PERGAMON

Phytochemistry 51 (1999) 987–990

PHYTOCHEMISTRY

(−)-Spirolepechinene, a spirosesquiterpene from *Lepechinia bullata* (Lamiaceae)

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Received 28 October 1998; received in revised form 11 January 1999; accepted 14 January 1999

Abstract

Spirolepechinene, a new sesquiterpene hydrocarbon, and the previously described spirovetivane premnaspirodiene were isolated from the essential oil of the leaves of *Lepechinia bullata* collected in Venezuela. Their structures have been established by means of spectroscopic methods. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: *Lepechinia bullata*; Lamiaceae; Essential oil; Sesquiterpenes; Spirolepechinene

1. Introduction

Lepechinia bullata (Briq.) Epl. (Lamiaceae) is a common medicinal plant in Columbia and Venezuela where the species is endemic (Hart, 1985; Barriga, 1992). Earlier investigations dealt with the occurrence of cytotoxic abietane diterpenes in the aerial parts of the plant (Jonathan, Che, Fong, & Farnsworth, 1989). The object of our studies was the volatile fraction of the leaves from *Lepechinia bullata* collected in the Venezuelan Andes. Two sesquiterpene hydrocarbons, which occurred in the essential oil to about 20% (**1**) and 45% (**2**), respectively, attracted our attention because of their unknown mass spectra. Both hydrocarbons even turned up in some of our samples of *L. salviaefolia* (Kunth) Epl. but in smaller amounts. Isolation and investigations by spectroscopic methods, namely EI-mass, ^{13}C NMR, ^{1}H NMR and 2D NMR (^{1}H–^{1}H COSY, NOESY, HMQC and HMBC) led to the structural elucidation of both compounds.

Compound (**1**) turned out to be a hitherto unknown spirosesquiterpene of a rare skeletal class, named (−)-spirolepechinene (**1**). We report here on its isolation and structural elucidation. Compound (**2**) is identical with (−)-premnaspirodiene, a spirovetivane hydrocarbon, which was isolated in the early eighties from two *Premna* species (Verbenaceae) (Rao, Raju, & Krishna, 1982; Rao, Krishna, & Suseela, 1985) but has never been reported for other plants. Its spectroscopic data are complemented in this paper.

2. Results and discussion

The hydrodistillate of *Lepechinia bullata* from Santo Domingo (Venezuela) was fractionated by column chromatography over silica gel into a polar and a non-polar hydrocarbonic fraction. From the latter fraction both sesquiterpenes were isolated by means of preparative GC on Carbowax 20M.

Spirolepechinene (**1**), with the elemental composition $C_{15}H_{24}$, was obtained as a yellowish viscous liquid. Optical rotation measurements showed the compound to be the (−)-enantiomer. The ^{1}H NMR indicated signals of four downfield shifted protons belonging to an *exo*-methylene group (δ 4.58, 1H, s and 4.49, 1H, s) and to an isopropenylic methylene (δ 4.62, 2H, br s) respectively, one secondary methyl (0.84, 3H, d, $J=6.3$) and one olefinic methyl (δ 1.65, 3H, s). ^{13}C

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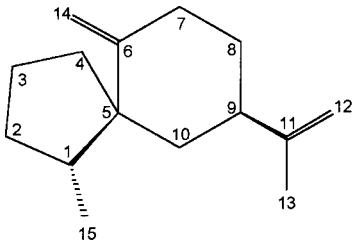
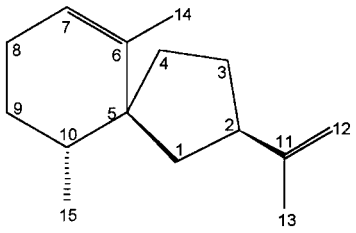
0031-9422/99/$ - see front matter
PII: S0031-9422(99)00048-5



1

(-)-Spirolepechinene



2

(-)-Premnaspirodiene

NMR-data showed the presence of four olefinic carbons (δ 104.6, 108.8, 150.9 and 154.7), two methyl carbons (δ 14.6 and 21.4), six methylene carbons (δ 21.4, 32.6, 33.8, 35.5, 35.6 and 37.2), two methine carbons (δ 39.7 and 41.5) and one quaternary carbon at δ 50.1. Taking into consideration the presented data including the 2D NMR-correlations, the quaternary carbon has to be a *spiro* C-atom that connects a five- and a six-membered ring.

(−)-Spirolepechinene (1-methyl-6-methylene-9-(1-methylene)-spiro[4.5]decane, **1**) is a new compound of a rare skeletal class that was designated as spirojatamane ring system by Huang and Forsyth (1995). His nomenclature refers to (+)-spirojatamol, a sesquiterpene alcohol of *Nardostachys jatamansi* (Bagchi, Oshima, & Hikino, 1990). The second hitherto known compound of this class is (−)-erythrodiene, which was isolated from the Caribbean coral *Erythropodium caribaeorum* (Coxon, Price, & Howard, 1974). The relative configuration of these compounds at C-5 is epimeric to (−)-spirolepechinene (**1**), which is most remarkable. The relative configuration could be definitely derived from its NOE correlations (Fig. 1) observed between H-14′ and H-1, H-14′ and the methylic protons H-15, as well as between H-15 and H-10′. Further NOEs between H-9 and H-4, along with effects between H-9 and H-10 confirm the *S*-configuration of the spiro C-atom.

(−)-Premnaspirodiene (**2**) is the main component of the essential oil of *L. bullata*. Its EIMS (70 eV) has not yet been published. Using NMR techniques we proved the skeleton to be a 6,10-dimethyl-2-(1-methylethenyl)-spiro[4.5]dec-6-ene. The ^{13}C NMR data in $CDCl_3$ turned out to be identical with the results published by Rao et al. (1982) except for his confusion of two methylene groups. Better resolved ^1H NMR spectra were achieved by measurements in C_6D_6. The NOESY data support the structure of premnaspirodiene (**2**) reported by Rao et al. (1982). The fact that premnaspirodiene causes a negative optical rotation is also in agreement with our structure. The sign of the optical rotation corresponds to that of (−)-solavetivone (6,10-dimethyl-2-(1-methylethenyl)-spiro[4.5]dec-6-en-8-one) (Coxon, Price, & Howard, 1974), also a natural product of higher plants, whose relative configuration is identical to that proposed for (−)-premnaspirodiene (**2**). This conclusion is supported by (**2**) occurring as a synthetic intermediate during a stereoselective synthesis of (−)-solavetivone (Hwu & Wenzel, 1992).

In order to explain the co-occurrence of compound **1** and **2** we suggest an identical biogenetic precursor. An eudesmane precursor with a *cis*-decalin skeleton (Scheme 1) can lead to the formation of both, **1** and **2**. Analogous to the biosynthetic pathway of the spirovetivane solavetivone (Coolbear & Threlfall, 1985; Zook, Chappell, & Kúc, 1992) we propose pathway **b** for premnaspirodiene (**2**) (Scheme 1). The eudesmane cation can rearrange via hydogenshift, ringcontraction and following proton loss to the spirovetivane hydrocarbon (**2**). The new compound spirolepechinene (**1**) can be generated via pathway **a** from the same eudesmane cation (Scheme 1). If the formation of this spirojatamane hydrocarbon is promoted by enzymatic reaction or simple chemical rearrangement remains an interesting question for further investigations.

3. Experimental

3.1. Plant material

Lepechinia bullata (Briq.) Epl. (Lamiaceae) was collected in Sto Domingo (Estate Mérida), Venezuela, in August 1996. Voucher specimens were deposited at the Botanical Centre of Mérida, Universidad de los Andes (Venezuela).

3.2. Essential oil

100 g of the air-dried leaves were hydrodistilled in a modified Karlsruher apparatus for 4 h using *n*-hexane as collecting solvent.

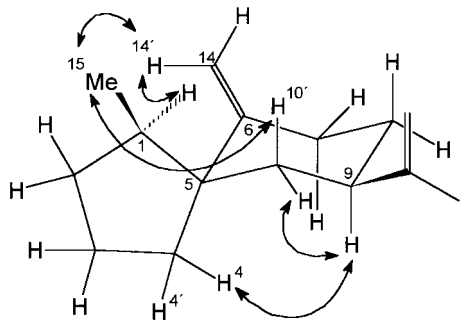


Fig. 1. Important NOE's for (−)-spirolepechinene (**1**).

3.3. Prep. GC

After fractionation of the essential oil over silica gel, the hydrocarbonic fraction was used for prep. GC on a modified Varian 1700 instrument, equipped with a stainless steel column (1.95 m × 0.5 mm) with Carbowax 20M on Chromosorb W-HP 80/100 mesh (20%). N_2 was used as carrier gas at 190 ml min^{-1}.

3.4. NMR-spectroscopy

NMR spectra were measured in C_6D_6 and $CDCl_3$ on a Bruker DRX 500 using TMS as internal standard.

3.5. GC–MS

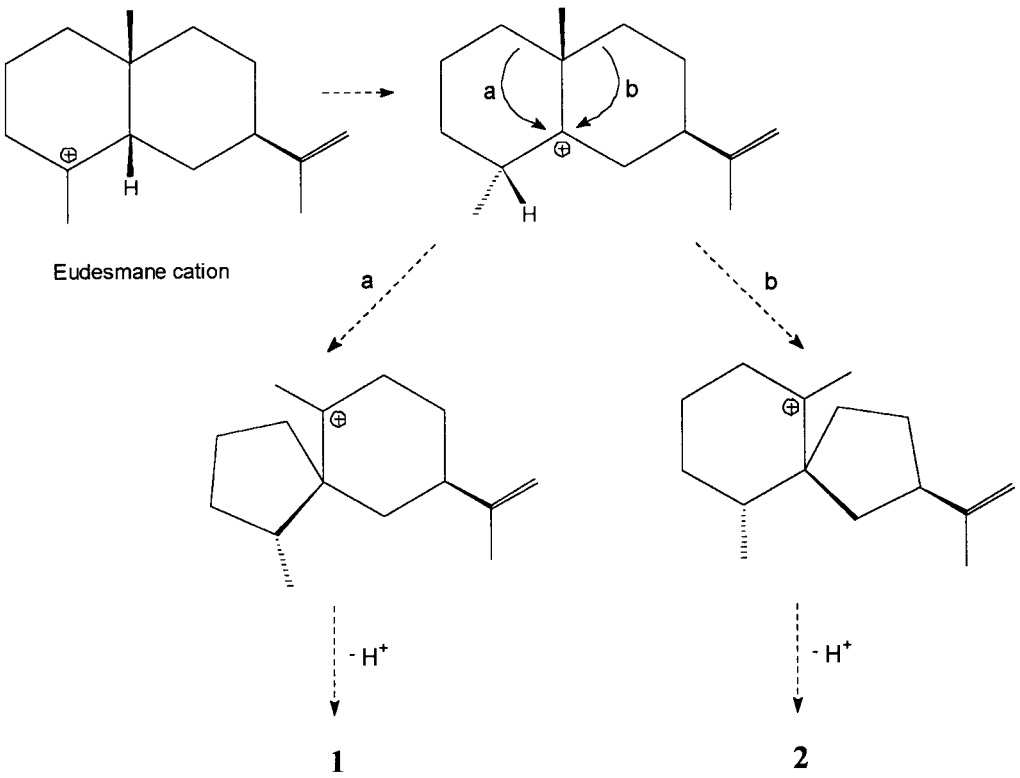
Electron impact (70 eV) GC–MS measurements were carried out on a Hewlett-Packard HP 5890 gas chromatograph (CP Sil 5 B capillary column) coupled with a VG Analytical VG 70-250S mass spectrometer.

3.6. Polarimetry

Optical rotation measurements were performed in $CHCl_3$ with a Perkin Elmer 241 polarimeter.

3.7. (−)Spirolepechinene (*1*) ((−)-(1R,5S,9R)-1-methyl-6-methylene-9-(1-methylene)-spiro[4.5]decane)

$[\alpha]_d^{20}$ −32 (*c* 0.125 $CHCl_3$); EIMS 70 eV, *m/z* (rel. int.): 204 [M^+] (28), 161 (40), 133 (56), 119 (42), 107 (76), 105 (90), 93 (91), 91 (100), 81 (48), 79 (75), 67 (50), 55 (45), 41 (72); ^{13}C NMR (125.76 MHz, $CDCl_3$): δ 14.6 (C-15), 21.4 (C-13), 21.4 (C-3), 32.6 (C-2), 33.8 (C-8), 35.5 (C-7), 35.6 (C-10), 37.2 (C-4), 39.7 (C-1), 41.5 (C-9), 50.1 (C-5), 104.6 (C-14), 108.8 (C-12), 150.9 (C-11), 154.7 (C-6); ^{13}C NMR (125.76 MHz, C_6D_6): δ 14.6 (C-15), 21.2 (C-13), 21.5 (C-3), 32.7 (C-2), 33.9 (C-8), 35.5 (C-7), 35.8 (C-10), 37.2 (C-4), 39.7 (C-1), 41.7 (C-9), 50.2 (C-5), 105.0 (C-14), 109.2 (C-12), 150.3 (C-11), 154.3 (C-6); ^{1}H NMR (500.13 MHz C_6D_6): δ 4.62 (2H, br s, H-12 and H-12′), 4.58 (1H, s, H-14),



Scheme 1. Possible route for the biosynthesis of spirolepechinene (**1**) and premnaspiodiene (**2**).

4.49 (1H, s, H-14′), 2.19 (2H, m, H-9, H-7), 2.15 (1H, m, H-7′), 2.12 (1H, m, H-1), 1.93 (1H, mc, H-4), 1.80 (1H, m, H-2), 1.74 (1H, m, H-8), 1.65 (3H, s, H-13), 1.54 (1H, m, H-3), 1.45 (1H, m, H-3′), 1.34 (1H, m, H-10), 1.29 (1H, m, H-4′), 1.25 (1H, m, H-2′), 1.18 (1H, m, H-8′), 1.05 (1H, t, $J_{10,10'}=12.65$ Hz, $J_{9,10}=12.65$ Hz, H-10′), 0.84 (3H, d, $J_{1,15}=6.3$ Hz, H-15).

*3.8. (−)-Premnaspirodiene (**2**) ((−)-(2R,5S,10R)-6,10-dimethyl-2-(1-methylethenyl)-spiro[4.5]dec-6-ene)*

$[\alpha]_d^{20}$ −88 (*c* 0.5011 $CHCl_3$); EIMS 70 eV, *m*/*z* (rel. int.): 204 [M^+] (23), 161 (48), 119 (70), 107 (100), 105 (45), 93 (85), 91 (56), 79 (58), 41 (60); ^{13}C NMR (125.76 MHz, C_6D_6): δ 14.9 (C-15), 20.3 (C-14), 21.3 (C-13), 22.0 (C-8), 27.3 (C-9), 33.1 (C-3), 34.3 (C-4), 38.0 (C-10), 44.1 (C-1), 47.0 (C-2), 48.7 (C-5), 108.7 (C-12), 121.3 (C-7), 139.1 (C-6), 148.3 (C-11); ^1H NMR (500.13 MHz C_6D_6): 5.41 (1H, s, H-7), 4.97, 4.91 (each 1H, br s, H-12), 2.50 (1H, tt, $J_{1',2}=6.95$ Hz, $J_{2,3}=6.95$ Hz, $J_{1,2}=11$ Hz, $J_{2,3'}=11$ Hz, H-2), 2.12 (1H, m, H-8), 1.95 (1H, m, H-8′), 1.83 (1H, m, H-1), 1.80 (3H, s, H-13), 1.78 (2H, m, H-4 and H-3), 1.76 (3H, m, H-14), 1.72 (1H, m, H-1′), 1.66 (3H, m, H-10, H-3′ and H-9), 1.56 (1H, m, H-4′), 1.46 (1H, m, H-9′), 1.01 (3H, d, $J_{10,15}=6.95$, H-15).

Acknowledgements

The authors are grateful to Professor Giovannina Orsini, Central University of Venezuela, Caracas, for her help in classifying the Lepechinia samples. This work was supported by the graduate sponsorship of the University of Hamburg.

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