



Chemistry of *Zataria multiflora* (Lamiaceae)

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Abstract

The hexane soluble part of *Zataria multiflora* afforded three new aromatic constituents which include two *p*-cymene derivatives: multiflotriol (**1**), multiflorol (**2**) and an aromatic ester of *p*-hydroxy benzoic acid (**3**). In addition to these three new compounds, three known compounds, dihydroxyaromadendrane, luteolin and α -tocopherolquinone, have also been isolated for the first time from the same source. The structures of all the isolated constituents were elucidated by means of spectroscopic methods including 2D-NMR techniques. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: *Zataria multiflora*; Lamiaceae; Multiflotriol; Multiflorol; Aromatic ester; Known constituents; Spectroscopy; Structure elucidation

1. Introduction

The family Lamiaceae previously called Labiatae is famous due to its members containing diterpenoids with antitumor activity (Fujita et al., 1976; Nagaa et al., 1982; Fujita and Node, 1984). Many biologically active essential oils have also been obtained from various Lamiaceous plants (Peana et al., 1994). *Salvia* is the largest genus of this family having about 800 species found throughout the world (Bruce et al., 1983). Another important genus of the same family is *Zataria*. The members of genus *Zataria* are widely distributed in Iran, Afghanistan and Pakistan. *Zataria multiflora*, an aromatic member of genus *Zataria* is used as a stimulant and also prescribed for the pre-mature labor pains (Gupta and Gupta, 1972). The biological and medicinal importance of the family Lamiaceae, genus *Zataria* and *Z. multiflora* prompted us to investigate *Z. multiflora* phytochemically. We have already reported some new and known constituents from *Z. multiflora* (Ali et al., 1999a,b). Now, we wish to compile our remaining phytochemical results of *Z. multiflora* in this last communication which describes the isolation and structure elucidation of two new *p*-cymene derivatives: multiflotriol (**1**), multiflorol (**2**), a new aromatic ester of *p*-hydroxy benzoic acid (**3**) and three known constituents:

dihydroxyaromadendrane (Bohlmann et al., 1983; Goldsby and Burke, 1987), luteolin (Sashida et al., 1983) and α -tocopherolquinone (Rasool et al., 1991) which have not been reported so far from this source.

2. Results and discussion

Among the new constituents (**1–3**), the molecular ion of **1** was observed in FDMS at m/z 182. The molecular formula of **1** was deduced from HREIMS as $C_{10}H_{14}O_3$ (m/z 182.09157 calcd, m/z 182.09428) with four degrees of unsaturation. The 1H NMR spectrum of **1** showed a pair of doublets ($J=6.8$ Hz) of three protons integration at δ 1.07 (H-9) and 1.03 (H-10), respectively, and a multiplet of one proton integration at δ 2.67 (H-8). The cross peak of the multiplet at δ 2.67 with the doublets at δ 1.07 and 1.03, respectively, in the COSY-45° experiment revealed the presence of an isopropyl moiety in the molecule. The downfield chemical shift of H-8 (δ 2.67), its HMBC correlations with carbons at δ 111.5 (C-5) and 132.6 (C-3) and comparative study (Ali et al., 1999a) confirmed the direct attachment of isopropyl moiety to the benzene ring. A downfield singlet of three protons at δ 2.20 was due to the Me-7 and the chemical shift of this signal showed the direct attachment to benzene ring. The Me-7 was placed *para* to the isopropyl unit on the bases of biogenetic grounds (Croteau, 1987) and also confirmed through HMBC interactions (Fig. 1). Only a singlet of one proton integration in the

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aromatic region at δ 6.74 (H-5) revealed the presence of five substitutions at benzene ring. The position of only one available methine and quaternary carbons in the benzene ring were attested on the basis of HMBC experiments (Fig. 1) and also this is in accordance with literature values (Ali et al., 1999a,b). This compound is a new addition in the list of constituents isolated from *Z. multiflora* and named multiflotriol.

The molecular ion of **2** appeared at m/z 194 in the EIMS which was counter checked by FDMS. The molecular formula $C_{12}H_{18}O_2$ was confirmed through HREIMS (m/z 194.13059, calcd m/z 194.13067) consistent with four degrees of unsaturation. Compound **2** was found different from **1** in two aspects: The appearance of three aromatic methines in the 1H NMR spectrum of **2** instead of one as observed in **1** revealed the absence of two substituents in the benzene ring and secondly, the presence of an ether function at C-3 position. The aromatic methines appeared in the 1H NMR spectrum at δ 6.83 (d, $J=1.5$ Hz, H-2) δ 7.02 (d, $J=7.5$ Hz, H-5) and δ 6.77 (dd, $J=7.5, 1.5$ Hz, H-6), respectively. The magnitudes of coupling constants showed that the methine at δ 6.77 (H-6) was *ortho* to the

methine resonated at δ 7.02 (H-5) and *meta* to the methine at δ 6.83 (H-2). A pair of triplets at δ 4.04 (H-1') and 3.62 (H-2') with a coupling constant of $J=7.0$ Hz showed their direct attachment to oxygen atom(s). The coupling constant (7.0 Hz) and their mutual interaction in COSY-45° spectrum confirmed that both the triplets were vicinal to each other. The HMBC correlation (Fig. 2) of a signal at δ 4.04 (H-1') with the quaternary carbon at δ 153.8 (C-3) confirmed the presence of ether linkage at C-3 position. The chemical shifts in both the 1H and ^{13}C NMR of **2** were assigned on the basis of HMBC, COSY-45° spectra (Fig. 2), and by comparison with the NMR data of **1** and literature values (Ali et al., 1999a). Compound **2** is another new constituent of *Z. multiflora* and named multiflorol.

The molecular ion peak of **3** was observed in EIMS and FDMS at m/z 348 corresponding to the molecular formula $C_{22}H_{36}O_3$ (m/z 348.26509, calcd m/z 348.26643) which was confirmed through HREIMS showing the presence of five degrees of unsaturation. The 1H NMR spectrum of **3** showed an AA'BB' system at δ 7.04 (H-2, H-6) and 6.73 (H-3, H-5) which was further confirmed through COSY-45° (Fig. 3) indicating *para* substitution in the benzene ring. The 1H NMR spectrum also showed two triplets, one with two protons integration at δ 4.21 ($J=7.1$ Hz, H-1') and the other with three protons integration at δ 0.86 ($J=6.6$ Hz, H-15') corresponding to the presence of a methylenoxy ($-OCH_2-$) and a methyl moiety, respectively, in the molecule. A broad singlet of 26 protons in the upfield region at δ 1.16–1.33 and its cross-peaks with the signals at δ 4.21 (H-1') and 0.86 (H-15') in COSY-45° spectrum (Fig. 3) showed the presence of a 15-carbon chain. This chain was found to be a part of an ester which was determined with the aid of HMBC correlation of methylenoxy protons (δ 4.21) to the ester carbonyl carbon (δ 174.3). All the chemical shifts were confirmed through HMBC, COSY-45° experiment (Fig. 3). This is another new secondary metabolite of *Z. multiflora* and assigned as pentadecanyl *p*-hydroxybenzoate.

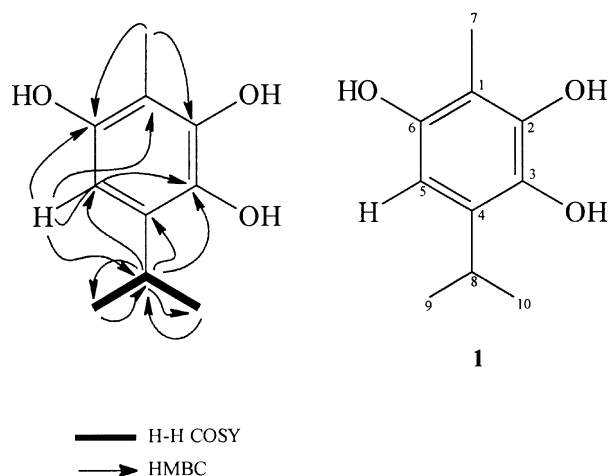


Fig. 1.

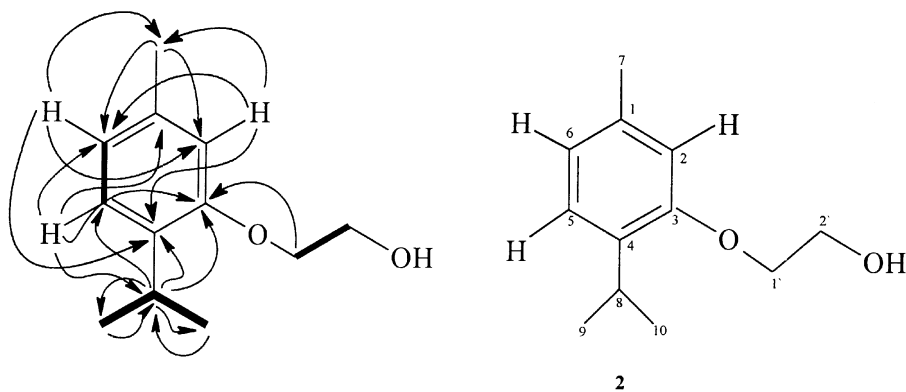


Fig. 2.

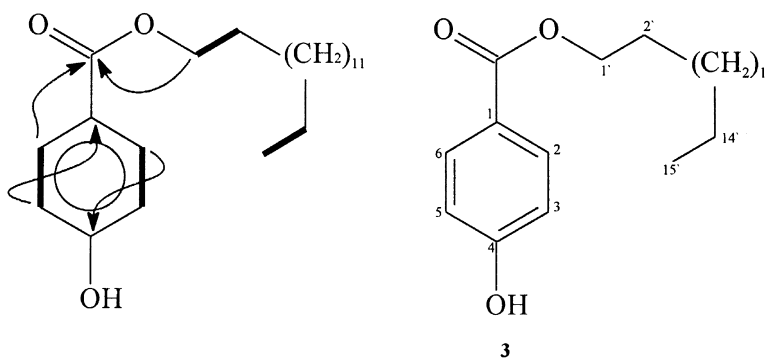


Fig. 3.

Z. multiflora is an aroma containing plant and the isolated *p*-cymene/thymol-type derivatives were expected from this source. Genus *Zataria* is comprised of only two species and not much work has been done on them (Gupta and Gupta, 1972) except our two recent reports (Ali et al., 1999a,b). Due to the lack of amounts of **1–3**, the biological study could not be performed. However, the chemical literature shows that several constituents with thymol-type skeleton, obtained from a Lamiaceous plant (*Monarda fistulosa*) have pesticidal, anti-tumor and anti-depressant activities (Johnson et al., 1998). In the light of this information, it can be forecasted that our isolated compounds of the same skeleton (**1–2**) may have the same properties. In addition to **1–3**, three known compounds: dihydroxyaromadendrane, luteolin and α -tocopherolquinone have also been isolated for the first time from the same source.

3. Experimental

The ^1H and ^{13}C NMR spectra were recorded in CDCl_3 , and CD_3OD at 300, 400, 500 MHz and 75, 100, 125 MHz, respectively.

3.1. Collection, identification and extraction

The plant material (whole parts) was collected from Quetta, Baluchistan, Pakistan, and identified by Dr. R.B. Tareen, Department of Botany, Baluchistan University, Pakistan, where the voucher specimen has been deposited in the herbarium (No. 367). The plant material was dried under shade for 2 weeks and the dried material (16 kg) was then chopped and soaked in hexane (28 l) twice. The combined hexane extracts were evaporated in vacuo at 28°C . The resulting gum thus obtained (415 g), was subjected to column chromatography (CC). Hexane, hexane: CHCl_3 , CHCl_3 , CHCl_3 : MeOH and finally, pure MeOH were used as mobile phase.

3.2. Isolation, purification and characterization

The fraction eluted with 5% CHCl_3 in hexane contained **3** which was further purified by repeated CC and then by TLC (CHCl_3 –hexane, 7:13) as a gum (13.1 mg). The fraction from the same column eluted with 25% CHCl_3 in hexane afforded **2**. After repeated CC and finally, by prep. TLC (EtOAc–hexane, 1:9) **2** was obtained as a light yellow oily mass (12.5 mg). With 30% CHCl_3 in hexane, some fractions were obtained having a lot of chlorophyll which on pooling, charcoaling and then repeated CC afforded dihydroxyaromadendrane as a green jelly like mass (15.2 mg). α -Tocopherolquinone was obtained with 40% CHCl_3 in hexane as a yellow mobile oil (17.3 mg). A solid material eluted with 70% CHCl_3 in hexane contained a single UV active compound **1** which was washed with a mixture of hexane– CHCl_3 (4:1) to afford **1** as a yellowish gum (11.3 mg). Elution with 5% MeOH in CHCl_3 yielded a semi-pure yellow powder which on washing with CHCl_3 gave luteolin (10.0 mg).

3.2.1. Multiflotriol (**1**)

EIMS m/z : 182 (M^+); FDMS m/z : 182; HREIMS m/z : 182.09157 ($\text{C}_{10}\text{H}_{14}\text{O}_3$ requires m/z 182.09428); ^1H NMR (CD_3OD 300 MHz): δ 1.03 and 1.07 (6H, *d*, $J=6.8$ Hz, isopropyl), 2.20 (3H, *s*, H-7), 2.67 (1H, *m*, H-8), 6.74 (1H, *s*, H-5); ^{13}C NMR (CD_3OD , 75 MHz): δ 120.17 (C-1), 154.2 (C-2), 132.6 (C-3), 146.4 (C-4), 111.5 (C-5), 153.4 (C-6), 15.3 (C-7), 29.2 (C-8), 23.1 (C-9), 24.8 (C-10).

3.2.2. Multiflorol (**2**)

EIMS m/z : 194 (M^+); FDMS m/z : 194; HREIMS m/z : 194.13059 ($\text{C}_{12}\text{H}_{18}\text{O}_2$ requires m/z 194.13067); ^1H NMR (CDCl_3 , 500 MHz): δ 1.13 (3H, *d*, $J=6.8$ Hz, isopropyl), 1.18 (3H, *d*, $J=6.8$ Hz, isopropyl), 2.18 (3H, *s*, H-7), 2.8 (1H, *m*, H-8), 3.62 (2H, *t*, $J=7.0$ Hz, H-2'), 4.04 (2H, *t*, $J=7.0$ Hz, H-1'), 6.77 (1H, *dd*, $J=7.5$, 1.5

Hz, H-6), 6.83 (1H, *d*, *J* = 1.5 Hz, H-2), 7.02 (1H, *d*, *J* = 7.5 Hz, H-5); ^{13}C NMR (CDCl_3 , 125 MHz): δ 120.3 (C-1, C-2), 153.8 (C-3), 146.9 (C-4), 120.8 (C-5), 130.9 (C-6), 16.4 (C-7), 64.4 (C-1'), 63.1 (C-2'), 33.7 (C-8), 24.1 (C-9), 24.0 (C-10).

3.2.3. Pentadecanoyl *p*-hydroxybenzoate (**3**)

EIMS m/z : 348 (M^+), 121 (100%); HRMS m/z : 348.26509 ($\text{C}_{22}\text{H}_{36}\text{O}_3$ requires m/z 348.26643); FDMS m/z : 348; IR ν_{max} (CHCl_3) cm^{-1} : 3540 (OH), 1715 (CO); ^1H NMR (CDCl_3 , 400 MHz): δ 0.86 (3H, *t*, *J* = 6.6 Hz, H-15'), 1.16–1.33 (26H, *br.s.*, 2'–13'), 4.21 (2H, *t*, *J* = 7.1 Hz, H-1'), 6.73 (2H, *d*, *J* = 8.5 Hz, H-3, H-5), 7.04 (2H, *d*, *J* = 8.5 Hz, H-2, H-6); ^{13}C NMR (CDCl_3 , 100 MHz): δ 128.6 (C-1), 129.6 (C-2, C-6), 115.1 (C-3, C-5), 174.3 (C-7), 155.3 (C-4), 65.1 (C-1'), 29.2 (C-2'–C-14'), 13.6 (C-15').

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