Phytochemistry xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Terpenoids, flavonoids and caffeic acid derivatives from *Salvia viridis* L. cvar. Blue Jeans

Supattra Rungsimakan [a,*], Michael G. Rowan [b]

[a] School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand
[b] Department of Pharmacy and Pharmacology, University of Bath, Bath BA2 7AY, UK

ARTICLE INFO

Article history:
Received 2 March 2014
Received in revised form 23 August 2014
Available online xxxx

Keywords:
Salvia viridis L.
Lamiaceae
Lupane
Triterpenoid
Diterpenoid
Abietane
Phenylpropanoid
Verbascoside
Flavonoid
Antibacterial activity

ABSTRACT

Three diterpenoids, 1-oxomicrostegiol (**1**), viroxocin (**2**), viridoquinone (**3**), were isolated from the roots of *Salvia viridis* L. cvar. Blue Jeans. Five known diterpenoids, microstegiol (**4**), 7α-acetoxy-14-hydroxy-8,13-abietadiene-11,12-dione (**5**; 7-*O*-acetylhorminone tautomer), 7α,14-dihydroxy-8,13-abietadiene-11,12-dione (**6;** horminone tautomer), ferruginol and salvinolonyl 12-methyl ether (**7**) were also found in the roots together with 1-docosyl ferulate (**8**), and a mixture of 2-(4′-alkoxyphenyl) ethyl alkanoates (**9**). Two lupane triterpenoids, 2α-acetoxy-lup-20(29)-en-3β-ol (**10**), and 3β-acetoxy-lup-20(29)-en-2α-ol (**11**) were found in the aerial parts together with known compounds, lup-20(29)-ene-2α,3β-diol (**12**), ursolic acid, oleanolic acid, β-sitosterol and β-sitosterol glucoside. A known phenylpropanoid, trans-verbascoside (or acteoside; **13**), was the main constituent in the polar fraction of the aerial part, and it is now reported in the genus *Salvia* for the first time. Other polyphenolic compounds were cis-verbascoside (**14**), leucosceptoside A (**15**), martynoside (**16**), caffeic acid, 6-*O*-caffeoyl-glucose (**18**), rosmarinic acid, salidroside, luteolin-7-*O*-α-rhamnopyranosyl-(1 → 6)-β-galactopyranoside, luteolin-7-*O*-β-galactopyranoside, luteolin-7-*O*-α-rhamnopyranosyl-(1 → 6)-β-glucopyranoside, luteolin-7-*O*-β-glucopyranoside, and apigenin-7-*O*-β-glucopyranoside. The structures were determined by 1D-, 2D-NMR and HR-ESI-MS techniques. Compounds **6**, **10**, ferruginol, ursolic acid and oleanolic acid exhibited antibacterial activity against *Enterococcus faecalis* (ATCC 775) with MIC 50 μM, 25 μM, 50 μM, 12.5 μM, 12.5 μM respectively. Ferruginol, ursolic acid and oleanolic acid were also active against *Staphylococcus aureus* (ATCC 6571), and *Bacillus cereus* (ATCC 2599) with MIC 12.5–50 μM. **4** was also active against *S. aureus* (ATCC 6571) with MIC 50 μM. These values are consistent with previous studies on the antimicrobial activity of *Salvia* diterpenoids.

1. Introduction

Various species of *Salvia* of the family Lamiaceae have long been used as traditional medicines and for culinary purposes. There are over 900 species of *Salvia* worldwide, about half of them in Central and South America (ca. 500 species) while the others are in Central Asia and the Mediterranean region (ca. 250 species), Eastern Asia (ca. 90 species), and Southern Africa (ca. 30 species) (Walker et al., 2004). Phytochemical studies on *Salvia* species have been extensively carried out, and their main chemical constituents can be classified as polyphenols and terpenoids. Various caffeic acid derivatives have been found in this genus (Lu and Foo, 2002). The major compounds of the aerial parts are flavonoids, triterpenoids and volatile substances, mainly monoterpenes, whereas diterpenoids are commonly found in the roots (Topcu, 2006).

Salvia viridis L. (Synonym *Salvia horminum* L.), or Red Topped Sage, is a perennial, annual, or biennial herb with erect stems up to 50 cm, 4–8 flowers in axillary verticillasters with various colored bracts (Hedge, 1972). Various cultivars with colored bracts are grown as garden ornamentals. It has been used in traditional medicine, for example, an infusion of leaves as a gargle for sore gums, and has also been employed to increase the quality of liquor by putting leaves and seeds into the fermentation tank (Dweck, 2000). Previous phytochemical reports on *S. viridis* L. and its synonym, *S. horminum* reported the presence of triterpenoids (Ulubelen and Brieskorn, 1975; Ulubelen et al., 1977), volatile oils (Kokkalou et al., 1982) and flavonoids (Kokkalou and Kapetanidis, 1988) in the aerial parts and diterpenoids in the roots (Ulubelen et al., 2000). These reports focussed on particular chemical groups in either roots or aerial parts of plants collected in the wild. The

* Corresponding author. Tel.: +66 83 123 4251.
E-mail address: rsupattra@outlook.com (S. Rungsimakan).

http://dx.doi.org/10.1016/j.phytochem.2014.08.029
0031-9422/

present study aimed to investigate the constituents of both the roots and aerial parts of the cultivar "Blue Jeans" to discover their respective secondary metabolite profiles across a range of biosynthetic pathways. The antibacterial activity of several of these chemical constituents was examined to complement previous reports (Moujir et al., 1993; Ulubelen et al., 2000).

2. Results and discussion

In the present study, roots and aerial parts of *S. viridis* L. were extracted and fractionated separately and gave a total of 31 compounds including those shown in Fig. 1. The non-polar fractions derived from roots of *S. viridis* L. yielded a series of abietane diterpenoids including three novel compounds **1**, **2**, **3**, and microstegiol (**4**), 7α-acetoxy-14-hydroxy-8,13-abietadiene-11,12-dione (**5**; 7-*O*-acetylhorminone tautomer), 7α,14-dihydroxy-8,13-abietadiene-11,12-dione (**6;** horminone tautomer), ferruginol and salvinolonyl 12-methyl ether (**7**) that had been previously reported.

The ^{1}H NMR spectra of all the diterpenoids showed signals for two overlapped methyl doublets coupled to a methine septet typical of an isopropyl group, and five methyl signals in all which suggests an abietane or rearranged abietane skeleton.

High resolution MS of compound **4** suggested a molecular formula of $C_{20}H_{26}O_2$ and its ^{1}H and ^{13}C NMR spectra (Table 1) were identical to those reported for microstegiol (Ulubelen et al., 1992).

Compound **1** ($C_{20}H_{24}O_3$) had similar ^{1}H and ^{13}C NMR spectra to microstegiol except for the presence of an additional ketone signal at δ 207.21 in the ^{13}C NMR spectrum and one fewer methylene signals in the DEPT spectra. The signal at δ 207.21 showed HMBC correlation (Fig. 2) to a proton at δ 3.11 (H-2 axial) while H-2 equatorial at δ 2.30 showed a long range HMBC correlation to an aromatic carbon signal at δ 138.14 (C-9). The presence of a ketone group at C-1 caused the adjacent aromatic carbon atom (C-10) to resonate at somewhat higher field (δ 137.56) than the equivalent carbon atom in microstegiol (δ 143.25). Using data tables published in the spectroscopic interpretation handbook (Williams and Fleming, 2008), a predicted chemical shift was calculated for C-10 in microstegiol as δ 141.6 and for 1-oxomicrostegiol as δ 135.2. When compared with the actual values of δ 143.3 and δ 137.6 respectively, both these values are well within the margin of error to be expected from the application of these empirical tables to a tetra-substituted aromatic compound. Moreover, the difference between the two pairs of values is 6.4 ppm for the calculated values and 5.7 ppm for the measured values. Other protons and carbon atoms were assigned by HMBC and NOESY correlations

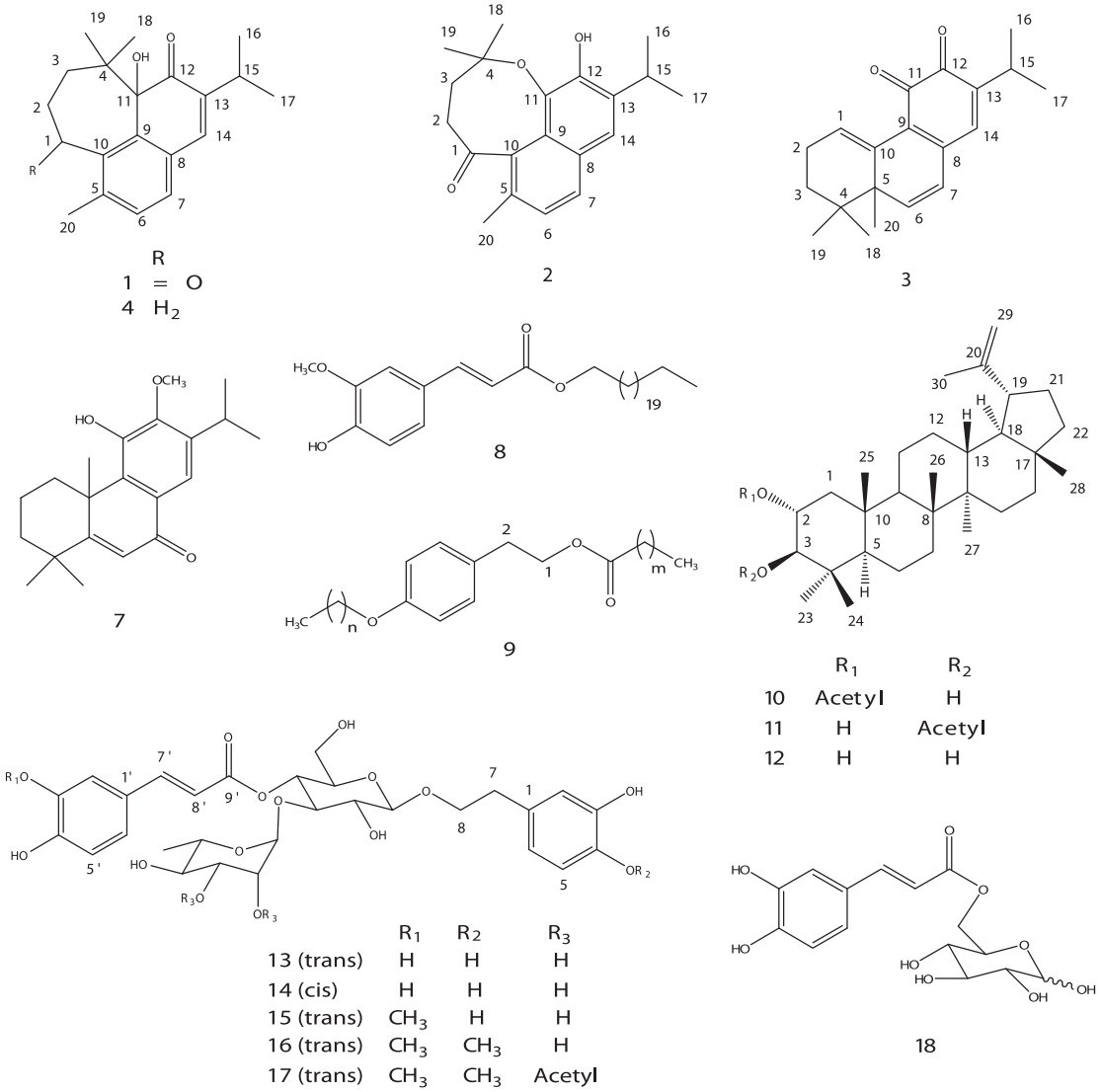

Fig. 1. Compounds isolated from *S. viridis* L. cvar. Blue Jeans (Diterpenoid numbering follows a biogenetic terpenoid system rather than the IUPAC system).

Please cite this article in press as: Rungsimakan, S., Rowan, M.G. Terpenoids, flavonoids and caffeic acid derivatives from *Salvia viridis* L. cvar. Blue Jeans. Phytochemistry (2014), http://dx.doi.org/10.1016/j.phytochem.2014.08.029