PERGAMON

Phytochemistry 52 (1999) 1319–1322

PHYTOCHEMISTRY

Buchariosside and buchariol from *Salvia bucharica*

Viqar Uddin Ahmad[a,*], Muhammad Zahid[b], Muhammad Shaiq Ali[a], Amir Reza Jassbi[a], Muhammad Abbas[a], Zulfiqar Ali[a], Muhammad Zafar Iqbal[b]

[a]*International Center for Chemical Sciences, H.E.J. Research Institute of Chemistry, University of Karachi, Karachi 75270, Pakistan*
[b]*Institute of Chemistry, University of the Punjab, Lahore, Pakistan*

Received 24 May 1999; accepted 9 July 1999

Abstract

A new monoterpene-glycoside (2-*exo*-β-D-glucopyranosyl-1,8-cineol) named buchariosside from the methanol-soluble part and a new sesquiterpenoid (4,10-epoxy-6α–hydroxyguaiane) named buchariol from the hexane-soluble part of *Salvia bucharica* were obtained. Their structures were elucidated with the help of NMR spectroscopy including 1D and 2D experiments. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: *Salvia bucharica*; Lamiaceae; Buchariosside; Buchariol; Spectroscopy; Structure elucidation

1. Introduction

Salvia is the largest genus of the family Lamiaceae having about 800 species throughout the world (Chadha, 1972). Most of the plants of this genus are well known for their constituents having biological activities, especially anti-tumor activity (Fujita & Node, 1984). The plants of this genus are rich in essential oils and among their constituents, 1,8-cineol and guaiane mono and sesquiterpenes are very common (Rustaiyan, Masoudi & Jassbi, 1997a; Rustaiyan, Komeilizadeh, Masoudi & Jassbi, 1997; Ahmad & Jassbi, 1999). *Salvia bucharica* is a conspicuous aromatic plant that grows in Pakistan, Afghanistan and central Asia (Hedge, 1990). It is locally called 'sursunda' and traditionally used for the treatment of liver disorders.

2. Results and discussion

The methanolic extract of *Salvia bucharica* was subjected to repeated column chromatography and then HPLC to purify **1**, which exhibited the molecular ion peak at *m/z* 332.1793 (calcd. *m/z* 332.1821 for $C_{16}H_{28}O_7$) in HREIMS. The EIMS of **1** showed in addition to the molecular ion peak at *m/z* 332, a prominent peak at *m/z* 170 (52%) due to the loss of glucose moiety from the molecular ion.

The ^{1}H-NMR spectrum suggested *p*-menthane monoterpene type aglycone. The signals at δ 1.39, 1.27 and 1.18 having integration of three protons each assigned to Me-9, Me-10 and Me-7, respectively. The signals at δ 4.05 (*dd*, J = 9.7, 3.2 Hz, H-2$_{endo}$), 2.37 (*dddd*, J = 14.0, 3.1, 3.1, 3.1 Hz, H-3$_{exo}$) and 1.80 (*ddd*, J = 14.0, 9.8, 3.1 Hz, H-3$_{endo}$) were clearly distinguished **1** from its *endo*-isomer (Orihara & Furuya, 1994). The double-doublet at δ 4.05 with coupling constant 9.7 and 3.2 Hz indicated the position of glucose moiety at C-2. The large vicinal coupling constant (9.8 Hz) in the signal of H-3$_{endo}$ at δ 1.80 and H-2$_{endo}$ at δ 4.05 clearly explained the orientation of glucose moiety as *exo* (Orihara & Furuya, 1994).

The ^{13}C-NMR of **1** showed sixteen signals due to aglycone and sugar moiety, which were resolved by DEPT experiments into three methyl, four methylene, seven methine and two quaternary carbons. Signals at δ 23.6, 29.1 and 28.4 were due to the Me-7, Me-9 and Me-10, respectively. The chemical shifts of these three

* Corresponding author.

0031-9422/99/$ - see front matter
PII: S0031-9422(99)00389-1

Table 1
Decoupling experiments of **1**

Number of experiments	Irradiation of ^{1}H	Collapsed ^{1}H
1	δ 4.05 (H-2$_{endo}$)	δ 1.80 (*ddd*, J = 14.0, 9.8 and 3.1 Hz, H-3$_{endo}$) → (*dd*, J = 12.0 and 3.0 Hz) δ 2.37(*dddd*, J = 14.0, 3.1, 3.1 and 3.1 Hz, H-3$_{exo}$) → (*ddd*, J = 14.0, 3.0 and 3.0 Hz)
2	δ 1.80 (H-3$_{endo}$)	δ 4.05 (*dd*, J = 9.7 and 3.2 Hz, H-2$_{endo}$) → (*d*, J = 4.0 Hz) δ 2.37(*dddd*, J = 14.0, 3.1, 3.1 and 3.1 Hz, H-3$_{exo}$) → (*br.s.*)
3	δ 1.30 (H-4)	δ 1.80 (*ddd*, J = 14.0, 9.8 and 3.1 Hz, H-3$_{endo}$) → (*dd*, J = 14.0 and 9.6 Hz) δ 2.37(*dddd*, J = 14.0, 3.1, 3.1 and 3.1 Hz, H-3$_{exo}$) → (*ddd*, J = 14.0, 3.0 and 3.0 Hz) δ 1.64 (*ddd*, J = 17.1, 11.8 and 6.1 Hz, H-5$_{exo}$) → (*dd*, J = 16.0 and 6.0 Hz)
4	δ 1.20(H-5$_{endo}$)	δ 1.64 (*ddd*, J = 17.1, 11.8 and 6.1 Hz, H-5$_{exo}$) → (*dd*, J = 12.0 and 8.0) δ 1.80 (*ddd*, J = 14.0, 9.8 and 3.1 Hz, H-3$_{endo}$) → (*br.dd*)

methyls concluded **1** as 1-8-cineol type skeleton (Formacek & Kubeczka, 1982). The remaining carbon signals were comparable with the reported data of *endo*-isomer of **1** (Orihara & Furuya, 1994).

The relative configuration of **1** was established through NOE experiments. The extensive decoupling experiments (Table 1) and NOE difference experiments (Table 2) suggested the structure of **1** as 2-*exo*-β-D-glucopyranosyl-1,8-cineol and named bucharioside. To the best of our knowledge this compound has not been isolated so far from any natural source. However, the *endo*-isomer of **1** had already been isolated from *Citrus unshiu* peel (Sawabe, Matsubara & Iizuka, 1988).

Compound **2** was purified from hexane soluble part of the same source. The molecular ion peak was observed in the HRMS and FDMS at m/z 238.1933 (calcd. m/z 238.1932) suggesting the molecular formula $C_{15}H_{26}O_2$ showing three degrees of unsaturation. In addition to molecular ion peak in the EIMS other peaks at m/z 223 $[M\text{-}CH_3]^+$, 220 $[M\text{-}H_2O]^+$, 195 $[M\text{-}C_3H_7]^+$, 167, 81 and 71 were also observed. Peaks at m/z 71(80%), 167(12%) and 81(100%) were due to the fragments A, B and C (Scheme 1) (Itigaka, Kurokawa, Moriyama, Sasaki & Watanase, 1985).

The ^{1}H-NMR spectrum of **2** displayed two secondary methyls due to isopropyl unit at δ 0.92 ($J = 6.7$ Hz), 0.93 ($J = 6.7$ Hz) and two tertiary methyls at δ 1.18, 1.42 due to Me-14 and Me-15. The chemical shifts of these methyls were compatible with guaiane type skeleton (Bohlmann & Jakupovic, 1979; Bruno, Torre, Rodriguez & Omar, 1993; Oshima, Iwakawa & Hikino, 1983). A broad doublet at δ 4.00 ($J = 1.5$ Hz) was observed in the spectrum due to hydroxyl bearing methine-carbon which was attested for C-6 (Bohlmann & Jakupovic, 1979).

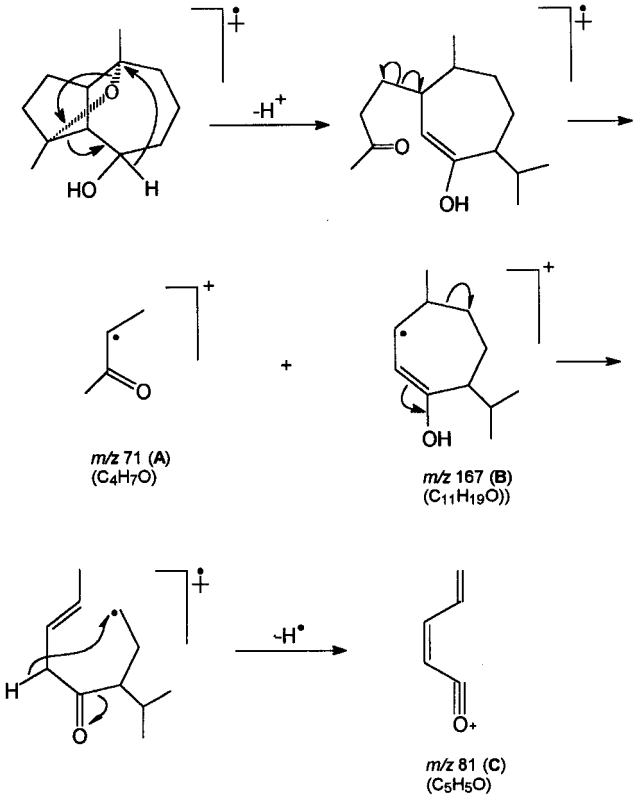
In the broad band spectrum of **2** fifteen signals were observed which were resolved into four methyl, four methylene, five methine and two quaternary carbons. The assignment of these signals were based on the reported data of the related compounds (Itigaka et al., 1985; Bohlmann & Jakupovic, 1979; Bruno et al., 1993; Ahmed, Ela, Adams & Mabry, 1996; Mahmud, 1997; Oshima et al., 1983; Yoshikawa, Hatakeyama, Tanak, Fukuda, Murakami & Yamahara, 1992).

The stereochemistry of **2** was established on the basis of comparative NMR data. A *cis*-fused-4,10 dihydroxy guaiane-type compound showed the same chemical shifts for Me-14 and Me-15 as in **2**, which have proved the stereochemistry of Me-14 and Me-15 as *cis* to each other (Bohlmann & Jakupovic, 1979).

The upfield shift of C-4 (δ 74.4) in comparison to other oxygen-bearing quaternary carbons which normally come at about δ 80.0 could be justified due to the γ-effect of the α-hydroxyl function situated at C-6. Similarly, the downfield shift of C-5 (δ 68.2) was due to β-effect of the same hydroxyl group (Bruno et al., 1993; Ahmed et al., 1996). The small coupling constant of H-6 ($J = 1.5$ Hz) was due to almost 90° dihedral

Table 2
NOE experiments of **1**

Number of experiments	Irradiation of ^1H	% NOE
1	δ 1.27 (H-10):	7.7% NOE at δ 2.37 (H-3$_{exo}$) 3.5% NOE at δ 4.93 (H-1′) 3.7% NOE at δ 1.10.-1.30 (H-6′)
2	δ 1.80 (H-3$_{endo}$):	38% NOE at δ 4.05 (H-2$_{endo}$) 61% NOE at δ 2.37 (H-3$_{exo}$)
3	δ 2.37 (H-3$_{exo}$):	30.1% NOE at δ 1.80 (H-3$_{endo}$) 23% NOE at δ 4.93 (H-1′)



Scheme 1. Proposed mass fragmentation pattern of **2**.

angle between H-5, H-6 and H-7β (Mahmud, 1997) which was confirmed by molecular model. So the orientation of isopropyl unit at C-7 must be α.

On biogenetic grounds it is proposed that **2** may be derived from alismoxide isolated from *Alisma plantago-aquatic* var *orientale* (Oshima et al., 1983) and

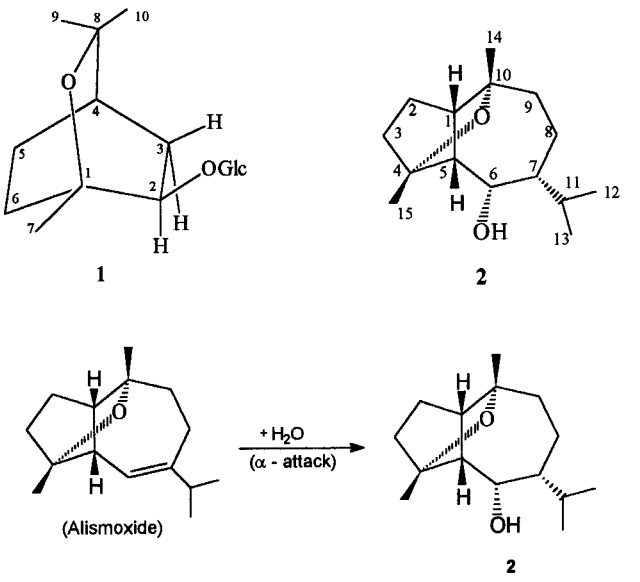


Fig. 1

Alisma orientale (Yoshikawa et al., 1992). On the basis of this suggested biogenetic route (Fig. 1), the hydroxyl function could be placed at C-6. The compound **2** was decomposed after spectroscopic techniques, because of the instability which may be due to the opening of the ether-bridge across C-4 and C-10. This compound is also a new addition in the natural products and named buchariol.

3. Experimental

3.1. General

^{1}H- and ^{13}C-NMR spectra were recorded on Bruker AM-500 and Bruker AM-300 spectrometers. Mass spectra were measured on JMSHX-110 (Jeol).The infra-red (IR) spectra were scanned on JASCO-A 302 spectrophotometer. Optical rotation was carried out on Schmidt and Haensch polartronic-D polarimeter at 25°.

3.2. Plant material

Salvia bucharica (whole plant) was collected from Quetta, Baluchistan, (Pakistan) in June, 1998 and was identified by Dr. Rasool Baksh Tareen, Department of Botany, Baluchistan University, Quetta, where the voucher specimen (No. 354) of the plant has been deposited in the herbarium.

3.3. Extraction and isolation

Shade-dried plant material (6.0 kg) was chopped into small pieces and soaked in hexane (15 l) and then in methanol (15 l) for a period of 10 days each. The solvents were removed at low temperature and pressure to afford gummy masses (hexane part: 92.71 g and methanolic part: 428.24 g). The methanolic extract was partitioned between aqueous and ethyl acetate soluble portions. The ethyl acetate portion (123.439) was subjected to column chromatography using hexane, hexane-chloroform, chloroform, chloroform-methanol and finally, pure methanol as mobile phase.

Compound **1** was obtained from column loaded with ethyl acetate crude extract. The fraction eluted with 10% methanol in chloroform give **1** as a semi-pure sample. After repeated column chromatography, **1** was finally (10.5 mg) purified by HPLC using MeOH–H_2O (9:1) (RP C-18, bondapak column with flow rate 1.5 ml/min. and pressure uptill 300 psi.).

Bucharioside (1): $[\alpha]_D^{25^\circ}$ -25.48^{O} (MeOH, c 2.29). IR ν_{max} (KBr) cm^{-1}: 3450 (OH). FDMS: *m/z* 332. EIMS: *m/z* 332 $[M]^+$, 170 [M-glucose]$^+$. Peak match: *m/z* 332.1793 (calcd. *m/z* 332.1821 for $C_{16}H_{28}O_7$). ^{1}H-NMR (C_5D_5N, 500 MHz): δ 4.05 (1H, *dd*, J = 9.7,

3.2 Hz, H-2$_{endo}$), 1.80 (1H, *ddd*, J = 14.0, 9.8, 3.1 Hz, H-3$_{endo}$), 2.37 (1H, *dddd*, J = 14.0, 3.1, 3.1, 3.1, 3.1 Hz, H-3$_{exo}$), 1.30 (1H, *m*, H-4), 1.64 (1H, *ddd*, J = 17.1, 11.8, 6.1 Hz, H-5$_{exo}$), 1.20 (1H, *m*, H-5$_{endo}$), 1.10–1.30 (1H, *m*, H-6$_{exo}$), 1.80 (1H, *m*, H-6$_{endo}$), 1.18 (3H, *s*, Me-7), 1.39 (3H, *s*, Me-9), 1.27 (3H, *s*, Me-10), 4.93 (1H, *d*, J = 7.8 Hz, H-1′), 3.92 (1H, *br.t*, J = 8.5 Hz, H-2′), 4.26 (1H, *t*, J = 9.6 Hz, H-3′), 4.14 (1H, *br.t*, J = 9.7 Hz, H-4′), 3.97 (1H, *ddd*, J = 10.0, 5.5, 2.5 Hz, H-5′), 4.33 (1H, *dd*, J = 11.7, 5.7 Hz, H-6′a) and 4.55 ((1H, *dd*, J = 11.7, 2.3 Hz, H-6′b). ^{13}C-NMR (C_5D_5N, 75 MHz): δ 71.7 (C-1), 74.5 (C-2), 31.4 (C-3), 33.5 (C-4), 30.5 (C-5), 20.1 (C-6), 23.6 (C-7), 73.4 (C-8), 29.1 (C-9), 28.4 (C-10), 101.4 (C-1′), 74.4 (C-2′), 78.5 (C-3′), 71.8 (C-4′), 78.4 (C-5′), 62.9 (C-6′).

Compound **2** was obtained from hexane soluble part of *Salvia bucharica*. It was eluted with 0.5% methanol in chloroform from silica gel column, which was further loaded on preparative plates using 2% methanol in chloroform as mobile phase. The UV active impurities were removed and finally, **2** was purified by repeated preparative layer chromatography as an oil (12.5 mg).

Buchariol (2): IR ν_{max} ($CHCl_3$) cm^{-1}: 3410 (OH). FDMS: m/z 238. EIMS: m/z 238 $[M]^+$, 223 $[M\text{-}CH_3]^+$, 220 $[M\text{-}H_2O]^+$, 195 $[M\text{-}C_3H_7]^+$, 169, 81 (100%). Peak match: m/z 238.1933 (calcd. m/z 238.1932 for $C_{15}H_{26}O_2$). ^{1}H-NMR ($CDCl_3$ 300 MHz): δ 4.00 (1H, *br.dd*, J = 1.5 Hz, H-6), 2.14 (1H, *br.dd*, J = 9.1,3.1 Hz, H-7), 0.93 (3H, *d*, J = 6.7 Hz, Me-12), 0.92 (3H, *d*, J = 6.7 Hz, Me-13), 1.18 (3H, *s*, Me-14), 1.42 (3H, *s*, Me-15). ^{13}C-NMR ($CDCl_3$ 75 MHz): δ 53.3 (C-1), 23.8 (C-2), 37.5 (C-3), 74.4 (C-4), 68.2 (C-5), 75.9 (C-6), 38.5 (C-7), 20.2 (C-8), 48.2 (C-9), 74.4 (C-10), 32.7 (C-11), 21.1 (C-12), 21.1 (C-13), 21.9 (C-14), 25.8 (C-15).

Acknowledgements

We thank Dr. R.B. Tareen, Department of Botany, Baluchistan University, Quetta, for the collection and identification of the plant material.

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