

## Labdane Diterpenes from *Stachys plumosa*

Maria P. Paternostro,<sup>†</sup> Antonella M. Maggio,<sup>†</sup> Franco Piozzi,<sup>\*,†,‡</sup> and Orietta Servettaz<sup>§</sup>

Dipartimento Chimica Organica, Università di Palermo, Viale delle Scienze, 90128 Palermo, Italy, Istituto Chimica e Tecnologia dei Prodotti Naturali, C.N.R., La Malfa 153, 90146 Palermo, Italy, and Dipartimento Biologia, Università di Milano, Celoria 3, 20133 Milano, Italy

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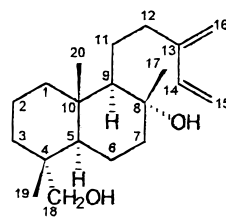
Three new labdane diterpenoids were isolated from the aerial parts of *Stachys plumosa*. The first two (**1**, **2**) were the dextrorotatory enantiomers of the known 6-deoxyandalusol and 13-epijabugodiol. Structures were determined using NMR and MS techniques. The absolute stereochemistry of the third compound (**3**) was not experimentally proved.

The genus *Stachys* (Labiatae) includes about 200–300 species. Neoclerodanes have been found in *S. recta*,<sup>1</sup> *S. annua*,<sup>2–6</sup> *S. aegyptiaca*,<sup>7</sup> and *S. rosea*;<sup>8,9</sup> kauranes in *S. lanata*<sup>10</sup> (now *S. byzantina*) and *S. sylvatica*;<sup>11</sup> and labdanes in *S. rosea*<sup>9</sup> and *S. mucronata*.<sup>12</sup> Usually, species containing diterpenoids are devoid of triterpenoids and vice versa. As a part of our ongoing search for new diterpenes with possible biological activity, we have investigated the aerial parts of *S. plumosa* Griseb., a species native to the Balkan peninsula.

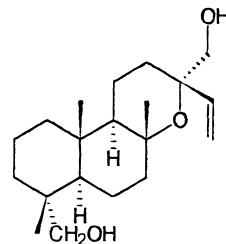
Extensive chromatographic separation of the acetone extract led to the isolation of three compounds (**1–3**). Compound **1** (C<sub>20</sub>H<sub>34</sub>O<sub>2</sub>), mp 62–65 °C, MW 306, showed <sup>1</sup>H and <sup>13</sup>C NMR spectra that matched with the reported<sup>13,14</sup> data of 6-deoxyandalusol, isolated from *Sideritis arborescens*. The absolute enantio configuration of the latter had been proved,<sup>13</sup> and the structure was correctly indicated as *ent*-8β,18-dihydroxy-labd-13(16),14-diene. However, [α]<sub>D</sub><sup>20</sup> of **1** gave a value of +16.4°, whereas the reported value<sup>13</sup> was –17.5°. Hence, **1** is 8α,18-dihydroxy-labd-13(16),14-diene with *normal* absolute configuration and has not been previously reported in nature. Thus, **1** is (+)-6-deoxyandalusol, and the product described previously is (–)-6-deoxyandalusol.

Compound **2** (C<sub>20</sub>H<sub>34</sub>O<sub>3</sub>) was obtained as a colorless amorphous solid, mp 190–192 °C, MW 322. The <sup>1</sup>H and <sup>13</sup>C NMR data of **2** were identical with those of 13-epijabugodiol, isolated<sup>14</sup> from *Sideritis arborescens* ssp. *paulii*. However, **2** showed [α]<sub>D</sub><sup>20</sup> +37.1°, whereas 13-epijabugodiol had [α]<sub>D</sub><sup>20</sup> –45°. Hence, **2** is 16,18-dihydroxy-13-epimanoyloxide with *normal* absolute configuration; it is a new natural product, and can be indicated as (+)-13-epijabugodiol, the enantiomer of (–)-13-epijabugodiol described by Spanish researchers.<sup>14</sup>

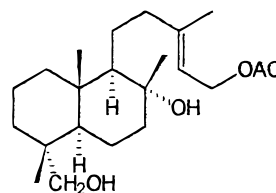
Compound **3** (C<sub>22</sub>H<sub>38</sub>O<sub>4</sub>), MW 366, [α]<sub>D</sub><sup>20</sup> +87.3°, had NMR spectra similar to those of **1** and **2**, but with some important differences. The <sup>1</sup>H spectrum showed three methyls (δ 0.73, 0.83, 1.13, each 3H, s), a –CH<sub>2</sub>OH group (δ 3.09 and 3.41, each d), an acetylic methyl (δ 2.05, s), an allylic methyl (δ 1.71, s), and a C=CH–CH<sub>2</sub>–O–CO–CH<sub>3</sub> system (δ 5.34 t, =CH–; 4.56 and 4.58, each d, –CH<sub>2</sub>–O–). The <sup>13</sup>C spectrum was consistent with the above attributions and showed three carbon atoms bonded to oxygen (two –CH<sub>2</sub>–O– and a quaternary –C–O–). These data



**1**



**2**



**3**

were consistent with a new labdane diterpenoid, represented by the structure **3** (15-acetoxy-labd-13-ene-8α,18-diol), which we gave the trivial name (+)-plumosol.

### Experimental Section

**General Experimental Procedures.** Optical rotations were determined on a Perkin-Elmer 241 polarimeter. LREIMS were collected on a Finnigan TSQ70 instrument (70 eV, direct inlet). <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra were obtained in CDCl<sub>3</sub> solution on a Bruker AC 250 E apparatus at 250 and 62.7 MHz, respectively, with chemical shifts referred to the solvent peaks (δ 7.27 and 77.0 ppm). <sup>13</sup>C NMR assignments were supported by a DEPT experiment. Merck Si gel no. 7734 (70–230 mesh) deactivated with 15% H<sub>2</sub>O was used for column chromatography (CC). Petrol refers to petroleum ether (bp 50–70 °C). Elemental analyses were made with Perkin-Elmer 240 apparatus. UV spectra (EtOH) were recorded on a JASCO 7800 instrument.

**Plant Material.** The plant used in this work, cultivated at Toscolano on Garda Lake (Experimental Field of the Botanic

\* To whom correspondence should be addressed. Tel.: 39-091-596905. Fax: 39-091-596825. E-mail: organica@unipa.it.

<sup>†</sup> Università di Palermo.

<sup>‡</sup> Istituto di Chimica e Tecnologia dei Prodotti Naturali–C.N.R.

<sup>§</sup> Università di Milano.

Garden of the University of Milano), was harvested in August 1996 and May 1997. Voucher specimens are deposited in the Herbarium of the Department of Biology, University of Milano.

**Extraction and Isolation.** Dried and finely powdered aerial parts (1 kg) were extracted with Me<sub>2</sub>CO (2.5 L × 3) at room temperature for one week. After removal of the solvent in vacuo, the residue was subjected to CC, eluting with petrol and a gradient of petrol/EtOAc. The fractions eluted with petrol/EtOAc (5:1) yielded sitosterol (1 g), identified by its physical (mp, [α]<sub>D</sub>) and spectroscopic (<sup>1</sup>H NMR, MS) data in comparison with an authentic sample. The fractions eluted with petrol/EtOAc (5:2) gave (+) 6-deoxy-andalusol (**1**) (500 mg, 0.05%), which was purified by CC with a gradient of CH<sub>2</sub>Cl<sub>2</sub>/MeOH. The fractions eluted with petrol/EtOAc (1:1) gave a mixture of two compounds, that was rechromatographed with a petrol/Me<sub>2</sub>CO gradient yielding, in order of increasing polarity, (+)-13-epijabugodiol (**2**) (5 mg, 0.0005%) and (+)-15-acetoxy-labd-13-ene-8α,18-diol (**3**) (30 mg, 0.003%).

**(+)-6-Deoxyandalusol [8α,18-dihydroxy-labda-13(16),-14-diene] (1):** amorphous solid, mp 62–65 °C; [α]<sub>D</sub><sup>20</sup> + 16.4° (c 1.014, CHCl<sub>3</sub>); UV (EtOH) λ<sub>max</sub> 227 (ε 11 220) nm; identified by the physical (mp, [α]<sub>D</sub>) and spectroscopic (<sup>1</sup>H NMR, <sup>13</sup>C NMR, MS) data previously reported<sup>13,14</sup> for the enantiomer (–)-6-deoxyandalusol; *anal.* C 78.22%, H 11.10%; calcd for C<sub>20</sub>H<sub>34</sub>O<sub>2</sub>, C 78.38%, H 11.18%.

**(+)-13-Epijabugodiol (16,18-dihydroxy-13-epimanoyle-oxide) (2):** colorless amorphous solid mp 190–192 °C, [α]<sub>D</sub><sup>20</sup> + 37.1° (c 0.491, CHCl<sub>3</sub>); identified by the physical (mp, [α]<sub>D</sub>) and spectroscopic (<sup>1</sup>H NMR, <sup>13</sup>C NMR, MS) data previously reported<sup>14</sup> for the enantiomer (–)-13-epijabugodiol; *anal.* C 74.35%, H 10.58%, calcd for C<sub>20</sub>H<sub>34</sub>O<sub>3</sub>, C 74.49%, H 10.63%.

**(+)-Plumosol (15-acetoxy-labd-13-ene-8α,18-diol) (3):** colorless thick oil, [α]<sub>D</sub><sup>20</sup> + 87.3° (c 0.369, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 250 MHz) δ 5.34 (1H, t, *J* = 7 Hz, H-14), 4.56 (1H, d, *J* = 7 Hz, H-15a), 4.58 (1H, t, *J* = 7 Hz, H-15b), 3.41 (1H, d, *J* = 10.8 Hz, H-18a), 3.09 (1H, d, *J* = 10.8 Hz, H-18b), 2.05 (3H, s, O–CO–Me), 1.71 (3H, s, 16-Me), 1.13 (3H, s, 17-Me), 0.83 (3H, s, 20-Me), 0.73 (3H, s, 19-Me); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 62.7 MHz) δ 171.3 (s, acetyl CO), 143.4 (s, C-13), 118.1 (d, C-14),

73.9 (s, C-8), 71.9 (t, C-18), 61.5 (t, C-15), 61.3 (d, C-9), 49.2 (d, C-5), 44.3 (t, C-12), 42.8 (t, C-7), 39.2 (t, C-1), 39.1 (s, C-10), 37.6 (s, C-4), 35.2 (t, C-3), 23.7 (q, C-17), 23.4 (t, C-11), 21.1 (q, acetyl Me), 20.3 (t, C-6), 17.7 (t, C-2), 17.4 (q, C-19), 16.6 (q, C-16), 15.9 (q, C-20); EIMS *m/z* 366 [M]<sup>+</sup> (1), 348 [M – H<sub>2</sub>O]<sup>+</sup> (9), 317 [M – H<sub>2</sub>O – CH<sub>2</sub>OH]<sup>+</sup> (2), 306 [M – HOAc]<sup>+</sup> (9), 289 [M – HOAc – H<sub>2</sub>O]<sup>+</sup> (76), 271 [M – HOAc – 2H<sub>2</sub>O]<sup>+</sup> (40), 207 (32), 177 (100), 135 (28), 123 (36), 121 (38), 95 (42), 81 (64), 67 (28); *anal.* C 72.16%, H 10.38%, calcd for C<sub>22</sub>H<sub>38</sub>O<sub>4</sub>, C 72.09%, H 10.45%.

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