) and **4**, isolated from *Calea rupicola* (Schmeda-Hirschmann *et al.*, 1986). The structures of the new compounds followed from their mass, ^{1}H and ^{13}C NMR spectra. All assignments were additionally confirmed by homo- and heteronuclear correlation experiments (2D-COSY, 2D-HMQC).

The mass spectrum of **3** was very similar to that of **1**. The presence of fragment ions at *m/z* 85 ($[C_5H_9CO]^+$) and 57 ($[85\text{-}CO]^+$) indicated the presence of an ester moiety build from a saturated five membered acid. In accordance with that, the ^{13}C NMR spectra of **3** contained signals for 20 carbons, fifteen of them found at shift values previously reported for C-1–C-15 of lobatin B (**1**).[9,14] Small differences only exist for C-8 and other carbons in this region of the molecule (see Experimental). The remaining five carbons due to the acid moiety were found at shift values characteristic for 2-methyl butyric acid (Passreiter *et al.*, 1999b; Budesinsky and Saman, 1995). The shift values and the respective coupling constants of the corresponding signals found in the ^{1}H NMR clearly indicated that all relative configurations in **3** are the same as found for **1** and similar furanoheliangolides (Passreiter *et al.*, 1995; Budesinsky

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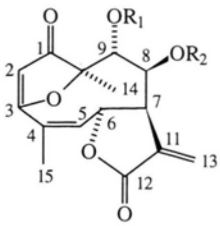
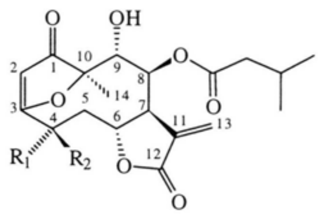


	R^1	R^2
1	H	iVal
2	Ac	iVal
3	H	2-Mebu
4	H	iBut



	R^1	R^2
5	H	Me
6	Me	H

and Saman, 1995). Thus, in analogy to the previously found pair of neurolenins (Passreiter *et al.*, 1999b), compound **3** should be 9α-hydroxy-atripliciolide-8-O-2-methylbutyrate, a derivative of lobatin B (**1**) containing 2-methylbutyric instead of 3-methylbutyric acid.

Compound **4** was previously reported from *Calea rupicola* (Schmeda-Hirschmann *et al.*, 1986) However, its structure as a derivative of **3** was elucidated by ^{1}H NMR only. The reported data were in full agreement with our data. Its structure as 9α-hydroxy-atripliciolide-8-O-isobutyrate was confirmed by its ^{13}C NMR data (see Experimental), which are reported here for the first time. The assignment of the NMR data was additionally confirmed by 2D-COSY and 2D-HMQC spectra.

Compound **3** is a new natural product and **4** was found for the second time in nature only, although other derivatives of the underlying sesquiterpene lactone have often been found in nature (Passreiter *et al.*, 1995; Budesinsky and Saman, 1995).

Analogous to **3,** the signals for 20 carbons were found in the ^{13}C NMR spectrum of **6**. In accord to its mass spectrum, **6** ($[M]^+$ 378) should be a dihydro derivative of **3** ($[M]^+$ 376), which was confirmed by the absence of the double bond signals at δ 131.36 (C-4) and 134.31 (C-5). Thus, **6** could be identical to **5**, but due to shift differences between the carbons C-3, C-4, C-5, and C-15 of **5** and **6** and by comparison to the similar 4,5-dihydro-15-desoxygoyazensolides isolated from *Eremanthus* species (Vichnewski *et al.*, 1989), it was obvious, that the methyl group attached to C-4 is α-oriented in **6,** while it is β-oriented in **5**. This was additionally confirmed by the ^{1}H NMR spectrum of **6**, in which a sharp singlet for H-2 and a subsequent missing allylic coupling between H-2 and H-4 is diagnostic for an α-oriented C-4 methyl group (Vichnewski *et al.*, 1989). The orientation of the methyl group is also evident from the shift values and couplings of H-5α and H-5β (Vichnewski *et al*, 1989). The C-9 hydroxylation clearly follows from the shift values for H-9 (δ 4.08) and C-9 (δ 72.87) and its α-orientation is evident from the couplings found for H-8 and H-9 (see Experimental). Compound **6** is therefore derived from the furanoheliangolide atripliciolide (Bohlmann and Dutta, 1979). Although 4αH,5-dihydro derivatives have already been reported (Gao *et al.* 1987; Fischer *et al.*, 1984), no 4β,5-dihydro derivative was found so far. However, several compounds have originally been reported as 4β,5-dihydro derivatives of atripliciolide (Bohlmann *et al.*, 1982; Lee *et al.*, 1982), but their stereochemistry at C-4 have been revised after X-ray analysis of 9α-acetoxyzexbrevin (Fronczek *et al.*, 1983).

Since all signals obtained from **6** were assigned by comparison with the corresponding 2D-COSY and HMBC spectra it is very likely, that the previously reported ^{13}C shift values for the signals of C-6 and C-9 in **5** have to be interchanged (Passreiter *et al.*, 1995).

N. macrocephala is the first plant in the genus *Neurolaena*, in which sesquiterpene lactones containing an α-oriented methyl substituent at C-4 were found. All other species investigated so far were only containing 4α,5-dihydro derivatives of atripliciolide, namely the calyculatolides with a β-oriented methyl group at C-4 (e.g. **5**). The other furanoheliangolides found in *N. macrocephala* were of the same type as found in other plants of the genus (Passreiter *et al.*, 1999a; 1998; 1995), but esters containing 2-methylbutyric and isobutyric acid attached to furanoheliangolides were exclusively found in *N. macrocephala*. This finding together with the possibly more valuable occurrence of 4β,5-dihydroatripliciolides underlines the outstanding position of this plant and let us assume, that the sesquiterpene lactone pattern of *N. macrocephala* together with the findings of Kerr *et al.* (1981) and Ulubelen *et al.* (1980), can possibly help to clarify the complex relationships inside the genus *Neurolaena* (Turner, 1982).

Experimental Section

General experimental procedures

NMR: Bruker DRX 500, 500 MHz (^1H) and 125 MHz (^{13}C) in $CDCl_3$. GC-MS: EI (70 eV) using the GC-MS mode on a MSD 5972 combined with a 5890 plus gas chromatograph (Hewlett-Packard); column 25m × 0.25 mm (Optima-1, Macherey & Nagel). Temperature progression: 150 °C (3 min) to 280 °C at 10°min^{-1}. HPLC: HP 1050 system, equipped with DAD detector. Detector channels set at 215 and 260 nm, with a RP_{18} Nucleosil 100 (5 μm) column (250 × 10 mm). Mobile phase: CH_3CN-H_2O (3:7 v/v) for isolation of compounds **3** and **4**; CH_3CN-H_2O (1:3 v/v) for isolation of compound **6**. TLC: Silica gel 60 F_{254} (Merck) toluene:EtOAc (3:2 v/v). Detection with anisealdehyde /H_2SO_4.

Plant material

Neurolaena macrocephala was collected during October 5th and 6th 1996 in Laguna Escondida, 2.5 km NW of the Estación de Biología 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 Biología, UNAM.

Extraction and isolation

Ground material (416 g) was extracted with CH_2Cl_2 in a Soxhlet apparatus. Evaporation of the solvent *in vacuo* 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 7 fractions (TLC monitored, toluene-EtOAc, 3:2 v/v). Fractions 4 and 5, respectively, were separated on silica gel 60 columns with toluene:EtOAc (3:2 v/v). The resulting subfractions were monitored using TLC. Subfractions 4.15, containing **6**, and 5.11, containing **3** and **4**, were further purified by prep. HPLC and gave pure **3** (4.1 mg), **4** (4.1 mg) and **6** (6.1 mg). Compounds **1**, **2** and **5** were isolated from our fractions as described previously (Passreiter *et al.*, 1995).

9α-Hydroxy-atripliciolide-8-O-2-methylbutyrate (**3**)

UV [MeOH-H_2O (9:11)] λ_{max} 216 nm, 267 nm; ^1H NMR ($CDCl_3$, 500 MHz) δ 6.33 (1H, d, J = 2.8 Hz, H-13a), 5.95 (1H, m, H-5), 5.72 (1H, d, J = 2.5 Hz, H-13b), 5.60 (1H, s, H-2), 5.26 (1H, m, H-6), 5.07 (1H, dd, J = 1.7, 5.0, H-8), 4.00 (1H, d, J = 5.1, H-9), 3.85 (1H, m, H-7), 2.27 (1H, m, H-2′), 2.04 (3H, s, H-15), 1.58 (1H, m, H-3′a), 1.54 (3H, s, H-14), 1.38 (1H, m, H-3′b), 1.06 (3H, d, J = 7.3, H-5′), 0.83 (3H, t, J = 7.6, H-4′); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 203.70 (C-1), 185.78 (C-3), 175.14 (C-1′), 168.76 (C-12), 138.93 (C-11), 134.31 (C-5), 131.36 (C-4), 124.39 (C-13), 103.99 (C-2), 89.86 (C-10), 77.73 (C-8), 75.04 (C-9), 73.51 (C-6), 44.55 (C-7), 40.98 (C-2′), 26.05 (C-3′), 19.55 (C-14), 17.61 (C-15), 16.27 (C-5′), 11.40 (C-4′); EIMS m/z 376 $[M]^+$ (1), 292 (1), 217 (3), 189 (4), 143 (15), 161 (100) 162 (12), 133 (22), 117 (9), 91 (11), 85(75), 57 (53), 43 (56).

9α-Hydroxy-atripliciolide-8-O-isobutyrate (**4**)

UV [MeOH-H_2O (9:11)] λ_{max} 216 nm, 267 nm; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 203.80 (C-1), 185.87 (C-3), 175.61 (C-1′), 168.97 (C-12), 139.10 (C-11), 134.23 (C-5), 131.36 (C-4), 124.47 (C-13), 104.11 (C-2), 89.95 (C-10), 77.85 (C-8), 75.05 (C-9), 73.29 (C-6), 44.26 (C-7), 33.97 (C-2′), 19.57 (C-15), 18.83 (C-3′), 18.26 (C-4′), 17.75 (C-14); EIMS m/z 362 $[M]^+$ (1), 217 (3), 189 (3), 162 (10), 161 (76), 146 (3), 133 (20), 129 (14), 118 (9), 105 (6), 101 (7), 91 (10), 71 (63), 43 (100).

4β,5-Dihydro-9α-hydroxy-atripliciolide-8-O-isovalerate (**6**)

UV [MeOH-H_2O (9:11)] λ_{max} 210 nm, 265 nm; ^1H NMR ($CDCl_3$, 500 MHz) δ 6.32 (1H, d, J = 3.5 Hz, H-13a), 5.72 (1H, d, J = 2.8 Hz, H-13b), 5.54 (1H, s, H-2), 5.04 (1H, d, J = 5.1 Hz, H-8), 4.49 (1H, dd, J = 5.0, 9.5 Hz, H-6), 4.08 (1H, d, J = 4.7 Hz, H-9), 3.59 (1H, m, H-7), 3.14 (1H, ddq, J = 6.6, 6.9, 10.7 Hz, H-4), 2.40 (1H, dd, J = 6.9, 13.7 Hz, H-5β), 2.06 (1H, dd, J= 3.5, 7.6 Hz, H-2′), 2.00 (1H, ddd, J = 9.5, 10.7, 13.7 Hz, H-5α), 1.95 (1H, m, H-3′), 1.47 (3H, s H-14), 1.29 (3H, d, J = 6.6 Hz, H-15), 0.87 (3H, d, J = 6.6 Hz, H-4′), 0.87 (3H, d, J = 6.6 Hz H-5′); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 204.16 (C-1), 191.67 (C-3), 175.54 (C-1′), 168.73 (C-12), 139.98 (C-11), 123.92 (C-13), 104.75 (C-2), 91.16 (C-10), 77.39 (C-8), 76.36 (C-6), 72.87 (C-9), 45.66 (C-7), 42.76 (C-2′), 41.68 (C-5), 33.76 (C-4), 25.16 (C-3′), 22.29 (C-4′), 22.23 (C-5′), 18.61 (C-14), 17.80 (C-15); EIMS m/z 378 $[M]^+$ (1), 350 (1), 322 (2), 288 (1), 273 (2), 248 (2), 235(6), 222 (4), 204 (5), 191 (51), 161 (19), 143 (19),139 (14), 126 (21), 105 (25), 91 (17), 85 (75), 57 (63), 43 (100).

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