

New Diacylated 2-Hydroxythymol Derivatives from *Melampodium divaricatum*

Juliane Schüngel and Claus M. Passreiter*

Institut für Pharmazeutische Biologie, Heinrich-Heine-Universität, Universitätsstr. 1,
D-40225 Düsseldorf, Germany. Fax: +49 21 18 11 19 23. E-mail: passreit@uni-duesseldorf.de

* Author for correspondence and reprint request

Z. Naturforsch. **57c**, 966–968 (2002); received July 24/August 19, 2002

Melampodium divaricatum, Thymol Derivatives, 2,5-Dihydroxythymol

Melampodium divaricatum is a medicinal plant, which occurs in Central America. In a recent paper we reported the occurrence of acylated 2-hydroxy thymol glycosides as main constituents in this plant. This paper deals with the isolation of two new 2,5-dihydroxythymol ester derivatives. The formerly reported sesquiterpene lactone mikanokryptin was not found in our plant material.

Introduction

Melampodium divaricatum is a member of the Asteraceae, which is widespread throughout Central America. Its leaves are used in Guatemala for treatment of malaria and stomach pains, and the whole plant is used against influenza (Giron *et al.*, 1991). In Mexico embolism is treated with an infusion of the whole plant (Martinez Alfaro, 1984) and it is used as remedy for wounds, as anti-dysenteric and anticonvulsive, taken orally or rectally (del Amo, 1980; Morton, 1981; Martinez, 1990).

It was reported that the terpenoid fraction of *M. divaricatum* contains several sesqui- and diterpenes as well as a variety of acylated 2-hydroxythymol 3-O-glycosides (Bohlmann and Le Van, 1977; Hubert and Wiemer, 1985). The latter group of compounds, only found in *M. divaricatum* so far, grew by addition of twelve acylated 2-hydroxythymol 3-O-diglycosides containing fucose and quinovose as sugar moiety (Hüther *et al.*, 1999).

The occurrence of the sesquiterpene lactone mikanokryptin (**3**), reported by Herz and Kalyanaraman (1975) in plant material collected in Panama, was challenged by Seaman *et al.* (1980), who could not find this compound in a collection from Costa Rica. They additionally argued that mikanokryptin is the only guaianolide found in *Melampodium* species and the plant material investigated by Herz and Kalyanaraman (1975) may therefore be mixed up with *Mikania micrantha*, which is rich in mikanokryptin (Herz *et al.*, 1975). Since sesquiterpene lactones have successfully been tested

against widespread vectors of dysentery (Passreiter *et al.*, 1999), and different *Plasmodium* species causing malaria (François *et al.*, Agtmael *et al.*, 1999), it was interesting to check, if such compounds and especially **3** are present in *M. divaricatum*.

Results and Discussion

In order to detect even the smallest quantities of mikanokryptin (**3**) in *M. divaricatum*, we firstly isolated this sesquiterpene lactone from *M. micrantha* collected in Mexico and proved its identity by NMR. The methanol-soluble parts of the dichloromethanic extract from *M. divaricatum* were then screened by GC-MS. Although no mikanokryptin was found, which confirmed the theory of Seaman *et al.* (1980), we found one fraction, possibly containing unsaturated C-5 acid ester derivatives of mikanokryptin, which could be assumed from the fragment ions found in their GC-MS spectrum (see Experimental). Although the ¹³C NMR spectra coincidentally showed 19 signals representing 20 carbons of a major compound **1**, which could be caused by fifteen carbons of a sesquiterpene lactone plus five carbons of a unsaturated acid, it was unambiguously clear by their shift values that the isolated compound was no lactone, but an aromatic monoterpene diester. The main compound **1** was additionally accompanied by 25% impurities consistent of a similar or even isomeric compound **2**. Since ten of the 20 major signals found for **1** were caused by the presence of two angelic acid ester groups (C-1'–C-5'

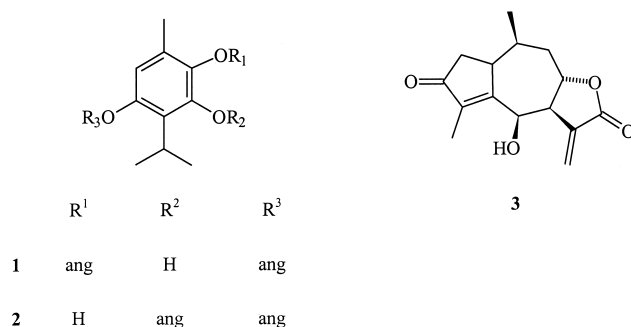


Fig. 1.

and C-1''–C-5'') (Budesinsky and Saman, 1987; Joseph-Nathan *et al.*, 1984), the remaining 9 signals representing ten carbons identified the alcohol component as a derivative of p-cymene. The signals were assigned to three methyl carbons at δ 16.5 (C-7) and 20.6 (C-9,C-10), one aliphatic methine carbon at δ 26.07 (C-8) and six aromatic carbons at δ values between 126.1 and 146.6 (C-1–C-6). Only one of the latter (C-6) showed a correlation to a proton (H-6, δ 6.5 ppm) in the HMQC spectrum, the other five were found to be quaternary, three of which by oxygenation (C-2, C-3, C-5). After interpretation of the signals found in the ^1H NMR and the corresponding cross peaks found in the 2D-COSY and HMQC spectra, **1** was unambiguously identified as 2,3,5-trihydroxy derivative of the monoterpene p-cymene, esterified twice by angelic acid. However, the positions of the angelic ester groups could only be deduced from the additionally recorded long-range HMBC spectrum, which also allowed the assignment of all aromatic carbon signals. From the cross peaks found, it was clear, that the two angelic acids are attached to hydroxyl groups at C-2 and C-5 while the C-3 hydroxyl group is free.

Compound **2** only differed from **1** by the position of one ester side chain. While both compounds had one angelic acid attached to the hydroxy group at C-5, the second ester in **2** was found at C-3, as seen from its HMBC spectrum.

Both compounds are new natural compounds. The 2,3,5-trihydroxy derivatives of the monoterpene p-cymene can also be interpreted as derivatives of 2-hydroxythymol, which was found as an aglycone in several glycosides isolated from *M. divaricatum*. However, trihydroxylated aromatic monoterpenes have never been found as a glycoside in *M. divaricatum*.

The presence of the sesquiterpene lactone mikanokryptin can be ruled out in our plant material after this investigation. The identity of the Panamanian plant material containing mikanokryptin is therefore questioned again, as much as Seaman (1980) suggested after investigation of plants from Costa Rica. The only excuse could be the occurrence of a chemical race, which has not proven yet.

Material and Methods

Plant material. *Melampodium divaricatum* (Rich. in Pers.) DC. cv. Medallion was cultivated in the New York Botanical Garden, Bronx, NY, and collected in summer 1994 at the flowering stage. A voucher specimen was deposited at the herbarium of the Institute for Pharmaceutical Biology at the Heinrich-Heine-University of Düsseldorf, Reg. No. 147.

Extraction and isolation. Dried and powdered aerial parts (1025 g) of *M. divaricatum* were extracted with CH_2Cl_2 and the extract (43 g) macerated 5 times with 100 ml MeOH. The extract was divided into nine fractions by gel chromatography of the soluble material (21 g) with Sephadex LH-20/MeOH. Fraction 4 containing **1** and **2** was further purified by CC on silica gel using toluene/EtOAc 9:1 v/v as mobile phase. After MPLC on a LiChroprep RP-18 column using a MeOH/ H_2O gradient a yellow oil (8.7 mg) was obtained consistent of an inseparable mixture of **1** and **2**.

MPLC: RP 18 (LiChroprep 15–25 μm), 150 \times 10 mm, H_2O /MeOH gradient starting at 0% MeOH. The medium was changed to H_2O /MeOH (40:60 v/v) by 6% per min, to H_2O /MeOH (10:90 v/v) by 0.33% per min and finally to 100% MeOH by 1% per min. Detection: UV 220 nm. TLC: silica gel 60 F₂₅₄, toluene-EtOAc (2:3); detection: anisaldehyde- H_2SO_4 , compounds **1–2** brown, later yellow.

NMR: Varian VXR 300 and Bruker ARX 400.

GC-MS: EI (70 eV) HP MSD 5972 with GC 5890 plus (HP); Optima-1 (MN), 25 m × 0.25 mm; 150 °C (3 min) to 280 °C at 10 ° min⁻¹; R_t (min): 13.57, not separated; 150 °C (1 min) to 280 °C at 10 ° min⁻¹; R_t (min): **1** and **2**: 10.3 min, not separated.

HPLC: HP 1050, DAD, 215 and 260 nm, Hypersil 120–5 ODS (5 µm, 125 × 4.6 mm), flow 3.0 ml min⁻¹. MeOH-H₂O (60:40) to MeOH (100%) at 2% per min. R_t (min): **1** and **2**: 8.3 min, not separated.

TLC: Silica gel 60 F₂₅₄; toluene/EtOAc (4:1); anisaldehyde/H₂SO₄; R_f: 0.7.

2,5-Diangeloyloxythymol (**1**) and 5-Angeloyloxy-2-hydroxythymol angeloat (**2**): λ_{max} MeOH/H₂O (1:1): 222 nm, 269 nm. EI-MS *m/z* (rel. int.): 346 [M]⁺ (1), 264 [M–C₄H₆CO]⁺ (1), 182 [M–2 × C₄H₆CO]⁺ (1), 167 [182–CH₃]⁺ (1), 91 (1), 83 [C₄H₇CO]⁺ (100), 69 (1), 55 [83–CO] (59), 53 (4), 41 (1).

IR (KBr), ν_{max} cm⁻¹: 3440 (OH), 1730 (CO, α,β-unsaturated ester), 1645 (C = C).

¹H NMR (300 MHz, CDCl₃, TMS): **1**: δ 6.50 (1H, d, *J* = 0.8 Hz, H-6), 6.36 (1H, m, H-3'), 6.26 (1H, m, H-3''), 3.20 (1H, m, H-8), 2.12 (3H, s (br.), H-7), 2.11 (3H, d, *J* = 6.0 Hz, H-4'), 2.10 (3H, s, H-5'), 2.08 (3H, d, *J* = 6.0 Hz, H-4''), 2.06 (3H, s, H-5''), 1.30 (6H, d, *J* = 7.1 Hz, H-9, H-10). **2**: δ 6.73

(1H, d, *J* = 0.7 Hz, H-6), 6.36 (1H, m, H-3'), 6.26 (1H, m, H-3''), 3.01 (1H, m, H-8), 2.23 (3H, s (br.), H-7), 2.10 (3H, d, *J* = 6.0 Hz, H-4'), 2.10 (3H, s, H-5'), 2.07 (3H, d, *J* = 5.9 Hz, H-4''), 2.06 (3H, s, H-5''), 1.22 (6H, d, *J* = 7.1 Hz, H-9, H-10).

¹³C NMR (300 MHz, CDCl₃, TMS): **1**: δ 166.41 (C-1'), 165.25 (C-1''), 146.61 (C-3), 146.28 (C-5), 142.60 (C-3'), 140.55 (C-3''), 135.47 (C-2), 128.41 (C-1), 127.17 (C-2'), 126.37 (C-2''), 126.05 (C-4), 116.38 (C-6), 26.07 (C-8), 20.76* (C-5'), 20.74* (C-5''), 20.62 (C-9, C-10), 16.47 (C-7), 16.25 (C-4'), 15.94 (C-4'') *assignment interchangeable. **2**: δ 166.62 (C-1'), 165.61 (C-1''), 137.50 (C-3), 141.74 (C-5), 142.30 (C-3'), 140.74 (C-3''), 143.64 (C-2), 124.36 (C-1), 127.13 (C-2'), 126.49 (C-2''), 130.08 (C-4), 122.44 (C-6), 26.46 (C-8), 20.76* (C-5'), 20.74* (C-5''), 21.02 (C-9, C-10), 15.70 (C-7), 16.13 (C-4'), 15.94 (C-4'') *assignment interchangeable.

Acknowledgements

We are grateful to Dr. A. Steigel, Institut für Organische und Makromolekulare Chemie, Heinrich-Heine-Universität Düsseldorf, for recording the 300 MHz NMR spectra and to Dr. Victor Wray, Gesellschaft für Biotechnologische Forschung, Braunschweig for recording the 400 MHz 2D-NMR spectra.

- Van Agtmael M. A., Eggelte T. A. and Van Boxtel C. J. (1999), Artemisinin drugs in the treatment of malaria: from medicinal herb to registered medication. *Trends Plant Sci.* **20**, 199–205.
- Amo S. del (1980), Plantas medicinales del Estado de Vera-Cruz, Insitituto Nacional de Investigaciones sobre Recursos Bióticos, Xalapa (Veracruz), 2^a edición.
- Bohlmann F. and Le Van N. (1977), Terpen-glykoside aus *Melampodium divaricatum*. *Phytochemistry* **16**, 1765–1768.
- Budesinsky M. and Saman D. (1987), Identification of acyl groups occurring in sesquiterpene lactone: Proton and carbon-13 NMR study. *Coll. Czech. Chem. Commun.* **52**, 453.
- François G., Passreiter C. M., Woerdenbag H. J. and Van Looveren M. (1996), Antiplasmodial activities and cytotoxic effects of aqueous extracts and sesquiterpene lactones from *Neurolaena lobata*. *Planta Med.* **62**, 126–129.
- Girón L. M., Freire V., Alonzo A. and Càceres A. (1991), Ethnobotanical survey of the medicinal flora used by the Caribs of Guatemala. *J. Ethnopharmacol.* **34**, 173–187.
- Herz W. and Kalyanaraman P. S. (1975), Mikanokryptin in *Melampodium divaricatum*, *Phytochemistry* **14**, 1664.
- Herz W., Srinivasan, A. and Kalyanaraman, P. S. (1975), Mikanokryptin, a new guaianolide from *Mikania*. *Phytochemistry* **14**, 233–237.
- Hubert T. D. and Wiemer D. F. (1985), Ant-repellent terpenoids from *Melampodium divaricatum*. *Phytochemistry* **24**, 1197–1198.
- Hüther J., Passreiter C. M., Wray V. and Willuhn, G. (1999), Acylated 2-Hydroxythymol 3-O-diglycosides from *Melampodium divaricatum*. *Phytochemistry* **51**, 979–986.
- Joseph-Nathan P., Wesener J. R. and Günther H. (1984), Two dimensional NMR study of angelic and tiglic acid. *Org. Magn. Res.* **22**, 190–191.
- Martinez Alfaro M. A. (1984), Medicinal plants used in a Totonac community of the Sierra Norte de Puebla: Tuzamapan de Galeana, Puebla, Mexico. *J. Ethnopharmacol.* **11**, 203–221.
- Morton J. (1981), Atlas of Medicinal Plants of Middle America, Bahamas to Yucatan, C. Thomas, Springfield (IL).
- Passreiter C. M., Sandoval-Ramirez J. and Wright, C. W. (1999), Sesquiterpene lactones from *Neurolaena oaxacana*, *J. Nat. Prod.* **62**, 1093–1095.
- Seaman F. C., Fischer N. H. and Stuessy T. F. (1980), Systematic implications of sesquiterpene lactones in the subtribe Melampodiinae. *Biochem. Syst. Ecol.* **8**, 263–271.