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Three *p*-cymene derivatives from *Zataria multiflora*

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Abstract

Two new *p*-cymene derivatives named zataroside-A and zataroside-B and a new triacetoxy derivative of the same skeleton have been detected from *Zataria multiflora* along with *p*-hydroxybenzoic acid. Their structures were elucidated with the aid of NMR spectroscopy. Zataroside-A was also confirmed by X-ray diffraction technique and the same compound was found to be a strong plant growth inhibiting agent.

Keywords: *Zataria multiflora*; Lamiaceae; *p*-Cymene derivatives; Spectroscopy; X-ray; Structure elucidation; Phytotoxicity

1. Introduction

The members of the Lamiaceae have been shown to possess aroma containing oils and diterpenoids having antitumor activity (Fujita, Nagao, Kaneko, Nakazawa & Kuroda, 1976; Fujita & Node, 1984; Nagao, Ito, Kohno, Kuroda & Fujita, 1982). The genus *Salvia,* the largest genus of this family, comprises of about 800 species containing mostly diterpenoids (Bruce, John, Peter & Dianne, 1983). After *Salvia*, the second famous genus of the same family is *Zataria* distributed in Iran, Afghanistan and Pakistan. Not many chemical constituents have been recorded from this genus. *Z. multiflora* is found abundantly in Quetta, Baluchistan (Pakistan). It is used as a stimulant and also prescribed for the treatment of premature labour pain (Gupta & Gupta, 1972). Previously, we have reported some known, synthetically known and new constituents from *Z. multiflora* (Ali, Saleem & Ahmed, 1999). Now, we wish to describe the isolation and structure elucidation of two new hydroxy-glycosides (**1**–**2**) of *p*-cymene (**5**), a new triacetoxy derivative (3) of same skeleton and *p*-hydroxybenzoic acid (**4**) from the same source. The X-ray structure and phytotoxicity of **1** are also described.

2. Results and discussion

Compound **1** was isolated from the hexane soluble part of *Z. multiflora* as colorless crystals. The molecular mass was observed in the FDMS at *m*/*z* 328 corresponding to the molecular formula $C_{16}H_{24}O_7$ (328.1519 calcd. 328.1521891) which was confirmed through HRMS with five degrees of unsaturation. The ^{1}H NMR spectrum of **1** showed the presence of two aromatic signals at δ 6.67 and 6.98 as singlets corresponding to H-5 and H-2 respectively. The same spectrum also showed three methyl signals. Two appeared at δ 1.15 and 1.13 having the integration of three protons each in the form of a pair of doublets having the coupling constants 6.9 Hz. This pair of doublets was found to be coupled with a multiplet appeared at δ 3.30. This pair of doublets and the related multiplet were due to the isopropyl moiety which is attached to the aromatic ring at position 4. In addition to methyls due to isopropyl unit, another methyl appeared as a

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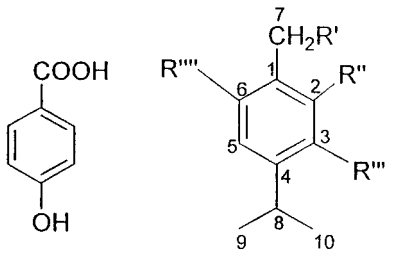
singlet at δ 2.12. The chemical shift of this methyl group reflects the direct attachment to the aromatic ring. The four degrees of unsaturation could be satisfied by the aromatic ring whereas, the fifth degree of unsaturation was due to the sugar moiety which was confirmed by the appearance of an anomeric signal resonated at δ 4.72 (J = 7.4 Hz.). The magnitude of coupling constant suggested the presence of glucose moiety in the molecule with β-D-configuration. The remaining glucose methine signals appeared between δ 3.45–3.71 as a multiplet with the over all integration of four protons.



4

	R′	R″	R‴	R⁗
1	H	H	OGlc	OH
2	H	H	OH	OGlc
3	OAc	OAc	OAc	H
3a	OH	OH	OH	H
5	H	H	H	H

The ^{13}C NMR of **1** accounted for sixteen carbon signals. Six were due to the glucose moiety. The multiplicities of the carbon signals were determined through DEPT experiment. The signal at δ 104.1 was due to the anomeric carbon. The remaining glucose carbon signals were displayed their presence at δ 75.0, 78.0, 71.7, 77.5 and 62.9. The two down field quaternary carbon signals resonated at δ 148.9 and 151.2 attested for the carbon attached to the glucose moiety and hydroxyl function. The aromatic methines exhibited their signals at δ 112.8 (C-5) and 120.2 (C-2). Methyls due to the isopropyl unit were found to exhibit their existence at δ 23.5 and 23.4. The signal at δ 16.0 was attributed to the only tertiary methyl attached to the aromatic ring.

Finally, the structure of **1** was confirmed through single-crystal X-ray diffraction technique as 3-*O*-β-D-glucopyranoxy-6-hydroxy-*p*-cymene (Fig. 1) and named as zataroside-A. **1** showed 100% inhibition with 50–500 μg/ml concentration, equal to standard inhibitor (Paraquat) when tested against *Lemna acqui-*

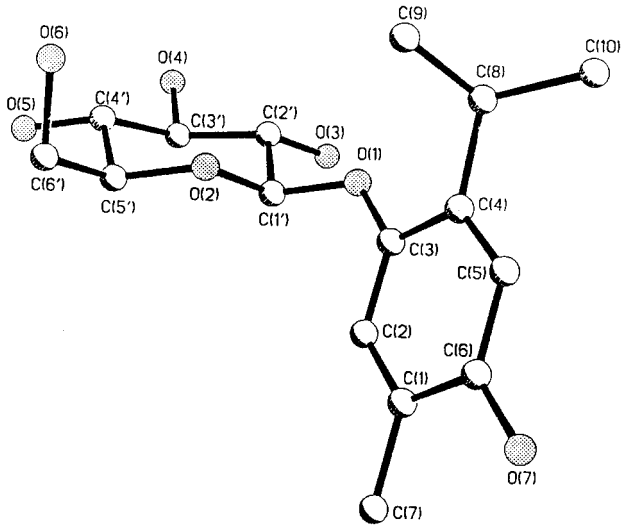


Fig. 1. A computer generated perspective drawing of the final X-ray model. Hydrogens are omitted for clarity.

noctialisn Welv. This compound has not been reported so far from any natural source. However, Shiow-yunn et al. reported the same compound after enzymatic hydrolysis of qerglanin (Shiow-yunn, Feng-lin & Yu-chan, 1992). Glycosides of a similar type have already been reported in the literature (Ahmed & Jakupovic, 1990).

Another glucoside (**2**) of the same skeleton was isolated just after **1**. The spectroscopic data were found to be almost similar to **1** and concluded that the only difference due to the position of glucose moiety. The change in position of glucose moiety was observed due to the shifting of signals in the ^{13}C NMR spectrum. The shifted chemical shifts in **2** compared to **1** are summarised in Fig. 2 and thus the structure is assigned as 6-*O*-β-D-glucopyranoxy-3-hydroxy-*p*-cymene and named zataroside-B. Finally, the structure of **2** was determined with the help of comparative spectral data of **1**. This is also a new natural constituent.

The fraction obtained with 10% methanol in chloroform gave **3** as a solid material. The molecular ion peak was observed in FDMS at m/z 308 corresponding to the molecular formula $C_{16}H_{20}O_6$ with seven degrees of unsaturation. The ^{1}H NMR of **3** displayed the pre-

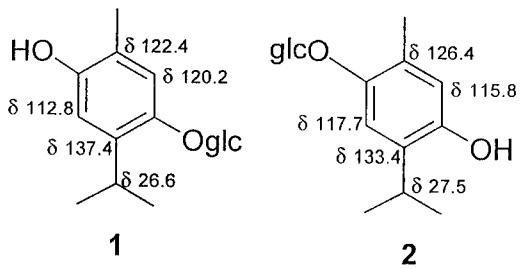


Fig. 2. Change in chemical shifts in both isomers 1 & 2

sence of three acetoxyl moieties and their corresponding methyls were appeared at δ 2.01, 2.02 and 2.03 as singlets. The only two aromatic protons appeared at δ 6.81 and 6.92 with 7.6 Hz coupling constant indicating the *ortho* relationship and thus assigned to H-5 and H-6, respectively.

This compound has recently been reported by us from the same source as a derivative of zatatriol (**3a**) (Ali et al., 1999) and now we wish to report the same derivative as a natural constituent. The ^{13}C NMR and other physical data of **3** exactly matched with the reported data (Ali, et al., 1999).

The last compound which could be isolated (**4**) was characterised as *p*-hydroxybenzoic acid which is a very common metabolite and isolated from various plants (Scott, 1972). The molecular mass was observed in EIMS at *m/z* 138. The base peak at *m/z* 121 was due to the loss of hydroxyl function from the molecule. The formula of **4** was determined with the aid of HRMS as $C_7H_6O_3$ with five degrees of unsaturation. The presence of hydroxyl function was confirmed by the broad absorption at 2400–3600 cm^{-1} in the IR spectrum. The broadness of this absorption band due to the overlapping of carboxyl (OH) function. The carboxyl function was confirmed in the molecule by the significant absorption band at 1695 cm^{-1} in the same spectrum. The ^1H NMR of **4** showed only two doublets in the aromatic region at δ 6.75 and 7.05 showing the same coupling constant (J = 8.5 Hz) with two protons integration each corresponding to the H-2, H-6 and H-3, H-5, respectively. On the basis of spectral information the structure of **4** assigned as *p*-hydroxybenzoic acid. This compound has not been reported so far from our investigated source.

3. Experimental

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

3.1. Collection, identification and extraction

The plant material was collected from Quetta, Baluchistan (Pakistan) and identified by Dr. R.B. Tareen, Department of Botany, Baluchistan University, where voucher specimen has been deposited (no. 367). The plant material was dried under shade for two weeks. The dried material (16 kg) was then soaked in hexane (28 l). The hexane extract was evaporated under reduced pressure and the resulting gum thus obtained (415 g), was subjected to silica gel column chromatography. Hexane, hexane:chloroform, chloroform, chloroform:methanol and finally, pure methanol were used as mobile phase.

3.2. Isolation, purification and characterization

The fraction eluted with 30% chloroform in hexane was further purified by repeated column chromatography and finally by preparative layer chromatography using 5% acetone in hexane as a mobile phase. The compound **4** was obtained as a white powder (7.0 mg).

EIMS: *m/z* 138 $[M]^+$, 121 (M–OH, 100%)$^+$; HRMS: *m/z* 138.03433 (calcd. 138.0316899 for $C_7H_6O_3$), 121.02974 (calcd. 121.0289512 for $C_7H_5O_2$); IR (KBr): 2400–3600 (OH), 1695 (CO) cm^{-1}; ^1H NMR (CD_3OD, 400 MHz): δ 6.75 (2H, d, J = 8.5 Hz, H-2 and H-6), 7.05 (2H, d, J = 8.5 Hz, H-3 and H-5).

A solid material (**3**) was obtained with 10% methanol in chloroform from the same column which was washed with hexane. The same material was later converted into an oily mass (14.8 mg).

FDMS: *m/z* 308 $(M)^+$; HRMS: *m/z* 308.124709 ($C_{16}H_{20}O_6$ requires 308.125978); ^1H NMR($CDCl_3$, 400 MHz): δ 1.16 and 1.17(3H each, d, J = 6.9 Hz, isopropyl), 2.01(3H, s, OAc), 2.02(3H, s, OAc), 2.03(3H, s, OAc), 2.92(1H, m, H-8), 4.26(2H, dd, J = 7.91, 5.56 Hz, H-7), 6.81(1H, d, J = 7.6Hz, H-5) and 6.92 (1H, d, 7.6 Hz, H-6).

Another fraction obtained with 15% methanol in chloroform was further cleaned through repeated column chromatography. As a result of this, **1** was isolated as colourless crystals (24.0 mg).

$[\alpha]_D$: −25.8° (c 1.65, methanol); M.P.: 80–82°C; EIMS: *m/z* 328 $[M]^+$, 166 $[M–C_6H_{10}O_5]^+$; FDMS: *m/z* 328; HRMS: *m/z* 328.1517(calcd 328.1521891 for $C_{16}H_{24}O_7$), 166.1007(calcd. 166.0993726 for $C_{10}H_{14}O_2$), 165.0885 (calcd. 165.0993726 for $C_{10}H_{13}O_2$); ^1H NMR (CD_3OD, 300 MHz): δ 1.13 and 1.15 (3H each, d, J = 6.9 Hz, isopropyl), 2.12 (3H, s, H-17), 3.30 (1H, m, H-8), 3.45–3.71 (4H, m, glucose-H), 3.67 (1H, dd, J = 12.2, 2.4 Hz, H-6′), 3.87 (1H, dd, J = 12.2, 6.1 Hz, H-6′), 4.72 (1H, d, J = 7.4 Hz, anomeric), 6.67 (1H, s, H-5), 6.98 (1H, s, H-2); ^{13}C NMR (CD_3OD, 75 MHz): δ 122.4 (C-1), 120.2 (C-2), 148.9 (C-3), 137.4 (C-4), 112.8 (C-5), 151.2 (C-6), 16.0 (C-7), 26.6 (C-8), 23.5 and 23.4 (isopropyl), 104.1 (C-1′), 75.0 (C-2′), 78.0 (C-3′), 71.7 (C-4′), 77.5 (C-5′) and 62.9 (C-6′).

3.3. X-ray data for zataroside-A (*1*)

The compound has a molecular formula $C_{16}H_{24}O_7$. Diffraction data were measured on a Nicolet X-ray diffractometer with graphite monochromated CuK radiation. The unit cell parameters were determined by a least squares fit of 20 reflections with θ range 3.35–67.47°. No crystal decay was observed. The structure was solved by direct method and the final R value was 0.0440. Crystals were monoclinic with cell parameters: A = 8.551 (2) A^o ; V = 874.6 (3) A^{o3} ; Space

Group = $P2_1$, $z = 2$. Data deposited with the X-ray Crystallographic Centre, Cambridge, UK.

During the repeated column chromatography after **1**, with the same polarity second compound **2** could be isolated in minor amount (11.1 mg) as an oil.

^{1}H NMR (CD_3OD, 300 MHz): δ 1.14 and 1.16 (3H each, d, J = 6.9 Hz, isopropyl), 2.12 (3H, s, H-7), 3.19 (1H, m, H-8), 4.68 (1H, d, J = 7.4 Hz, anomeric), 6.52 (1H, s, H-5), 6.97 (1H, s,. H-2); ^{13}C NMR (CD_3OD, 75 MHz): δ 126.4(C-1), 115.8(C-2), 150.3(C-3), 133.4(C-4), 117.7(C-5), 15.0(C-6), 16.0(C-7), 27.5(C-8), 22.9 and 23.0(isopropyl), 103.9(C-1′), 74.9(C-2′), 78.2(C-3′), 71.7(C-4′), 77.5(C-5′) and 62.4(C-6′).

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